

TRIAL PROTOCOL

**Prospective, Multicentre Trial to
Assess the Diagnostic Accuracy of
the Truenat Assays at Intended
Settings of Use**

Protocol Number: 7212-03/2

Amendment Number: NA

Short title:
Truenat Evaluation

Version1.0

Date: 07 SEP 2018

Sponsor Name: FIND INDIA

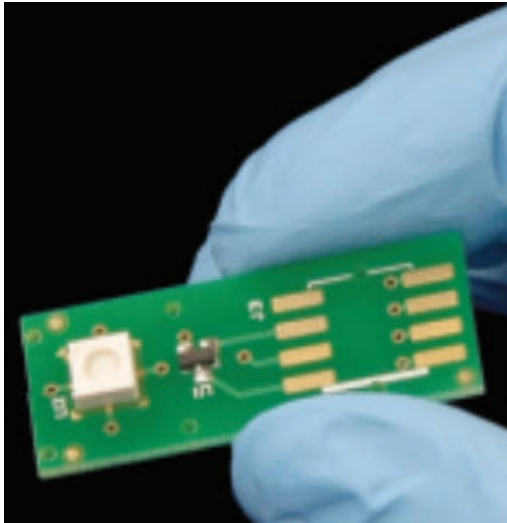
Funder: BMGF and ICMR

Disease Programme: Tuberculosis

Regulatory Agency Identifying Number(s):

www.ClinicalTrials.gov: NCT03712709

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Site 2 (Investigational Site)

To be determined

Site 3 (Investigational Site)

To be determined

Site 4 (Investigational Site)

To be determined

*Terms of references and nature of agreements are available from FIND on request.

Signature Page (Sponsor)

We, the undersigned, have reviewed and approved this Protocol, including Appendices. We will supervise and coordinate the clinical trial as described and ensure adherence to GCP/GCLP, the principles outlined in the Declaration of Helsinki and applicable regulatory requirements.

HEAD OF TUBERCULOSIS PROGRAMME

Name:

Institution:

Signature: _____

Date: _____

DD/MMM/YYYY

HEAD OF CLINICAL & REGULATORY AFFAIRS

Name:

Institution:

Signature: _____

Date: _____

DD/MMM/YYYY

Statement of Principal Investigator

All studies sponsored by FIND will be conducted in accordance with ICH-GCP Guidelines, to the extent possible in the research setting.

In signing this page, I, the undersigned, agree to conduct the trial in compliance with all applicable regulations and guidelines as stated in the protocol and other information supplied to me.

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 Individuals involved with the conduct of this trial have completed GCP training within the past 3 years 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 subject's names or personal identifying information may be disclosed. All subject data will be anonymized and identified by assigned numbers on all Case Report Forms, laboratory samples and source 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.

Name of Principal Investigator: _____

(Print)

Signature: _____

Date: _____

DD/MMM/YYYY

Protocol History/Amendment Summary*

Protocol Version	Date	Description of changes	Brief rationale
Approved 1.0	28 Dec-2018	<ul style="list-style-type: none"> - Changed Draft versions to approved version 1.0 (Common protocol) 	<ul style="list-style-type: none"> - IRB approval at NIRT, Peru, EPHI and Burnet. Pending PNG and Hinduja.
Draft 3.3	07 Sep-2018	<ul style="list-style-type: none"> - Updated section 8.1, study flow, narrative and figure - Placement of a cap of 250 enrolled participants in the drug resistance group. 	<ul style="list-style-type: none"> - Sputum collection and test were altered to pool and homogenise S1 and S2 on Day 1 (allow better distribution of bacilli) and separate S4 to prevent splitting sputum in the microscopy centre, thereby reducing risk of contamination. - Capped to not undermine enrolment of smear-negative culture positive participants for primary aim analysis.
Draft 3.2	27-Aug-2018	<ul style="list-style-type: none"> - Updated section 8.1, study flow, narrative and figure 	<ul style="list-style-type: none"> - Sputum collection and test were altered to i) fit with national guidelines and ii) allow head-to-head comparison of Truenat assays to Xpert on unprocessed sputum.
Draft 3.1	05-Jul-2018	<ul style="list-style-type: none"> - Removed use of frozen samples (RIF resistant). - Introduced differentiation of "Case Detection" group and "Drug Resistant TB" group. 	<ul style="list-style-type: none"> - Full RIF resistance will be performed in a separate laboratory study. - Increased enrolment of participants with presumed RIF resistance.
Draft 3.0	28-Jun-2018	<ul style="list-style-type: none"> - Revised sample size - Added use of frozen samples (RIF-resistant) to supplement testing - No differentiation of trial participants in two groups - Trial to be part of global trial 	<ul style="list-style-type: none"> - TB positivity increased based on pilot data Phase 1b - Mitigate the risk of not meeting targets to determine accuracy of RIF assay - A sub-analysis will be done based on prior history of TB independent of the patient group

		<ul style="list-style-type: none"> - Updated timeline & list of investigators - Format changes - "Trial" replaces "study" - Modified exclusion criteria to having received TB treatment within the last 60 days prior to enrolment. 	<ul style="list-style-type: none"> - Results to be submitted to Indian health authorities as well as WHO, so need collect data outside India - Revised site selection - Changes in protocol template that was developed based on TransCelerate's Common Protocol Template and modified for IVD clinical studies. - Updated FIND definition - Allows enhanced enrolment rate and inclusion of possible TB relapse cases.
Draft 2.1	05-Sep-2017	<ul style="list-style-type: none"> - Truenat MTB & MTB Plus assays 	<ul style="list-style-type: none"> - Side by side assessment of MTB and MTB plus assays to complement India in-country trial
Draft 2.0	22-Aug-2017	<ul style="list-style-type: none"> - Revised sample size - Removed global trial - Removed statistical analysis details - Added potential use of well-characterised samples in future - Updated timeline & list of investigators - Format changes 	<ul style="list-style-type: none"> - Preliminary data Phase 1a - Trial will be conducted only in India - Detailed information to be included in SAP - Better use of well-characterised specimens and reduce need for ad-hoc collection - Update from IRB & site selection completed - Changes in protocol template
Draft Version 1.0 (Original Protocol)	27-Oct-2016	NA	NA

*Refer to Appendix 3 for Protocol Amendment History

List of Abbreviations and Acronyms

Abbreviation/acronym	Meaning
AE	Adverse Event
CRF	Case Report Form
DMC	Designated Microscopy Centres
DNA	Deoxyribonucleic acid
DR TB	Drug Resistant Tuberculosis
DST	Drug Susceptibility Testing
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
GDP	Good Documentation Practice
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
IDMC	Independent Data-Monitoring Committee
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IQC	Income Quality Check
ISF	Investigator Site File
ISO	International Organization for Standardization
MDR TB	Multidrug Resistant Tuberculosis
MGIT (SIRE)	Mycobacterial Growth Indicator Tube (Streptomycin, Isoniazid, Rifampicin, Ethambutol)
MTB	<i>Mycobacterium tuberculosis</i>
NAT	Nucleic acid tests
QA	Quality Assurance
QC	Quality Control
QMS	Quality Management System
RA	Regulatory Authority
RBM	Risk Based Monitoring
RIF	Rifampicin

RM	Risk Management
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SOA	Schedule of Activities
SOP	Standard Operating Procedure
TB	Tuberculosis
TMF	Trial Master File
UPHC	Urban Primary Health Centre

Protocol Synopsis

Title	Prospective, multicentre trial to assess the diagnostic accuracy of the Truenat assays at intended settings of use.
Short title	Truenat Evaluation
Protocol version and date	V3.1, 05-July-2018
Background and rationale	<p>The Truenat MTB (including both MTB and MTB plus) and the MTB-RIF Dx reflex assays (Molbio Diagnostics; Bangalore, India) utilize chip-based real-time micro PCR for detection of tuberculosis (TB) and rifampicin (RIF) resistance from DNA extracted from sputum samples in about 25 minutes.</p> <p>A pilot trial conducted in India of the Truenat MTB assay found the assay to achieve high clinical performance. However, further evidence of the Truenat MTB as well as the Truenat MTB-RIF Dx assay, is needed prior to recommending the clinical use of the assays.</p> <p>Prior studies have been conducted as part of a three-phase/stage approach i) a validation of the Truenat MTB assays and MTB-RIF Dx assay diagnostic performance on frozen samples and ii) an operational assessment of the assays performance. The current trial corresponds to iii) the evaluation trial to confirm the diagnostic accuracy and to ensure that the performance characteristics will be consistent in the sites of intended use in a geographical diverse population (microscopy centre level).</p> <p>Further evidence on the diagnostic performance and patient important outcomes on the use of the Truenat MTB assays and MTB-RIF Dx assay at the microscopy centre level is needed to inform potential policy recommendations.</p>
Objectives	<ol style="list-style-type: none"> 1. Primary objectives: to determine the diagnostic accuracy of the Truenat MTB assays and MTB-RIF Dx assay using culture and phenotypic/genotypic drug susceptibility test (DST) as gold standard in the intended setting of use. 2. Secondary objectives: to determine the diagnostic accuracy of the Truenat MTB assays and MTB-RIF Dx assay compared to Xpert MTB/RIF using culture and phenotypic/genotypic DST as gold standard and to assess patient important outcomes.

Trial design	<p>Prospective, multicentre assessment of Truenat MTB assays and MTB-RIF Dx assay diagnostic accuracy in the intended settings of use.</p> <p>The sample size has been calculated at 1,666 enrolled participants. Sites in India will enrol n= 1,110 participants. Other three global sites will enrol n = 556 participants overall.</p>
Trial sites/setting	India and three other global sites. In India the trial will be conducted at 9 designated microscopy centres (DMC) and 1 private laboratory.
Trial population	Adults with pulmonary tuberculosis symptoms.
Eligibility criteria	<p>Individuals who have symptoms consistent with pulmonary TB presenting to participating centres will be assessed and asked to participate. Participants will be categorised into 2 groups:</p> <p><u>Case Detection group</u> - Only participants who have not received any form of TB treatment within the prior 60 days will be enrolled.</p> <p><u>Drug Resistant group</u> – Non-converting TB cases presumed to be at risk of drug-resistance.</p>
Primary outcomes	<p>1. Primary outcomes</p> <p>1.1. Sensitivity of the Truenat MTB assays (including analysis by smear-status and by sample).</p> <p>1.2. Specificity of the Truenat MTB assays (including analysis by sample).</p> <p>1.3. Sensitivity of the Truenat MTB-RIF Dx assay.</p> <p>1.4. Specificity of the Truenat MTB-RIF Dx assay.</p>
Secondary outcomes	<p>2. Secondary outcomes</p> <p>2.1. Sensitivity of the Truenat MTB assays and MTB-RIF Dx assay compared to Xpert MTB/RIF.</p> <p>2.2. Specificity of the Truenat MTB assays and MTB-RIF Dx assay compared to Xpert MTB/RIF.</p> <p>2.3. Patient important outcomes (see details in section Patient important outcomes under Statistical analysis plan).</p>
Trial duration	Approximately 12 months
Time schedule	Trial start expected Q4-2018 with enrolment completion by Q2-2019. Follow-up after 2 months and testing of discordant cases completed Q4-2019.
GCP statement	All studies sponsored by FIND will be conducted in accordance with ICH-GCP Guidelines (ICH-GCP E6 R2), to the extent possible in the research setting as well as all national legal and regulatory requirements (as apply).

Schedule of Activities

Procedure	Enrolment		Follow-up visit 1	Notes
	Day 1	Day 2	Day 56 (±14)	
Informed consent	X			
Inclusion and exclusion criteria	X			
Demography	X			
Medical history (includes prior history of TB)	X			
Sputum sample collection	X	X*		
Blood sample collection (optional)	X			HIV testing will be offered to all participants as part of the trial procedures, unless a prior result is available (see Section 8)
AE/SAE review	←=====→			
Assess interval medication history			X	<ul style="list-style-type: none"> • Culture-negative, Truenat MTB (and/or MTB plus) and Xpert discordant cases. • 20% of Case Detection Group participants with negative results for all test, including culture
Spot sputum (microscopy and culture)			X [#]	<ul style="list-style-type: none"> • Culture-negative, Truenat MTB (and/or MTB plus) and Xpert discordant cases. • 20% of Case Detection Group participants with negative culture

* Two sputum samples are collected on both Day 1 and Day 2. On Day 1, two spot sputa are collected. On Day 2, firstly, a morning sputum is self-collected in a pot taken home on Day 1. Secondly, an additional sputum is collected when the participant returns to the clinic to deliver the sputum pot from home.

[#] An additional spot sputum will be obtained at follow-up for smear microscopy and culture if i) not on TB treatment and ii) sputum can be produced spontaneously.

1 Introduction

The Truenat MTB, MTB plus and MTB-RIF Dx assays are a set of new tests developed for the diagnosis of tuberculosis (TB) and detection of resistance to rifampicin (RIF), a commonly used drug for treatment of active TB.

1.1 Trial Rationale

An estimated 4.1 million tuberculosis (TB) cases go undiagnosed globally each year¹, leading to substantial morbidity and mortality. In most countries smear microscopy remains the only option for the rapid diagnosis of TB, though it detects only 45% of infections²⁻⁶. For these reasons, new molecular TB diagnostics that can be instituted at the microscopy level are a research and implementation priority.

One such new molecular TB diagnostic is the Truenat including the MTB, MTB Plus and MTB-RIF Dx assays. Further evidence on the diagnostic performance and patient important outcomes on the use of the Truenat MTB assays and the MTB-RIF Dx assay at the microscopy centre level is needed to inform potential policy recommendations.

1.2 Background

The Truenat MTB and MTB plus assays and the MTB-RIF Dx (Truenat RIF or RIF assay) assay (Molbio Diagnostics; Bangalore, India) utilize chip-based real-time micro PCR for detection of TB and rifampicin (RIF) resistance from DNA extracted from sputum samples in about 25 minutes⁷. Briefly, automated DNA extraction is performed using the Trueprep Auto sample prep device (Figure 1). The extracted DNA is then added onto the Truenat MTB (or MTB plus) chip which is pre-loaded with stabilized reagents. Automatic amplification and analysis is then done using a handheld battery-operated device, the Truelab UnoDx real time PCR analyser. If the Truenat MTB (or MTB plus) chip is positive- and dependent on the diagnostic algorithm being employed- the user can then take another aliquot of extracted DNA and add it onto a separate Truenat MTB-RIF Dx chip to detect the presence of selected mutations associated with rifampicin resistance. A pilot trial conducted in India of the Truenat MTB assay found the assay to achieve 91% sensitivity and 100% specificity against a composite reference standard, including 99% sensitivity for smear-positive, culture-positive samples and 76% sensitivity for smear-negative, culture-positive samples⁸. However, further evidence of the performance and operational characteristics of the Truenat MTB assay, as well as the Truenat MTB plus (alternative version including an additional gene target

for TB detection) and RIF Reflex assay, is needed prior to recommending the clinical use of the assays.



Figure 1. Truenat MTB assays steps. A mixture of raw sputum and liquefaction buffer is directly loaded onto the Trueprep Auto chip interface, which extracts MTB DNA in 25 minutes. The extracted DNA is transferred to the Truenat MTB (or Truenat MTB Plus) chip and then onto the Truelab UnoDx PCR machine, which detects the presence of MTB DNA, and provides an automated result as either MTB-detected, MTB not detected or indeterminate result. For MTB positive results, another aliquot of the same DNA extracted is then transferred (reflex) to the Truenat MTB-RIF Dx chip.

To comprehensively evaluate the performance of the Truenat MTB assays and RIF assay, prior studies have been conducted as part of a three-phase/stage approach: i) a validation of the Truenat MTB assays and RIF assay diagnostic performance on frozen samples and ii) an operational assessment of the assays performance. This approach will allow for a rapid, direct assessment of assay performance against characterized specimens from diverse clinical sites (Africa, Eastern Europe and Asia) to confirm adequate performance of the Truenat MTB assays and RIF assay prior to the large clinical evaluation. The current trial corresponds to iii) the evaluation trial to confirm the diagnostic accuracy and to ensure that the performance characteristics will be consistent in the sites of intended use in a geographical diverse population (microscopy centre level) as well as patient important outcome will be assessed during this trial.

1.3 Benefit/Risk Assessment

Knowledge gained from this trial may benefit society by improving TB diagnosis. Trial participants may directly benefit from the trial because they will be provided with a higher standard of TB diagnostic care than may be routinely available to them.

Given the minimal risks associated with this trial and the potential benefits to society and individuals, the benefits outweigh the aggregated risks. As for any clinical trial, there is a possibility of unknown and unforeseen risk; that possibility is small for this trial. If unforeseen risks are recognized during the trial, then FIND, trial partners, IRBs/ethics committees, and participants must be provided with relevant information.

2 Trial Objectives and Endpoints

Table 1. Trial objectives and endpoints

Objectives	Endpoints
1. Primary	
1.1. Estimate diagnostic accuracy of the Truenat assays (MTB and MTB Plus) for Mycobacterium tuberculosis (MTB) detection among individuals undergoing evaluation for pulmonary TB, overall and per sample, separately for smear-positive and smear-negative TB samples using a culture reference standard.	1.1.1. Sensitivity of the Truenat MTB and MTB Plus assays (including analysis by smear-status and by sample). 1.1.2. Specificity of the Truenat MTB and MTB Plus assays (including analysis by sample).
1.2. Estimate diagnostic accuracy of the Truenat MTB-RIF Dx assay for RIF resistance detection among individuals undergoing evaluation for pulmonary TB and DR TB, using phenotypic/genotypic drug susceptibility testing (DST) ¹ .	1.2.1 Sensitivity of the Truenat MTB-RIF Dx assay 1.2.2 Specificity of the Truenat MTB-RIF Dx assay
2. Secondary	
2.1. Compare the diagnostic accuracy of the Truenat assays (MTB and MTB Plus) and MTB-RIF Dx assay to that of Xpert MTB/RIF, using a reference standard of culture for TB diagnosis	2.1.1. Sensitivity of the Truenat MTB and MTB Plus assays and MTB-RIF Dx assay compared to Xpert MTB/RIF

¹ All samples that exhibit RIF resistance, as determined by either phenotypic DST or Truenat RIF, will be sent for DNA sequencing to determine the mutations responsible for drug resistance. A subset of drug sensitive samples, of approximately matching sample size, will be randomly selected for sequencing, to act as a comparator sequence

and phenotypic/genotypic DST for detection of RIF resistance.	2.1.2. Specificity of the Truenat MTB and MTB Plus assays and MTB-RIF Dx assay compared to Xpert MTB/RIF
2.2. Assess patient important outcomes, including time to detection of TB and RIF resistance.	2.2.1. Patient important outcomes (see details in section Patient important outcomes under Statistical analysis plan)

3. Trial Design

3.1. General Design

This will be a prospective, multicentre, diagnostic accuracy trial in which the performance of an investigational rapid molecular diagnostic test (index test) on sputum samples (Truenat MTB assays and RIF assay) will be assessed in India, using solid and liquid culture as reference standard for the diagnosis of TB and MGIT SIRE as reference standard for the detection of RIF resistance.

3.2. Scientific Rationale for Trial Design

Subjects undergoing evaluation for TB and DR will be enrolled after informed consent is obtained. Results of the investigational Truenat assays will not be used for clinical care and will not be provided to clinicians or participants.

Laboratory technicians performing Truenat testing will be blinded to the results of conventional tests results. HIV testing or information about the status will be obtained in order to provide estimates of test performance in the important subgroup of HIV-infected cases with TB/DR symptoms, and also since HIV testing is considered standard of care for TB cases.

3.3. End of Trial Definition

A participant is considered to have completed the trial if he/she has completed the last visit. The end of the trial is defined as the date of the last visit of the last participant in the trial globally.

4. Trial Population and Eligibility

Trial population: Adults with presumptive tuberculosis disease.

Trial/sample size: Target enrolment for the multicentre trial is 1,666 participants enrolled.

Setting: In India, a total of 9 DMC will represent the intended settings of use including urban, peri-urban/hilly, tribal and rural sites with low and high throughput laboratories in India. Based on these characteristics the following numbers of presumptive TB cases, including smear-positive cases, are anticipated per DMC type (Table 2).

Table 2. Types of designated microscopy centres in India

S.No	DMC type	Nr. subjects with TB symptoms with sputum examination/month*	% Smear-positive among subjects with TB symptoms/month*
1	Low volume DMC (in health posts/UPHC)	40 (30 - 50)	2% - 3%
2	High volume DMC (in UPHC)	120 (100 - 150)	6% - 9%
3	High volume DMC (in Tertiary care/hospitals for chest diseases or TB)	300 (200 - 500)	10% - 12%

*Under routine conditions.

Participating DMC have been selected based on the following requirements:

- Smear microscopy must be available
- Xpert MTB/RIF must not be available
- At least 10 hours of electricity available (Trueprep Auto 10h per charge for 16 runs; Truelab UnoDx 4h per charge for 8-10 runs)
- At least 10 sputum samples collected per week, i.e. ~2 samples per day
- Have easy access to a reference laboratory with capacity to perform reference standard tests to ensure timely and high quality results

Given the important role of the private sector in India, 1 private laboratory will be included in the trial which is an MDR reference hospital.

Additionally, 3 other global sites will be setup in South America, Eastern Europe and Asia/Africa to achieve a wider geographic variation. These sites will be outpatient TB clinics at district or regional health facilities.

In order to meet enrolment targets (n= 1,666 total enrolled participants: India will enrol n = 1,110; other global sites will enrol n = 556).

All sites will need to be able to attain informed consent and obtain four sputum samples per patient for index and reference tests (as per sample flow in Figure 2). Participating centres must also have sufficient space to accommodate the Truenat instruments.

Location: Trial sites in India are listed in Table 3. Global sites will be determined among sites in South America, Eastern Europe and Asia/Africa.

Table 3. List of trial sites in India

Site name	Location
Madhupura Urban Health Centre, Ahmedabad	Urban
CHC Chhala, Ahmedabad	Rural
PHC Kuha, Ahmedabad	Rural
Kamrup Metro, Guwahati	Hilly/Peri-urban
Sonapur District Hospital, Guwahati	Tribal belt
Railway Hospital, Guwahati	Hilly/Peri-urban
Ayanavaram, Chennai	Urban
Villiwakkam, Chennai	Peri-urban
Thanthai Periyar, Chennai	Urban
PD Hinduja Hospital, Mumbai*	Urban

*Private sector

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

4.1. Inclusion Criteria

- Age 18 years or above;
- Clinical suspicion of pulmonary TB (including cough ≥ 2 week and at least 1 other symptom typical of TB);
- Willingness to provide 3 sputum specimens at enrolment;
- Willingness to have a trial follow-up visit approximately 2 months after enrolment;
- Provision of informed consent.

Two groups of participants will be enrolled. Namely a “Case Detection Group” and a “Drug Resistant Group”.

Case Detection Group: Participants are eligible to be included only if they meet all the conditions above.

Drug Resistant TB Group: In addition to the criteria of the Case Detection Group, participants should also meet the following conditions:

- Non-converting pulmonary TB cases (category I and category II failures)

4.2. Exclusion Criteria

Participants are excluded from the trial in case of:

Case Detection Group:

- Receipt of any dose of TB treatment within 60 days prior to enrolment (even if within last two days only).

Drug Resistant TB Group:

- Receipt of any dose of MDR-TB treatment within 60 days prior to enrolment (even if within last two days only).

4.3. Lifestyle Considerations

None

4.4. Screen Failures

Screen failures are defined as participants who consent to participate in the trial but are not subsequently entered in the trial. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Early exclusions

Incomplete sputum sample set

Participants who have provided consent and who are enrolled, but who do not provide a total of 3 sputum specimens of sufficient volume will be classified as early exclusions; each will be removed from the trial and a new trial subject enrolled. Participants who are classified as early exclusions will be referred to the appropriate local health service for TB evaluation and care. If positive for

M. tuberculosis, results of conventional microbiological tests (e.g., sputum smear microscopy, mycobacterial cultures, and Xpert MTB/RIF) will be reported to appropriate local health authorities in accordance with local public health reporting regulations. Trial data for participants who are classified as early exclusions will not be used for any final analyses.

Individuals who do not meet the criteria for participation in the trial (screen failure) may not be rescreened.

5. Trial Intervention

Trial Intervention is defined as any investigational intervention(s), marketed product(s), or medical device(s) intended to be used with a trial participant according to the trial protocol. Nevertheless in the current trial, the results of the intervention i.e. Truenat MTB, MTB Plus and RIF will not inform patient care decisions.

5.1. Medical Devices

The devices and diagnostic tests manufactured for sponsor use in this trial are:

- TruePrep Auto
- Truenat MTB chip
- Truenat MTB Plus chip
- Truenat UnoDx
- Truenat MTB-RIF Dx chip

Other medical devices (not manufactured by or for sponsor) to be used in this trial are:

- GeneXpert IV
- MGIT 960

Instructions for medical device use are provided in the Manual of Procedures.

Any medical device incidents, including those resulting from malfunctions of the device, must be detected, documented and reported by the investigator throughout the trial (see section 9 Safety and Incident Reporting).

5.2. Preparation/Handling/Storage/Accountability

The investigational products are intended for the detection of MTB in patient samples as well as the detection of MTB mutations associated with resistance to Rifampicin. The investigational products will be strictly accounted for, including receipt and inventory, storage, use during the trial, and return or disposal, as detailed in the Manual of Procedures provided by FIND.

Acquisition: Procurement of the investigational products will be done through FIND, which 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.

Storage: Procedures for product storage and disposal will be described in the Manual of Procedures. Briefly, investigational products will be checked for quality at the time of receipt at the site. Investigational products will be stored according to the manufacturer's instructions; expired or unused investigational products will be destroyed with documentation. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all devices, reagents and samples received and any discrepancies are reported and resolved before use of the trial intervention.

Test handling and performance: Testing using the investigational products will be performed according to the manufacturer's instructions outlined within the Manual of Procedures. Only participants enrolled in the trial may have samples evaluated using the medical devices described herein, and only authorized site staff may process samples and perform the diagnostic test. All trial medical devices, reagents and samples 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.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for trial intervention accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

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.

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 Manual of Procedures. New lots may only be used after this quality check is successfully passed.

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.

5.3. Minimization of Error and Bias

5.3.1. Patient selection

Spectrum bias will be avoided by enrolling a consecutive series of subjects and using a cross-sectional trial design. Enrolment will be based on clearly defined eligibility criteria, targeting patients suspected to have TB as defined by WHO12, thus representing future target populations. Descriptive statistics on patient characteristics and estimates of diagnostic accuracy stratified by site, smear and HIV status to further ensure the validity and generalizability of trial results will be provided.

5.3.2. Index test

The risk of review bias is minimal. Interpretation of results from the index tests does not require interpretation by the end-users and is based on pre-defined and automatically implemented thresholds. Further, results will become available and will be recorded before those of the reference standard (culture or DST). Where other rapid molecular assays are performed on the same sample, laboratory staff members will be instructed to record results independently of other test results.

5.3.3. Reference standard

Each participating laboratory will undergo an on-site laboratory evaluation to ensure standardized and high-quality performance of culture across sites (see also section Quality Assurance). Results from reference standard testing will be recorded blinded to index test results, eliminating the risk of review bias. Presence of MTB complex will be confirmed for all patients from a positive culture using MPT64 identification test and/or Line Probe Assay.

A reference standard of MTB culture (using two MGIT and two LJ cultures) will be used for TB detection. For RIF resistance detection a composite reference standard of phenotypic and genotypic RIF DST by sequencing will be used (as detailed in section Case definitions). This will allow for a high confidence in the interpretation of Truenat RIF resistant assay results. RIF sequencing will be done at a central laboratory for standardisation purposes (where possible).

5.3.4. Flow and timing

The planned patient and sample flow includes testing of three samples per patient with the index test and reference standard and there is thus little risk of disease progression bias. Manual mixing or splitting of decontaminated sample (pellet) together with random allocation of pellet aliquots (i.e. pipetting order) will also ensure that neither the index test nor the reference standard test are given an artificial advantage by virtue of being used on a higher quality sample.

5.3.5. Handling of indeterminate results

Samples for which the index test is indeterminate or cultures are contaminated will be excluded from the main accuracy analyses but reported separately. Results based on initial testing only as well as those obtained upon repeat-testing (for indeterminate results, where possible) will be presented.

5.3.6. Blinding Procedure

This is an open-label trial. The results of the Truenat MTB and RIF assays are generated automatically. Nevertheless, there is a potential bias of laboratory tests that are subject to operator's interpretation such as smear microscopy and LJ culture.

- Different operators will be assigned to Xpert MTB/RIF and smear microscopy. Similarly for MGIT and LJ culture.
- Technicians will be instructed to record results independently of other test results.

6. Discontinuation of Trial Intervention and Participant Discontinuation/Withdrawal

6.1. Participant Discontinuation/Withdrawal from the Trial

- A participant may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance, or administrative reasons.
- Participants who withdraw will be referred to the appropriate local health service for TB evaluation and care. If positivity for MTB is confirmed (through sputum smear microscopy, mycobacterial cultures or Xpert MTB/RIF), results will be reported to appropriate local health authorities in accordance with local public health reporting regulations.

- If the participant withdraws consent for disclosure of future information, FIND may retain and continue to use any data collected before such a withdrawal of consent.
- If a participant withdraws from the trial, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site trial records.
- See Manual of Procedures for data to be collected at the time of trial discontinuation and follow-up.

6.2. Lost to Follow Up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the trial site.

The following actions must be taken if a participant fails to return to the clinic for a required trial visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the trial.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant. These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable after 3 attempts at different time points, he/she will be considered to have withdrawn from the trial.

7. Trial Procedures

- Trial procedures and their timing are summarized in the SOA. Protocol waivers or exemptions are not allowed.
- Adherence to the trial design requirements, including those specified in the SOA, is essential and required for trial conduct.
- Eligibility criteria must be completed and reviewed to confirm that potential participants meet the requirements. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (e.g. chest X-ray) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SOA.

7.1. Trial Workflow

Following initial screening, individuals will be referred to trial personnel for additional information and to review eligibility (if initial assessment was done by non-trial clinicians). If appropriate, samples will be obtained following the informed consent process. Information on signs/symptoms (duration of cough, fever, haemoptysis, night sweats and weight loss) and TB history (previous TB episodes, dates of treatment, treatment outcome) will be obtained. Information on TB history will be critical given the potential for molecular tests to detect DNA from nonviable, non-intact bacilli in patients with recent history of TB¹⁰.

7.2. Efficacy Assessments

See section 10.4 Efficacy Analyses.

7.3. Safety Assessments

See Section 9, Safety and Incident Reporting.

7.4. Health Economics

The data collected may be used to conduct exploratory economic analyses and a specific study protocol will be prepared.

8. Specimen Collection, Handling, Transport and Storage

Participants will be asked to provide a total of four sputum samples at enrolment and over 2 days. The intent is for all samples to be collected before the participant starts any form of TB treatment, unless the participant is part of the Drug Resistant TB group.

Each specimen should be of 2 ml or greater in volume. On Day 1, each participant will be asked to submit two spot sputa (S1 and S2, approximately 30-60min apart) after enrolment. Participants will be given a labelled sputum pot and instructions for use, and instructed to collect a second sputum (S3) the next morning (Day 2) before going to the clinic. At the clinic participants will be asked to provide a third spot sputum (S4). In the event that a participant fails to return on Day 2, S3 and S4 may be collected a maximum of 7 days after enrolment, provided that no TB treatment has been initiated (the Case Detection group).

HIV testing will be offered to all participants as part of the trial procedures unless any one or more of the following are present:

- written results of a positive HIV antibody test;
- written results of a positive HIV viral load;
- documentation in the medical record of positive HIV status by a treating clinician;
- immediate/verifiable documentation of HIV negativity within the preceding one month.

HIV testing can be performed using any test method approved by local health authorities. Depending on the test method used, this test will require approximately 1 ml of saliva (rapid oral test), or up to approximately 5 ml of blood. Participants refusing to undergo HIV testing will not be excluded from the trial.

8.1. Reference Standard Test and Index Test Procedures

At each site, as per laboratory flow (Figure 2), standard diagnostic algorithms will be followed according to national guidelines and policies of each participating country. If national guidelines cannot be fulfilled using study-specific samples and test results, then an additional spot sputum should be collected.

Sites: All

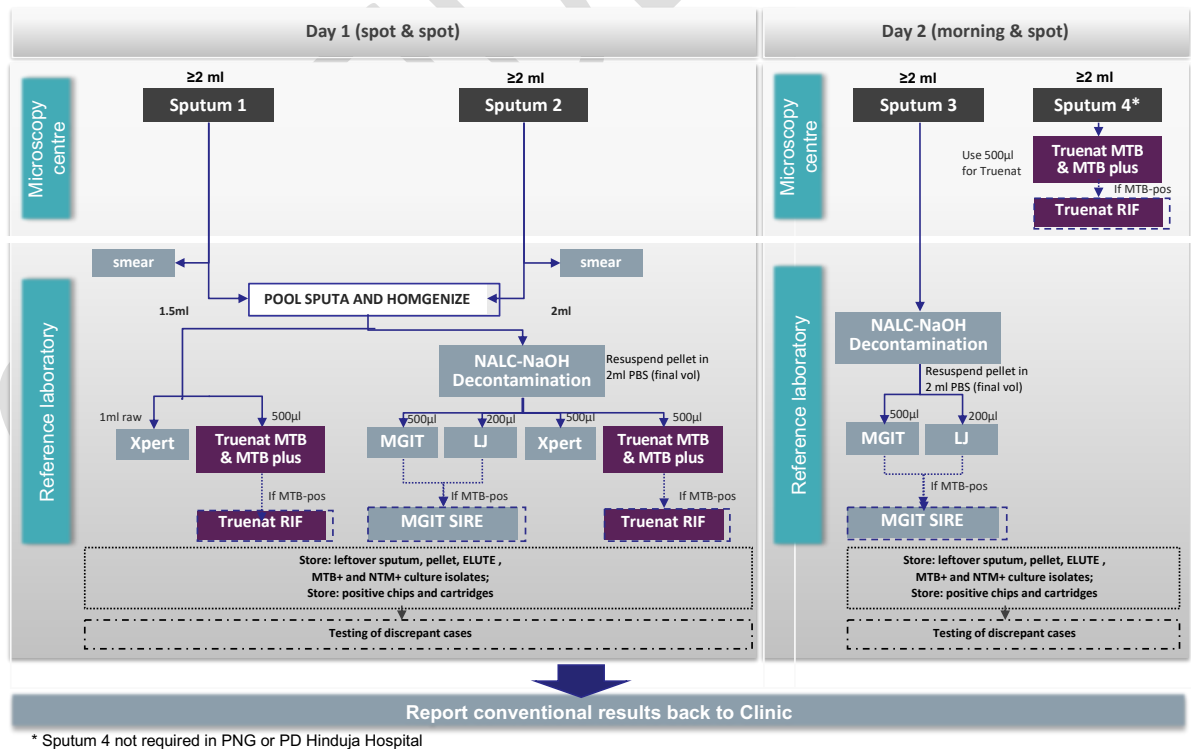


Figure 2. Laboratory workflow

Study spot Sputum 1 (S1) will be collected on Day 1. Approximately 30-60 minutes later, a second spot sputum (S2) will be collected and sent directly to the reference laboratory.

Participants will be given a labelled sputum pot and instructions on how to use it to collect an early-morning sputum (S3), and asked to bring this back to the clinic the next day (Day 2), and provide another spot sputum (S4).

On Day 1, smear microscopy will be performed on Sputum S1 and S2 in the reference laboratory or, if required, in the microscopy centre. In the reference lab, S1 and S2 will be pooled together and homogenised using glass beads and a vortex. Homogenised sputa will be further split: 1.5ml will be used for analysis on raw sputa, and at least 2ml used for NALC-NaOH decontamination.

Briefly, DNA will be extracted independently from i) raw sputum and ii) decontaminated pellet, by the Trueprep Auto device and tested by both the Truenat MTB and the MTB Plus chips, both of which are read by the Truelab UnoDx real-time PCR analyser. All DNA samples testing positive by the MTB assay will be subsequently tested by the Truenat MTB-RIF Dx assay (reflex) which is also read by Truelab UnoDx analyser.

Xpert assays will be performed on the same raw and decontaminated samples.

MGIT and LJ culture will be performed on only on the decontaminated sample.

On Day 2, morning sputum S3 will be returned to the clinic in a labelled sputum pot and the participant will be asked to provide spot sputum S4. S3 will be sent to the reference laboratory and a second round of MGIT and LJ culture will be performed on decontaminated sample. As for S1 and S2, each positive culture will be identified for MTB complex using MPT64 identification test and/or Line Probe Assay. MGIT SIRE will be used to determine the phenotypic DST for RIF. All samples that exhibit RIF resistance, as determined by either phenotypic DST or Truenat RIF, will be sent for DNA sequencing to determine the mutations responsible for drug resistance. A subset of drug sensitive samples, of approximately matching sample size, will be randomly selected for sequencing, to act as a comparator sequence.

Spot sputum S4 will be processed in the microscopy centre: 500ul raw sputum will be used for DNA extraction by Trueprep Auto and MTB detection by the Truenat assays. Any MTB-positive samples will be subsequently tested by the Truenat MTB-RIF Dx assay (reflex).

The intended objective is to test the Truenat assay in the setting of use (i.e. microscopy centre). If the microscopy centre has an Xpert machine (or if the

microscopy centre and the reference lab are the same), then the Truenat assay need only be performed once along side Xpert (on Day 1).

All leftover pellet, eluate and culture isolates will be stored as per the Manual of Procedures for further testing in case of discordant results e.g. result of Truenat RIF differs from that of phenotypic DST or Xpert MTB/RIF.

Importantly, laboratory technicians performing smear microscopy will be blinded to the results of Truenat MTB assays and other conventional test results. Moreover, the results of the Truenat MTB assays and Truenat RIF will not be reported back to the clinic i.e. will not be used for patient management.

8.2. Trial supplies and incoming quality check

- A list of materials for Truenat MTB and RIF testing is provided in Appendix 1.
- Income Quality Check (IQC) procedures are described in the Manual of Procedures. This will be conducted upon arrival of each new shipment of assays. New lots may only be used after this quality check is successfully passed.

8.3. Other Trial Procedures

8.3.1. Assessment of patient important outcomes

The following will be assessed as patient important outcomes using the Truenat MTB assays and RIF assay compared to routine tests

- Time to detection of TB and susceptibility to RIF.
- Yield over smear microscopy and Xpert MTB/RIF.
- Upfront diagnosis of RIF (reflex on all Truenat MTB or MTB Plus positive versus current standard of smear-positive reflexed to Xpert MTB/RIF based on risk factors).

See Patient important outcomes under Statistical analysis plan.

8.3.2. Participant follow-up

A follow-up visit at Day 56 (+/- 14 days) post-enrolment will be conducted to a subset of participants in order to collect additional information on the TB status.

- **Culture-negative, Truenat MTB (and/or MTB plus) and Xpert discordant cases.** During the prospective assessment, Truenat results will not be provided to clinicians or participants or used for

decision-making. Thus participants who are Xpert-negative but Truenat-positive at enrolment (“discordant”) will not be treated on the basis of Truenat results. All culture negative cases with discrepant Truenat and Xpert results will undergo a follow-up visit performed at Day 56 (+/-14) post-enrolment to assess:

- Interval medication history, including TB treatment and clinical evolution.
- An additional spot sputum will be obtained for smear microscopy and culture (LJ and MGIT) provided the patient was not started on therapy and is able to provide a spontaneously produced sputum sample.

This will aid in the identification of patients who would be diagnosed (in the absence of Truenat MTB assays being available for decision making) on clinical grounds.

- **Cases Detection group with negative results.** The first 267 patients who are negative on all tests (approximately 20%) will be followed at Day 56 (+/-14) post-enrolment to assess:
 - Interval medication history, including TB treatment and clinical evolution
 - An additional spot sputum will be obtained for smear microscopy and culture (LJ and MGIT) provided the pulmonary symptoms persist and the patient was not started on therapy

The purpose of this follow-up visit is to identify the participants in this subset who are diagnosed/initiated on treatment on clinical grounds and those that are missed completely.

A follow-up visit is not required for participants who are started on treatment (based on Xpert or culture results).

8.3.3. Analysis of discordant results

Given that the sensitivity and specificity of culture and culture-based DST for Rifampicin is not perfect, misclassification of samples by these reference standards may introduce bias into the sensitivity and specificity estimates for the Truenat assays and underestimate their diagnostic performance.

In order to address this bias, cases with discordant results (and a subset of cases with concordant results) will be further assessed following a Manual of Procedures and described separately in the final trial report. The same number of non-discardant cases will be tested to avoid introduction of additional bias.

8.4. Genetics

Besides sequencing of *M. tuberculosis* to identify mutations recognized to be associated with drug resistance, no other genetic tests are evaluated in this trial.

8.5. Biomarkers

Biomarkers are not evaluated in this trial.

9. Safety and Incident Reporting

Given the nature of this trial i.e. diagnostic accuracy and comparison of diagnostic solutions, the probability of an AE and SAE to be associated with the investigational products is exceedingly rare.

Safety reporting is therefore limited in scope to events associated with the collection of samples and those that occur at participating laboratories using the investigational medical device i.e. TruePrep Auto and Truenat UnoDx. For the purposes of this trial only fatal SAEs and medical device incidents fulfilling the definition of AE or SAE (see section 9.2 Medical Device Incidents) will be reported.

The definitions can be found in Appendices 2 and 3.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up all AEs that are serious, considered related to the trial intervention or trial procedures, or that caused the participant to discontinue the trial. If the AE meets the definition of a SAE it must be reported to FIND regardless if associated with and of the investigational diagnostic devices.

9.1. Time Period for Collecting SAE Information

Relevant AE or SAEs will be collected from the signing of the informed consent form (ICF) until the follow-up visit.

Fatal SAEs will be recorded and reported to FIND or designee within 24 hours, as indicated in Appendix 2. The investigator will submit any updated SAE data to FIND within 24 hours of it being available.

Investigators are not obligated to actively seek SAEs after conclusion of the trial participation.

The method of recording, evaluating, and assessing causality of the SAE and the procedures for completing and transmitting SAE reports are provided in Appendix 2.

9.1.1. Reporting and Follow up of SAEs

Further information on follow-up procedures is given in Appendix 2.

9.1.2. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to FIND of a fatal SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and investigational medical device operators are met.
- FIND has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a trial intervention under clinical investigation. FIND will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- An investigator who receives an investigator safety report describing a fatal SAE or other specific safety information (e.g. summary or listing of SAEs) from FIND will review and then file it in the ISF and will notify the IRB/IEC, if appropriate according to local requirements.

9.2. Medical Device Incidents (including Malfunctions)

Medical devices are being provided for use in this trial for the purpose of evaluating diagnosis of TB and detecting RIF resistance. In order to fulfil regulatory reporting obligations worldwide, the investigator is responsible for the detection and documentation of events meeting the definitions of incident or malfunction that occur during the trial with such devices.

The definition of a Medical Device Incident can be found in Appendix 3.

NOTE: Incidents fulfilling the definition of an AE/SAE (e.g. any event that occurs as a result of insufficient or inadequate instructions for use, installation, or operation, or any malfunction of the investigational device, as well as any event resulting from operator error(s), intentional misuse of the investigational medical device or protocol violation) will follow the processes outlined above and in Appendix 3 of the protocol.

9.2.1. Time Period for Detecting Medical Device Incidents

Medical device incidents or malfunctions of the device that result in an incident will be detected, documented, and reported during all periods of the trial in which the medical device is used.

Medical device incidents meeting the definition of AE or SAE will be reported to FIND or designee within 24 hours, as indicated in Appendix 3. The investigator will submit any updated SAE data to FIND within 24 hours of it being available.

If the investigator learns of any incident at any time after a participant has been discharged from the trial, and such incident is considered reasonably related to a medical device provided for the trial, the investigator will promptly notify FIND.

The method of documenting Medical Device Incidents is provided in Appendix 3.

9.2.2. Follow-up of Medical Device Incidents

- All medical device incidents involving an AE will be followed and reported in the same manner as other AEs (see Appendix 2). This applies to all participants, including those who discontinue trial intervention as well as medical device operators.
- The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the incident.
- New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

9.2.3. Reporting of Medical Device Incidents to Sponsor

- Medical device incidents will be reported to FIND within 24 hours after the investigator determines that the event meets the protocol definition of a medical device incident.
- The Medical Device Incident Report Form will be sent to FIND by email.
- The same individual will be the contact for the receipt of medical device reports and SAE.

9.2.4. Regulatory Reporting Requirements for Medical Device Incidents

- The investigator will promptly report all incidents occurring with any medical device provided for use in the trial in order for FIND to fulfil the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.
- The investigator, or responsible person according to local requirements (e.g. the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of incidents to the IRB/IEC.

10. Statistical Considerations

This trial is designed to assess diagnostic accuracy of the index test compared to culture reference standard, and to assess the diagnostic accuracy of RIF resistance detection compared to a reference standard of phenotypic drug susceptibility testing. Further this trial will compare diagnostic accuracy and RIF detection of the index tests to that of Xpert MTB/RIF. As such, the trial has been designed to capture a sufficient number of smear negative TB cases to be able to provide estimates of sensitivity with a confidence interval width of approximately 20%.

This section provides an overview of the data analysis strategy and methodology. A detailed Statistical Analysis Plan (SAP) will be written before the start of recruitment.

10.1. Statistical Hypotheses

Primary endpoint 1.1 is to assess the diagnostic accuracy of the Truenat MTB assays for MTB detection among individuals undergoing evaluation for pulmonary TB, overall and per sample, separately for smear-positive and smear-negative TB samples using a culture reference standard.

Primary endpoint 1.2 will estimate diagnostic accuracy of the Truenat RIF assay for RIF resistance detection among individuals undergoing evaluation for pulmonary TB and DR TB, using a reference standard of phenotypic/genotypic DST i.e. MGIT SIRE and sequencing for RIF resistance.

Secondary endpoints will compare the diagnostic accuracy of the Truenat MTB assays and RIF assay to that of Xpert MTB/RIF, using a reference standard of culture for TB diagnosis and MGIT SIRE and sequencing for detection of RIF

resistance. Additionally, the trial will assess patient important outcomes, including: i) time to detection of TB and RIF resistance, ii) yield over smear microscopy and Xpert MTB/RIF and iii) upfront diagnosis of RIF (reflex testing on all Truenat MTB or MTB Plus positive versus current standard of smear-positive reflexed to Xpert MTB/RIF based on risk factors).

10.2. Sample Size Determination

The sample size was chosen to achieve high confidence in the accuracy estimates for MTB-detection and RIF resistance detection for the overall multi-country trial for which the Indian trial is one of the countries.

Based on an expected sensitivity of Truenat MTB plus for detection of TB among smear-negative/culture-positive cases of 67% (based on preliminary data), 80 smear-negative/culture-positive cases would be required to achieve a total width of the 95% confidence interval of 20% (95%CI: 57 to 77). Assuming a TB prevalence of 20% and a prevalence of smear-negative/culture-positive cases among TB cases of 30%, the total number of subjects to be enrolled would be 1,333. To account for losses, this is inflated by 20%, yielding a final sample size of 1,666 participants under investigation for TB overall.

In India, two thirds of these trial participants will be recruited i.e. $n = 1,110$ thus 67 smear-negative/culture-positive cases and a 95% confidence interval of 21% could be achieved (95%CI 55 to 79).

The other one-third of enrolled participants ($n = 556$) will be recruited in three other countries in order to provide geographic variation.

The secondary objective of determining diagnostic accuracy of RIF resistance by Truenat MTB is based upon an expected Truenat RIF sensitivity of 95% with a confidence interval of 10% (90-100%), requiring $n=37$ RIF-resistant participants detected. Assuming a prevalence of 20% culture-positive TB cases detected across all presumed TB cases, and 2.8% RIF resistance amongst all culture-positive TB cases and 12% prevalence of RIF resistance amongst TB retreatment cases, we predict 1,542 re-treatment patients would thus need to be enrolled. While the prevalence of culture-positive TB cases may be higher if enrolment is conducted at a drug-resistance TB referral clinic, we conservatively accept that this may not be the case. As such, we may not reach sufficient sample size to allow analysis of secondary objectives in this trial. Detection of RIF resistance will be continually monitored throughout the trial, and any possible shortfall will be supplemented with a future sub-study, if needed, using confirmed RIF-resistant sputum samples from the FIND specimen bank of cryopreserved samples. We will place an enrolment cap of 200 enrolled participants in the drug resistance group for the entire trial, in order to not undermine the primary objective of enrolment of smear-negative culture

positive TB cases through inadvertent over-enrolment of DR cases who are more likely to be smear-positive culture-positive.

10.3. Populations for Analyses

For purposes of analysis, the following participant populations are defined:

Table 4. Populations for analyses

Population	Description
Enrolled	All participants who sign the ICF
Evaluable	All participants with a culture positive or culture negative results. Contaminated cultures will be excluded. Contamination is defined as below:
Smear-positive	≥ 1 positive smear (inclusive of scanty positive smears). Smear positives with only negative or contaminated cultures will be excluded from analysis.
Culture-positive	≥ 1 LJ and/or MGIT culture growth confirmed MTB complex. Cross-Contamination: A single LJ culture with ≤ 20 colonies or a single MGIT culture with MTB growth ≥28 days per patient will be excluded from analysis.
Culture-negative	At least 2 LJ or MGIT have no culture growth after >56 days and >42 days
Contaminated culture	LJ: Cultures completely overgrown by bacterial or fungal contaminations within 3 weeks (discarded). In case of mixed cultures, isolated MTB colonies transferred to new LJ tube (repeat culture). MGIT: Instrument positivity tests for confirmation of MTB complex negative. 2 contaminated cultures will lead to exclusion of this patient from analysis unless other criteria for culture-positivity/negativity are met
Xpert-positive	MTB positive on Xpert® MTB/RIF.
Xpert-negative	MTB negative on Xpert® MTB/RIF.
Xpert-indeterminate	Any indeterminate, error, or inability to produce a result from a single Xpert® MTB/RIF run.
Non-TB case	Smear-negative, Xpert-negative and culture-negative and not started on TB treatment on the basis of clinical criteria. NTM: Specimens with growth of mycobacteria other than MTB complex only.
Clinical TB case	Any participant who tests smear-negative, Xpert-negative, culture-negative but is started on TB treatment on the basis of clinical criteria.

Phenotypic RIF resistant	Culture-positive and growth for RIF on MGIT SIRE DST testing.
Phenotypic RIF sensitive	Culture-positive and no growth for RIF on MGIT SIRE DST testing
Genotypic RIF resistant	Sequencing identifies mutations recognized to be associated with RIF-resistance (defined based on consultation with WHO prior to analysis)
Genotypic RIF sensitive	Sequencing identifies no mutations recognized to be associated with RIF-resistance (defined based on consultation with WHO prior to analysis)
Composite reference standard RIF resistant	<p>If phenotypic DST shows sensitivity but sequencing identifies mutations recognized to be associated with resistance, the composite reference standard will be considered RIF-resistant.</p> <p>If phenotypic DST shows resistance but sequencing does not identify mutations to be associated with resistance, the composite reference standard will be considered RIF-resistant (as mutations will be assumed outside of the region sequenced).</p>
Safety	All participants assigned to trial intervention.

10.4. Statistical Analysis Plan

The statistical analysis plan (SAP) will be developed and finalized before database lock and will describe the participant populations to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a high-level summary of the planned statistical analyses of the primary and secondary endpoints.

10.5. Efficacy Analyses

Table 5. Statistical analysis methods

Endpoint	Statistical Analysis Methods
Primary	Sensitivity and specificity, and ANOVA comparison of Likelihood Ratio test.
Secondary	Difference in sensitivity and specificity between each index assay and Xpert with 95%CI around the difference using methods appropriate for paired data (e.g. Tango score CI)

10.5.1. General Approach

For each test, estimates of sensitivity and specificity will be derived together with 95% confidence intervals. Estimates will be calculated on the overall dataset and by subgroups defined below.

10.5.2. Analysis of Primary Outcomes

Analyses of the diagnostic accuracy of the index test will be done according to the case definitions. Table 6 summarizes the way diagnostic tests results are usually reported when comparing the index test results to a reference standard. Based on the definitions in the table, the following values are defined:

Sensitivity = $TP / (TP + FN)$

Specificity = $TN / (FP + TN)$

Table 6. Definition of classification metrics

Test result	Case definition			
		TB	Non-TB	Total
	Detected	TP	FP	TP + FP
	Not detected	FN	TN	FN + TN
	Total	TP + FP	FP + TN	All

Abbreviations: TB=true positive, FP=false positive, FN=false negative, TN=true negative

Only patients with uncontaminated culture results and without indeterminate test results will be included in the analysis of performance characteristics for MTB detection. Similarly, such patients will contribute to the analysis of the performance characteristics for RIF detection. For each point estimate, a 95% confidence interval will be derived based on Wilson score. Missing data and invalid results will be reported in the descriptive tables, and will not be imputed.

10.5.3. Analysis of Secondary Outcomes

- Accuracy of Truenat MTB assays and RIF assay compared to Xpert MTB/RIF.

The same parameters and sub-groups as for the analysis of primary outcomes will be determined for Xpert MTB/RIF and compared to those of Truenat MTB assays and RIF assay side by side.

- Patient-important outcomes
 - The median time to detection of TB using the Truenat MTB assays will be determined and compared to that of smear, Xpert MTB/RIF, liquid and solid cultures. Furthermore, the median time to detection of RIF resistance using the Truenat RIF assay will be determined and compared to that of phenotypic DST.
 - The number of additional TB (culture-positive) cases detected by the Truenat MTB assays compared to smear and Xpert MTB/RIF.
 - The number of RIF-resistant cases detected using the Truenat RIF assay compared to those detected following the current standard of smear-positive reflexed to Xpert MTB/RIF.

10.5.4. Descriptive statistics

Descriptive statistics tables will be generated to summarize the characteristics of the participants. The number of participants included and excluded will be reported. Among the included participants, the information will be broken down by site, gender, age group, HIV status, history of TB and enrolment group (Case Detection Group and Drug Resistant Group). Results will be reported either in absolute numbers (e.g. number of subjects in a group) or summarized by IQR, percentage.

10.5.5. Additional sub-group analyses

The trial outcomes defined will be also evaluated on the following subpopulations:

- Sensitivity for MTB detection:
 - Per-patient: Truenat MTB assays on S1 and S2 against culture status (based on cultures from S2 and S3)
 - Per-sample processing method: Truenat MTB assays on S1 against culture result on S2 and Truenat MTB assays on S2 against culture result on S2 (same specimen)
 - By smear status
 - By HIV status (where available)
 - On unprocessed (S1) vs decontaminated specimen (S2)
 - After repetition (if result invalid)
 - Compared to Xpert MTB/RIF on S2 (based on culture from S2 and S3)
- Specificity for MTB detection:
 - By TB history
 - By prior TB treatment (> 60 days ago)
 - Compared to Xpert MTB/RIF (based on cultures from S2 and S3)
- Sensitivity and specificity for rifampin resistance detection:
 - Against phenotypic DST
 - Compared to Xpert MTB/RIF on S2 (based on DST from S2 and S3)
- Intermediate/invalid/erroneous results
 - For MTB detection
 - For RIF resistance detection
- Quantitative ability (based on assay cycle threshold (Ct) values)
 - In comparison to culture (positivity and time to positivity, grade for solid culture)
 - In comparison to smear (positivity and grade)

Additional subgroups will be defined and specified in the final SAP.

10.5.6. Planned Interim Analyses

No interim analysis is planned. Continued data monitoring will be performed (see section 12 Data handling and record keeping).

10.6. Safety Analyses

Not applicable. Given the nature of this trial i.e. diagnostic accuracy and comparison of diagnostic solutions, the probability of an AE and SAE to be associated with the investigational products is exceedingly rare. Therefore, no safety analysis will be conducted. However, AE and SAE will be reported as per Section 9 Safety and incident reporting.

10.7. Independent Data Monitoring Committee (IDMC)

Not applicable. Based on FIND internal procedures, an IDMC will be established only for trials where participants are at greater than minimal risk e.g. invasive techniques for specimen collection or use of index test results to inform patient care.

11. Regulatory and Ethical Considerations

11.1. Regulatory and Ethics Approvals

This trial will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the trial is initiated.

- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the trial design, except for changes necessary to eliminate an immediate hazard to trial participants.
- The investigator will be responsible for the following:

- Providing written summaries of the status of the trial to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the trial at the site and adherence to requirements of ICH guidelines, the IRB/IEC, the WHO Good Clinical Laboratory Practice (GCLP), European regulation 536/2014 for clinical studies (if applicable), and with applicable national regulations.

11.2. Financial Disclosure

Investigators and sub-investigators will provide FIND with sufficient, accurate financial information as requested to allow FIND to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the trial and for 1 year after completion of the trial.

11.3. Informed Consent Process

- The investigator or his/her representative will explain the nature of the trial to the participant or his/her legally authorized representative and answer all questions regarding the trial.
- Prospective participants must be informed that their participation is voluntary and will be required to sign and date a statement of informed consent that meets the requirements of local regulations and/or ICH guidelines where applicable, and the IRB/IEC or trial centre.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the trial and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Illiterate patients must provide a thumbprint on the ICF and the ICF must be signed and dated by an impartial witness.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the trial.

11.4. Data Protection

- Participants will be assigned a unique identifier by FIND. Any participant records or datasets that are transferred to FIND will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal trial-related data will be used by FIND in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by FIND, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

11.5. Subject Confidentiality

The Principal Investigator will be responsible for the recording of all subject's personal details, screening number and unique trial number in the Subjects Identification List. To ensure confidentiality, this list must be kept in a place, with access restricted to authorized trial staff. All laboratory specimens, including stored specimens, as well as trial reports, data collection tools, and administrative forms will be identified by a using the patient's unique trial number. Names will not be used on any of these documents. All local databases will be secured with password-protected access systems. The Investigator must ensure anonymity of the patient and ensure that all documents are anonymised before being transmitted to FIND.

11.6. Other ethical considerations

11.6.1. Use of stored specimens:

Sputum volume after processing as well as Trueprep used chips will be stored temporarily in case further testing is deemed to be necessary. Samples will be labelled with a trial identifier; the specimens will be linked to results of other mycobacteriology tests, the final trial diagnosis (e.g. non-TB case, culture-positive TB case, clinical TB case, Indeterminate) of the participant. Specimens will be stored temporarily at the sites. Further testing may be required at a Reference Laboratory or specialized research laboratories. An export permit will be obtained accordingly if required. Moreover, after trial completion leftover well-characterised specimens could be used for future research of product development as long as trial participants have agreed to this during the initial visit.

11.6.2. Protocol deviations

Protocol deviations occur when there is non-adherence to the Protocol and includes Informed Consent, enrolment, and other occurrences of non-adherence to the Protocol. Protocol deviations should be sent to the local IRB/IEC per the local IRB/IEC guidelines. Any protocol deviation that meets reporting requirements of the local IRB/IEC will also be reported with the same timeliness to the FIND project manager.

11.6.3. Termination of the Trial

Given the nature of the trial, termination due to safety or other reasons is not anticipated. However, should the trial be terminated, participants will be referred to the appropriate local health service for TB evaluation and care. If positive for MTB, results of conventional microbiological tests (i.e., sputum smear microscopy and mycobacterial cultures) will be reported to appropriate local health authorities in accordance with local public health reporting regulations.

11.6.4. Site Performance and Data Submission

In the event that one site does not meet enrolment targets within the trial timelines, the trial will go forward at the other sites and the investigators will proceed with data submission as per trial plan.

12. Data Handling and Record Keeping

- All participant data relating to the trial will be recorded on printed or electronic CRF unless transmitted to FIND or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The investigator must permit trial-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- FIND or designee is responsible for the data management of this trial including quality checking of the data.

- Trial monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the trial is being conducted in accordance with the currently approved protocol and any other trial agreements, ICH GCP, and all applicable regulatory requirements.

12.1. Source Data and Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site and will include testing logs, registers, screening forms, laboratory forms, operating characteristics surveys, and data collected through direct patient interviews.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or reports if available.
- The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trials subjects. Source data should be attributable, legible, contemporaneous, original, accurate and complete. Changes to source data should be traceable, should not obscure the original entry and should be explained if necessary.

12.2. Data to be captured

Demographic data, treatment and testing records, clinical status, comparator and reference test results, index test results and selected operational and environmental data will be collected using paper and/or eCRF and transmitted electronically to FIND using the OpenClinica.

12.3. Data Management

FIND is responsible for the data management for the trial, including the setup of the database, programming, editing and range checking. Trial site staff will be responsible for entering trial data from a paper CRF into OpenClinica within 10 working days of a participant's visit or the availability of results. The site will be provided with individual password-protected accounts to access OpenClinica, following a training session given by FIND. Data entry training will be provided by FIND, either on site or remotely sharing screen through Skype

or any other similar system. OpenClinica provides an audit trail system recording all data entries/changes and queries between FIND and the site.

No information concerning the trial or the data generated from the trial will be released to any unauthorized third party without prior written approval of FIND and manufacturers.

12.4. Data Archiving

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.

13. Quality Management

Quality Control and Quality Assurance systems with written Standard Operating Procedures will be established to ensure that the trial is conducted and the data are generated, documented and reported in compliance with this protocol, good clinical practice (GCP), good clinical laboratory practice (GCLP) and applicable regulatory requirements. Data should be collected according to trial specific Data Management plan.

13.1. Quality Control

Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

Risk Based Monitoring will be done and the trial will be monitored remotely (centrally) as well as on-site. Further details on monitoring visits will be included in the Monitoring Plan.

13.2. Quality assurance

FIND will conduct clinical and laboratory assessments at the DMC, selected private laboratories and reference laboratory sites prior to trial start. On-site visits will include observation of routine procedures, assessment of quality management systems as well as review of historical data such as quality indicators (including but not limited to smear- and culture-positivity, smear-positive/culture-negative, Xpert-positive/culture-negative rates). Following the initial assessments and implementation of any recommendations, on-site training will be conducted by FIND (see section Training plan and piloting of laboratory and data collection procedures).

The FIND Clinical Trial Standard will be implemented to verify the clinical and laboratory quality prior to initiation of the trial and the data quality, applicable regulatory documentation, and subject safety throughout the trial.

The trial co-investigators are responsible for conducting routine quality assurance and quality control activities to internally monitor trial progress and protocol compliance in addition to procedures in the FIND Manual of Procedures. The co-investigators will ensure that all trial personnel are appropriately trained and applicable documentations are maintained on site.

13.3. Training plan and piloting of laboratory and data collection procedures

The principal investigators should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s) and their trial-related duties and functions.

The principal investigators are responsible for supervising any individual or party to whom the investigator delegates trial-related duties and functions conducted at the trial site. The principal investigators should ensure this individual or party is qualified to perform those trial-related duties and functions and should implement procedures to ensure the integrity of the trial-related duties and functions performed and any data generated. All key trial staff shall be trained and certified in GCP and certificates provided to FIND.

On-site training on all relevant trial procedures that are not part of routine care will be provided by FIND to the relevant staff, i.e. the trial coordinator at each site, the data entry personnel, the staff at sputum collection sites and the DMC and reference laboratory staff. The training will include clinical and laboratory aspects with focus on hands-on training on the Truenat MTB assays and RIF assay. Proficiency to perform the Truenat MTB assays and RIF assay will be assessed using a proficiency testing tool designed for this trial.

13.4. Trial and Site Closure

FIND designee reserves the right to close the trial site or terminate the trial at any time for any reason at the sole discretion of FIND. Trial sites will be closed upon trial completion. A trial site is considered closed when all required documents and trial supplies have been collected and a trial-site closure visit has been performed.

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 the early closure of a trial site by FIND or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, FIND's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further trial intervention development

14. Work plan & timeline

Trial preparation activities start with IRB approval. Trial initiation followed by enrolment will last for 32 weeks and details of the trial timeline are shown below.

Table 7. Trial timeline

Phase	Activity	Duration	Estimated timeline
Preparation	IRB approval	10 weeks	Q3-2018
	Training	3-4 days	Q4-2018
Implementation	Enrolment	32 weeks	Q4-2018 to Q2-2019
	Follow-up	8 weeks	Q3-2019
	Laboratory testing (enrolment, FU, discordant cases)	26 weeks	Q4-2019
Reporting	Data analysis	3 weeks	Q4-2019
	Report write up	4 weeks	Q4-2019

15. Publication Policy

- The results of this trial may be published or presented at scientific meetings. Details are provided in the Trial Agreement (e.g. Memorandum of Understanding, Collaboration Agreement).
- FIND will comply with the requirements for publication of trial results. In accordance with standard editorial and ethical practice.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

16. References

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17. Appendices

Appendix 1: Supply requirements for Truenat testing

Appendix 2: Safety Definitions and Reporting

Appendix 3: Incident Definitions and Reporting

Appendix 3: Protocol Amendment Summary

Appendix 1: Supply requirements for Truenat testing

Item
Trueprep Auto Sample Prep
Truelab Uno DxAnalyzer
Truenat MTB chip
Truenat MTB Plus chip
Truenat MTB-RIF Dx chip
Additional Trueprep Supplies
Trueprep Auto Sputum sample prep kit (cartridges, reagents)
Additional Truenat Supplies
Truenat Universal Control Kit
Precision micropipettes (fixed volume) including micropipette tips
Additional Truelab Supplies
Truelab AC adapter
Other Supplies <u>not provided</u>
- 70% isopropyl alcohol
- Gloves
- Paper towel
- Squash bottles
- Discard bin for waste (dry)
- Discard bin for waste (wet)
- 0.5% sodium hypochlorite solution
- Mask
- Gown
- Timer

All the Molbio instruments and reagents will be supplied including package inserts and user manuals

Appendix 2: Safety Definitions and Reporting

AE Definition

- An AE is any untoward medical occurrence in a patient or clinical trial participant, temporally associated with the use of trial intervention, whether or not considered related to the trial intervention.
- NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of trial intervention.

SAE Definition

a. Results in death

b. Is life-threatening

The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

SAE Reporting to FIND via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to FIND will be the electronic data collection tool.
- If the electronic system is unavailable for more than 24 hours, then the site will use the paper SAE data collection tool (see next section).
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the trial is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a trial participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the [X/medical monitor/SAE coordinator] by telephone.
- Contacts for SAE reporting can be found in the Manual of Procedures.

SAE Reporting to FIND via Paper CRF

- E-mail transmission of the SAE paper CRF is the preferred method to transmit this information to FIND.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in the Manual of Procedures.

Appendix 3: Incident Definition and Reporting

Medical Device Incident Definition

- A medical device incident is any malfunction or deterioration in the characteristics and/or performance of a device as well as any inadequacy in the labeling or the instructions for use which, directly or indirectly, might lead to or might have led to the death of a participant/user/other person or to a serious deterioration in his/her state of health.
- Not all incidents lead to death or serious deterioration in health. The nonoccurrence of such a result might have been due to other fortunate circumstances or to the intervention of health care personnel.

It is sufficient that:

- An **incident** associated with a device happened.

AND

- The **incident** was such that, if it occurred again, might lead to death or a serious deterioration in health.

A serious deterioration in state of health can include any of the following:

- Life-threatening illness
- Permanent impairment of body function or permanent damage to body structure
- Condition necessitating medical or surgical intervention to prevent one of the above
- Foetal distress, foetal death, or any congenital abnormality or birth defects

Medical Device Incident Documenting

- Any medical device incident occurring during the trial will be documented in the participant's medical records, in accordance with the investigator's normal clinical practice, and on the appropriate form of the CRF.
- For incidents fulfilling the definition of an AE or an SAE, the appropriate AE/SAE CRF page will be completed as described in Appendix 3.
- The CRF will be completed as thoroughly as possible and signed by the investigator before transmittal to FIND or designee.
- It is very important that the investigator provides his/her assessment of causality (relationship to the medical device provided by FIND) at the time of the initial SAE report and describes any corrective or remedial actions taken to prevent recurrence of the incident.
- A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of an incident. This includes any amendment to the device design to prevent recurrence.

Appendix 4: Protocol Amendment Summary

The Protocol Amendment Summary of Changes Table is located directly before the Table of Contents (TOC).

Amendment Number 1 (India only).

Overall Rationale for the Amendment

Since the first submission of the trial protocol to the Ethics Committee in India, updates were needed given that: i) new results on Truenat became available, ii) further assessment of trial risks and revision of mitigation strategies including enrolment targets to determine the performance of the Truenat MTB-RIF Dx assay, adoption of the Common Protocol Template (TransCelerate).

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Institutions/Partners Involved in the Trial*

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Veer Sawarkar Marg, Asavari

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District TB Centre, Chennai (Investigational Site)

Kanakaraya Thottam

Pulianthope, Chennai

Tamil Nadu 600012, India

Commissionerate of Health Medical Services & Me (Hs), Gujarat (Investigational Site)

Sector 10B, Sector 10

Gandhinagar

Gujarat 382010, India

Directorate of Health Services, Assam (Investigational Site)

Hengrabari Health Complex

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Assam 781036, India

Revised National TB Control Programme

State TB Control Department

2nd Floor, Delhi Govt. Dispensary Building

Gulabi Bagh, Delhi-110007

Site 2 (Investigational Site)

To be determined

Site 3 (Investigational Site)

To be determined

Site 4 (Investigational Site)

To be determined

*Terms of references and nature of agreements are available from FIND on request.

Signature Page (Sponsor)

We, the undersigned, have reviewed and approved this Protocol, including Appendices. We will supervise and coordinate the clinical trial as described and ensure adherence to GCP/GCLP, the principles outlined in the Declaration of Helsinki and applicable regulatory requirements.

HEAD OF TUBERCULOSIS PROGRAMME

Name:

Institution:

Signature: _____

Date: _____

DD/MMM/YYYY

HEAD OF CLINICAL & REGULATORY AFFAIRS

Name:

Institution:

Signature: _____

Date: _____

DD/MMM/YYYY

Statement of Principal Investigator

All studies sponsored by FIND will be conducted in accordance with ICH-GCP Guidelines, to the extent possible in the research setting.

In signing this page, I, the undersigned, agree to conduct the trial in compliance with all applicable regulations and guidelines as stated in the protocol and other information supplied to me.

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 Individuals involved with the conduct of this trial have completed GCP training within the past 3 years 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 subject's names or personal identifying information may be disclosed. All subject data will be anonymized and identified by assigned numbers on all Case Report Forms, laboratory samples and source 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.

Name of Principal Investigator: _____

(Print)

Signature: _____

Date: _____

DD/MMM/YYYY

Protocol History/Amendment Summary*

Protocol Version	Date	Description of changes	Brief rationale
Approved 1.0	28 Dec-2018	<ul style="list-style-type: none"> - Changed Draft versions to approved version 1.0 (Common protocol) 	<ul style="list-style-type: none"> - IRB approval at NIRT, Peru, EPHI and Burnet. Pending PNG and Hinduja.
Draft 3.3	07 Sep-2018	<ul style="list-style-type: none"> - Updated section 8.1, study flow, narrative and figure - Placement of a cap of 250 enrolled participants in the drug resistance group. 	<ul style="list-style-type: none"> - Sputum collection and test were altered to pool and homogenise S1 and S2 on Day 1 (allow better distribution of bacilli) and separate S4 to prevent splitting sputum in the microscopy centre, thereby reducing risk of contamination. - Capped to not undermine enrolment of smear-negative culture positive participants for primary aim analysis.
Draft 3.2	27-Aug-2018	<ul style="list-style-type: none"> - Updated section 8.1, study flow, narrative and figure 	<ul style="list-style-type: none"> - Sputum collection and test were altered to i) fit with national guidelines and ii) allow head-to-head comparison of Truenat assays to Xpert on unprocessed sputum.
Draft 3.1	05-Jul-2018	<ul style="list-style-type: none"> - Removed use of frozen samples (RIF resistant). - Introduced differentiation of "Case Detection" group and "Drug Resistant TB" group. 	<ul style="list-style-type: none"> - Full RIF resistance will be performed in a separate laboratory study. - Increased enrolment of participants with presumed RIF resistance.
Draft 3.0	28-Jun-2018	<ul style="list-style-type: none"> - Revised sample size - Added use of frozen samples (RIF-resistant) to supplement testing - No differentiation of trial participants in two groups - Trial to be part of global trial 	<ul style="list-style-type: none"> - TB positivity increased based on pilot data Phase 1b - Mitigate the risk of not meeting targets to determine accuracy of RIF assay - A sub-analysis will be done based on prior history of TB independent of the patient group

		<ul style="list-style-type: none"> - Updated timeline & list of investigators - Format changes - "Trial" replaces "study" - Modified exclusion criteria to having received TB treatment within the last 60 days prior to enrolment. 	<ul style="list-style-type: none"> - Results to be submitted to Indian health authorities as well as WHO, so need collect data outside India - Revised site selection - Changes in protocol template that was developed based on TransCelerate's Common Protocol Template and modified for IVD clinical studies. - Updated FIND definition - Allows enhanced enrolment rate and inclusion of possible TB relapse cases.
Draft 2.1	05-Sep-2017	<ul style="list-style-type: none"> - Truenat MTB & MTB Plus assays 	<ul style="list-style-type: none"> - Side by side assessment of MTB and MTB plus assays to complement India in-country trial
Draft 2.0	22-Aug-2017	<ul style="list-style-type: none"> - Revised sample size - Removed global trial - Removed statistical analysis details - Added potential use of well-characterised samples in future - Updated timeline & list of investigators - Format changes 	<ul style="list-style-type: none"> - Preliminary data Phase 1a - Trial will be conducted only in India - Detailed information to be included in SAP - Better use of well-characterised specimens and reduce need for ad-hoc collection - Update from IRB & site selection completed - Changes in protocol template
Draft Version 1.0 (Original Protocol)	27-Oct-2016	NA	NA

*Refer to Appendix 3 for Protocol Amendment History

List of Abbreviations and Acronyms

Abbreviation/acronym	Meaning
AE	Adverse Event
CRF	Case Report Form
DMC	Designated Microscopy Centres
DNA	Deoxyribonucleic acid
DR TB	Drug Resistant Tuberculosis
DST	Drug Susceptibility Testing
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
GDP	Good Documentation Practice
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
IDMC	Independent Data-Monitoring Committee
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IQC	Income Quality Check
ISF	Investigator Site File
ISO	International Organization for Standardization
MDR TB	Multidrug Resistant Tuberculosis
MGIT (SIRE)	Mycobacterial Growth Indicator Tube (Streptomycin, Isoniazid, Rifampicin, Ethambutol)
MTB	<i>Mycobacterium tuberculosis</i>
NAT	Nucleic acid tests
QA	Quality Assurance
QC	Quality Control
QMS	Quality Management System
RA	Regulatory Authority
RBM	Risk Based Monitoring
RIF	Rifampicin

RM	Risk Management
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SOA	Schedule of Activities
SOP	Standard Operating Procedure
TB	Tuberculosis
TMF	Trial Master File
UPHC	Urban Primary Health Centre

Protocol Synopsis

Title	Prospective, multicentre trial to assess the diagnostic accuracy of the Truenat assays at intended settings of use.
Short title	Truenat Evaluation
Protocol version and date	V3.1, 05-July-2018
Background and rationale	<p>The Truenat MTB (including both MTB and MTB plus) and the MTB-RIF Dx reflex assays (Molbio Diagnostics; Bangalore, India) utilize chip-based real-time micro PCR for detection of tuberculosis (TB) and rifampicin (RIF) resistance from DNA extracted from sputum samples in about 25 minutes.</p> <p>A pilot trial conducted in India of the Truenat MTB assay found the assay to achieve high clinical performance. However, further evidence of the Truenat MTB as well as the Truenat MTB-RIF Dx assay, is needed prior to recommending the clinical use of the assays.</p> <p>Prior studies have been conducted as part of a three-phase/stage approach i) a validation of the Truenat MTB assays and MTB-RIF Dx assay diagnostic performance on frozen samples and ii) an operational assessment of the assays performance. The current trial corresponds to iii) the evaluation trial to confirm the diagnostic accuracy and to ensure that the performance characteristics will be consistent in the sites of intended use in a geographical diverse population (microscopy centre level).</p> <p>Further evidence on the diagnostic performance and patient important outcomes on the use of the Truenat MTB assays and MTB-RIF Dx assay at the microscopy centre level is needed to inform potential policy recommendations.</p>
Objectives	<ol style="list-style-type: none"> 1. Primary objectives: to determine the diagnostic accuracy of the Truenat MTB assays and MTB-RIF Dx assay using culture and phenotypic/genotypic drug susceptibility test (DST) as gold standard in the intended setting of use. 2. Secondary objectives: to determine the diagnostic accuracy of the Truenat MTB assays and MTB-RIF Dx assay compared to Xpert MTB/RIF using culture and phenotypic/genotypic DST as gold standard and to assess patient important outcomes.

Trial design	<p>Prospective, multicentre assessment of Truenat MTB assays and MTB-RIF Dx assay diagnostic accuracy in the intended settings of use.</p> <p>The sample size has been calculated at 1,666 enrolled participants. Sites in India will enrol n= 1,110 participants. Other three global sites will enrol n = 556 participants overall.</p>
Trial sites/setting	India and three other global sites. In India the trial will be conducted at 9 designated microscopy centres (DMC) and 1 private laboratory.
Trial population	Adults with pulmonary tuberculosis symptoms.
Eligibility criteria	<p>Individuals who have symptoms consistent with pulmonary TB presenting to participating centres will be assessed and asked to participate. Participants will be categorised into 2 groups:</p> <p><u>Case Detection group</u> - Only participants who have not received any form of TB treatment within the prior 60 days will be enrolled.</p> <p><u>Drug Resistant group</u> – Non-converting TB cases presumed to be at risk of drug-resistance.</p>
Primary outcomes	<p>1. Primary outcomes</p> <p>1.1. Sensitivity of the Truenat MTB assays (including analysis by smear-status and by sample).</p> <p>1.2. Specificity of the Truenat MTB assays (including analysis by sample).</p> <p>1.3. Sensitivity of the Truenat MTB-RIF Dx assay.</p> <p>1.4. Specificity of the Truenat MTB-RIF Dx assay.</p>
Secondary outcomes	<p>2. Secondary outcomes</p> <p>2.1. Sensitivity of the Truenat MTB assays and MTB-RIF Dx assay compared to Xpert MTB/RIF.</p> <p>2.2. Specificity of the Truenat MTB assays and MTB-RIF Dx assay compared to Xpert MTB/RIF.</p> <p>2.3. Patient important outcomes (see details in section Patient important outcomes under Statistical analysis plan).</p>
Trial duration	Approximately 12 months
Time schedule	Trial start expected Q4-2018 with enrolment completion by Q2-2019. Follow-up after 2 months and testing of discordant cases completed Q4-2019.
GCP statement	All studies sponsored by FIND will be conducted in accordance with ICH-GCP Guidelines (ICH-GCP E6 R2), to the extent possible in the research setting as well as all national legal and regulatory requirements (as apply).

Schedule of Activities

Procedure	Enrolment		Follow-up visit 1	Notes
	Day 1	Day 2	Day 56 (±14)	
Informed consent	X			
Inclusion and exclusion criteria	X			
Demography	X			
Medical history (includes prior history of TB)	X			
Sputum sample collection	X	X*		
Blood sample collection (optional)	X			HIV testing will be offered to all participants as part of the trial procedures, unless a prior result is available (see Section 8)
AE/SAE review	←=====→			
Assess interval medication history			X	<ul style="list-style-type: none"> • Culture-negative, Truenat MTB (and/or MTB plus) and Xpert discordant cases. • 20% of Case Detection Group participants with negative results for all test, including culture
Spot sputum (microscopy and culture)			X [#]	<ul style="list-style-type: none"> • Culture-negative, Truenat MTB (and/or MTB plus) and Xpert discordant cases. • 20% of Case Detection Group participants with negative culture

* Two sputum samples are collected on both Day 1 and Day 2. On Day 1, two spot sputa are collected. On Day 2, firstly, a morning sputum is self-collected in a pot taken home on Day 1. Secondly, an additional sputum is collected when the participant returns to the clinic to deliver the sputum pot from home.

[#] An additional spot sputum will be obtained at follow-up for smear microscopy and culture if i) not on TB treatment and ii) sputum can be produced spontaneously.

1 Introduction

The Truenat MTB, MTB plus and MTB-RIF Dx assays are a set of new tests developed for the diagnosis of tuberculosis (TB) and detection of resistance to rifampicin (RIF), a commonly used drug for treatment of active TB.

1.1 Trial Rationale

An estimated 4.1 million tuberculosis (TB) cases go undiagnosed globally each year¹, leading to substantial morbidity and mortality. In most countries smear microscopy remains the only option for the rapid diagnosis of TB, though it detects only 45% of infections²⁻⁶. For these reasons, new molecular TB diagnostics that can be instituted at the microscopy level are a research and implementation priority.

One such new molecular TB diagnostic is the Truenat including the MTB, MTB Plus and MTB-RIF Dx assays. Further evidence on the diagnostic performance and patient important outcomes on the use of the Truenat MTB assays and the MTB-RIF Dx assay at the microscopy centre level is needed to inform potential policy recommendations.

1.2 Background

The Truenat MTB and MTB plus assays and the MTB-RIF Dx (Truenat RIF or RIF assay) assay (Molbio Diagnostics; Bangalore, India) utilize chip-based real-time micro PCR for detection of TB and rifampicin (RIF) resistance from DNA extracted from sputum samples in about 25 minutes⁷. Briefly, automated DNA extraction is performed using the Trueprep Auto sample prep device (Figure 1). The extracted DNA is then added onto the Truenat MTB (or MTB plus) chip which is pre-loaded with stabilized reagents. Automatic amplification and analysis is then done using a handheld battery-operated device, the Truelab UnoDx real time PCR analyser. If the Truenat MTB (or MTB plus) chip is positive- and dependent on the diagnostic algorithm being employed- the user can then take another aliquot of extracted DNA and add it onto a separate Truenat MTB-RIF Dx chip to detect the presence of selected mutations associated with rifampicin resistance. A pilot trial conducted in India of the Truenat MTB assay found the assay to achieve 91% sensitivity and 100% specificity against a composite reference standard, including 99% sensitivity for smear-positive, culture-positive samples and 76% sensitivity for smear-negative, culture-positive samples⁸. However, further evidence of the performance and operational characteristics of the Truenat MTB assay, as well as the Truenat MTB plus (alternative version including an additional gene target

for TB detection) and RIF Reflex assay, is needed prior to recommending the clinical use of the assays.



Figure 1. Truenat MTB assays steps. A mixture of raw sputum and liquefaction buffer is directly loaded onto the Trueprep Auto chip interface, which extracts MTB DNA in 25 minutes. The extracted DNA is transferred to the Truenat MTB (or Truenat MTB Plus) chip and then onto the Truelab UnoDx PCR machine, which detects the presence of MTB DNA, and provides an automated result as either MTB-detected, MTB not detected or indeterminate result. For MTB positive results, another aliquot of the same DNA extracted is then transferred (reflex) to the Truenat MTB-RIF Dx chip.

To comprehensively evaluate the performance of the Truenat MTB assays and RIF assay, prior studies have been conducted as part of a three-phase/stage approach: i) a validation of the Truenat MTB assays and RIF assay diagnostic performance on frozen samples and ii) an operational assessment of the assays performance. This approach will allow for a rapid, direct assessment of assay performance against characterized specimens from diverse clinical sites (Africa, Eastern Europe and Asia) to confirm adequate performance of the Truenat MTB assays and RIF assay prior to the large clinical evaluation. The current trial corresponds to iii) the evaluation trial to confirm the diagnostic accuracy and to ensure that the performance characteristics will be consistent in the sites of intended use in a geographical diverse population (microscopy centre level) as well as patient important outcome will be assessed during this trial.

1.3 Benefit/Risk Assessment

Knowledge gained from this trial may benefit society by improving TB diagnosis. Trial participants may directly benefit from the trial because they will be provided with a higher standard of TB diagnostic care than may be routinely available to them.

Given the minimal risks associated with this trial and the potential benefits to society and individuals, the benefits outweigh the aggregated risks. As for any clinical trial, there is a possibility of unknown and unforeseen risk; that possibility is small for this trial. If unforeseen risks are recognized during the trial, then FIND, trial partners, IRBs/ethics committees, and participants must be provided with relevant information.

2 Trial Objectives and Endpoints

Table 1. Trial objectives and endpoints

Objectives	Endpoints
1. Primary	
<p>1.1. Estimate diagnostic accuracy of the Truenat assays (MTB and MTB Plus) for Mycobacterium tuberculosis (MTB) detection among individuals undergoing evaluation for pulmonary TB, overall and per sample, separately for smear-positive and smear-negative TB samples using a culture reference standard.</p> <p>1.2. Estimate diagnostic accuracy of the Truenat MTB-RIF Dx assay for RIF resistance detection among individuals undergoing evaluation for pulmonary TB and DR TB, using phenotypic/genotypic drug susceptibility testing (DST)¹.</p>	<p>1.1.1. Sensitivity of the Truenat MTB and MTB Plus assays (including analysis by smear-status and by sample).</p> <p>1.1.2. Specificity of the Truenat MTB and MTB Plus assays (including analysis by sample).</p> <p>1.2.1 Sensitivity of the Truenat MTB-RIF Dx assay</p> <p>1.2.2 Specificity of the Truenat MTB-RIF Dx assay</p>
2. Secondary	
2.1. Compare the diagnostic accuracy of the Truenat assays (MTB and MTB Plus) and MTB-RIF Dx assay to that of Xpert MTB/RIF, using a reference standard of culture for TB diagnosis	2.1.1. Sensitivity of the Truenat MTB and MTB Plus assays and MTB-RIF Dx assay compared to Xpert MTB/RIF

¹ All samples that exhibit RIF resistance, as determined by either phenotypic DST or Truenat RIF, will be sent for DNA sequencing to determine the mutations responsible for drug resistance. A subset of drug sensitive samples, of approximately matching sample size, will be randomly selected for sequencing, to act as a comparator sequence

and phenotypic/genotypic DST for detection of RIF resistance.	2.1.2. Specificity of the Truenat MTB and MTB Plus assays and MTB-RIF Dx assay compared to Xpert MTB/RIF
2.2. Assess patient important outcomes, including time to detection of TB and RIF resistance.	2.2.1. Patient important outcomes (see details in section Patient important outcomes under Statistical analysis plan)

3. Trial Design

3.1. General Design

This will be a prospective, multicentre, diagnostic accuracy trial in which the performance of an investigational rapid molecular diagnostic test (index test) on sputum samples (Truenat MTB assays and RIF assay) will be assessed in India, using solid and liquid culture as reference standard for the diagnosis of TB and MGIT SIRE as reference standard for the detection of RIF resistance.

3.2. Scientific Rationale for Trial Design

Subjects undergoing evaluation for TB and DR will be enrolled after informed consent is obtained. Results of the investigational Truenat assays will not be used for clinical care and will not be provided to clinicians or participants.

Laboratory technicians performing Truenat testing will be blinded to the results of conventional tests results. HIV testing or information about the status will be obtained in order to provide estimates of test performance in the important subgroup of HIV-infected cases with TB/DR symptoms, and also since HIV testing is considered standard of care for TB cases.

3.3. End of Trial Definition

A participant is considered to have completed the trial if he/she has completed the last visit. The end of the trial is defined as the date of the last visit of the last participant in the trial globally.

4. Trial Population and Eligibility

Trial population: Adults with presumptive tuberculosis disease.

Trial/sample size: Target enrolment for the multicentre trial is 1,666 participants enrolled.

Setting: In India, a total of 9 DMC will represent the intended settings of use including urban, peri-urban/hilly, tribal and rural sites with low and high throughput laboratories in India. Based on these characteristics the following numbers of presumptive TB cases, including smear-positive cases, are anticipated per DMC type (Table 2).

Table 2. Types of designated microscopy centres in India

S.No	DMC type	Nr. subjects with TB symptoms with sputum examination/month*	% Smear-positive among subjects with TB symptoms/month*
1	Low volume DMC (in health posts/UPHC)	40 (30 - 50)	2% - 3%
2	High volume DMC (in UPHC)	120 (100 - 150)	6% - 9%
3	High volume DMC (in Tertiary care/hospitals for chest diseases or TB)	300 (200 - 500)	10% - 12%

*Under routine conditions.

Participating DMC have been selected based on the following requirements:

- Smear microscopy must be available
- Xpert MTB/RIF must not be available
- At least 10 hours of electricity available (Trueprep Auto 10h per charge for 16 runs; Truelab UnoDx 4h per charge for 8-10 runs)
- At least 10 sputum samples collected per week, i.e. ~2 samples per day
- Have easy access to a reference laboratory with capacity to perform reference standard tests to ensure timely and high quality results

Given the important role of the private sector in India, 1 private laboratory will be included in the trial which is an MDR reference hospital.

Additionally, 3 other global sites will be setup in South America, Eastern Europe and Asia/Africa to achieve a wider geographic variation. These sites will be outpatient TB clinics at district or regional health facilities.

In order to meet enrolment targets (n= 1,666 total enrolled participants: India will enrol n = 1,110; other global sites will enrol n = 556).

All sites will need to be able to attain informed consent and obtain four sputum samples per patient for index and reference tests (as per sample flow in Figure 2). Participating centres must also have sufficient space to accommodate the Truenat instruments.

Location: Trial sites in India are listed in Table 3. Global sites will be determined among sites in South America, Eastern Europe and Asia/Africa.

Table 3. List of trial sites in India

Site name	Location
Madhupura Urban Health Centre, Ahmedabad	Urban
CHC Chhala, Ahmedabad	Rural
PHC Kuha, Ahmedabad	Rural
Kamrup Metro, Guwahati	Hilly/Peri-urban
Sonapur District Hospital, Guwahati	Tribal belt
Railway Hospital, Guwahati	Hilly/Peri-urban
Ayanavaram, Chennai	Urban
Villiwakkam, Chennai	Peri-urban
Thanthai Periyar, Chennai	Urban
PD Hinduja Hospital, Mumbai*	Urban

*Private sector

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

4.1. Inclusion Criteria

- Age 18 years or above;
- Clinical suspicion of pulmonary TB (including cough ≥ 2 week and at least 1 other symptom typical of TB);
- Willingness to provide 3 sputum specimens at enrolment;
- Willingness to have a trial follow-up visit approximately 2 months after enrolment;
- Provision of informed consent.

Two groups of participants will be enrolled. Namely a “Case Detection Group” and a “Drug Resistant Group”.

Case Detection Group: Participants are eligible to be included only if they meet all the conditions above.

Drug Resistant TB Group: In addition to the criteria of the Case Detection Group, participants should also meet the following conditions:

- Non-converting pulmonary TB cases (category I and category II failures)

4.2. Exclusion Criteria

Participants are excluded from the trial in case of:

Case Detection Group:

- Receipt of any dose of TB treatment within 60 days prior to enrolment (even if within last two days only).

Drug Resistant TB Group:

- Receipt of any dose of MDR-TB treatment within 60 days prior to enrolment (even if within last two days only).

4.3. Lifestyle Considerations

None

4.4. Screen Failures

Screen failures are defined as participants who consent to participate in the trial but are not subsequently entered in the trial. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Early exclusions

Incomplete sputum sample set

Participants who have provided consent and who are enrolled, but who do not provide a total of 3 sputum specimens of sufficient volume will be classified as early exclusions; each will be removed from the trial and a new trial subject enrolled. Participants who are classified as early exclusions will be referred to the appropriate local health service for TB evaluation and care. If positive for

M. tuberculosis, results of conventional microbiological tests (e.g., sputum smear microscopy, mycobacterial cultures, and Xpert MTB/RIF) will be reported to appropriate local health authorities in accordance with local public health reporting regulations. Trial data for participants who are classified as early exclusions will not be used for any final analyses.

Individuals who do not meet the criteria for participation in the trial (screen failure) may not be rescreened.

5. Trial Intervention

Trial Intervention is defined as any investigational intervention(s), marketed product(s), or medical device(s) intended to be used with a trial participant according to the trial protocol. Nevertheless in the current trial, the results of the intervention i.e. Truenat MTB, MTB Plus and RIF will not inform patient care decisions.

5.1. Medical Devices

The devices and diagnostic tests manufactured for sponsor use in this trial are:

- TruePrep Auto
- Truenat MTB chip
- Truenat MTB Plus chip
- Truenat UnoDx
- Truenat MTB-RIF Dx chip

Other medical devices (not manufactured by or for sponsor) to be used in this trial are:

- GeneXpert IV
- MGIT 960

Instructions for medical device use are provided in the Manual of Procedures.

Any medical device incidents, including those resulting from malfunctions of the device, must be detected, documented and reported by the investigator throughout the trial (see section 9 Safety and Incident Reporting).

5.2. Preparation/Handling/Storage/Accountability

The investigational products are intended for the detection of MTB in patient samples as well as the detection of MTB mutations associated with resistance to Rifampicin. The investigational products will be strictly accounted for, including receipt and inventory, storage, use during the trial, and return or disposal, as detailed in the Manual of Procedures provided by FIND.

Acquisition: Procurement of the investigational products will be done through FIND, which 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.

Storage: Procedures for product storage and disposal will be described in the Manual of Procedures. Briefly, investigational products will be checked for quality at the time of receipt at the site. Investigational products will be stored according to the manufacturer's instructions; expired or unused investigational products will be destroyed with documentation. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all devices, reagents and samples received and any discrepancies are reported and resolved before use of the trial intervention.

Test handling and performance: Testing using the investigational products will be performed according to the manufacturer's instructions outlined within the Manual of Procedures. Only participants enrolled in the trial may have samples evaluated using the medical devices described herein, and only authorized site staff may process samples and perform the diagnostic test. All trial medical devices, reagents and samples 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.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for trial intervention accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

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.

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 Manual of Procedures. New lots may only be used after this quality check is successfully passed.

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.

5.3. Minimization of Error and Bias

5.3.1. Patient selection

Spectrum bias will be avoided by enrolling a consecutive series of subjects and using a cross-sectional trial design. Enrolment will be based on clearly defined eligibility criteria, targeting patients suspected to have TB as defined by WHO12, thus representing future target populations. Descriptive statistics on patient characteristics and estimates of diagnostic accuracy stratified by site, smear and HIV status to further ensure the validity and generalizability of trial results will be provided.

5.3.2. Index test

The risk of review bias is minimal. Interpretation of results from the index tests does not require interpretation by the end-users and is based on pre-defined and automatically implemented thresholds. Further, results will become available and will be recorded before those of the reference standard (culture or DST). Where other rapid molecular assays are performed on the same sample, laboratory staff members will be instructed to record results independently of other test results.

5.3.3. Reference standard

Each participating laboratory will undergo an on-site laboratory evaluation to ensure standardized and high-quality performance of culture across sites (see also section Quality Assurance). Results from reference standard testing will be recorded blinded to index test results, eliminating the risk of review bias. Presence of MTB complex will be confirmed for all patients from a positive culture using MPT64 identification test and/or Line Probe Assay.

A reference standard of MTB culture (using two MGIT and two LJ cultures) will be used for TB detection. For RIF resistance detection a composite reference standard of phenotypic and genotypic RIF DST by sequencing will be used (as detailed in section Case definitions). This will allow for a high confidence in the interpretation of Truenat RIF resistant assay results. RIF sequencing will be done at a central laboratory for standardisation purposes (where possible).

5.3.4. Flow and timing

The planned patient and sample flow includes testing of three samples per patient with the index test and reference standard and there is thus little risk of disease progression bias. Manual mixing or splitting of decontaminated sample (pellet) together with random allocation of pellet aliquots (i.e. pipetting order) will also ensure that neither the index test nor the reference standard test are given an artificial advantage by virtue of being used on a higher quality sample.

5.3.5. Handling of indeterminate results

Samples for which the index test is indeterminate or cultures are contaminated will be excluded from the main accuracy analyses but reported separately. Results based on initial testing only as well as those obtained upon repeat-testing (for indeterminate results, where possible) will be presented.

5.3.6. Blinding Procedure

This is an open-label trial. The results of the Truenat MTB and RIF assays are generated automatically. Nevertheless, there is a potential bias of laboratory tests that are subject to operator's interpretation such as smear microscopy and LJ culture.

- Different operators will be assigned to Xpert MTB/RIF and smear microscopy. Similarly for MGIT and LJ culture.
- Technicians will be instructed to record results independently of other test results.

6. Discontinuation of Trial Intervention and Participant Discontinuation/Withdrawal

6.1. Participant Discontinuation/Withdrawal from the Trial

- A participant may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance, or administrative reasons.
- Participants who withdraw will be referred to the appropriate local health service for TB evaluation and care. If positivity for MTB is confirmed (through sputum smear microscopy, mycobacterial cultures or Xpert MTB/RIF), results will be reported to appropriate local health authorities in accordance with local public health reporting regulations.

- If the participant withdraws consent for disclosure of future information, FIND may retain and continue to use any data collected before such a withdrawal of consent.
- If a participant withdraws from the trial, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site trial records.
- See Manual of Procedures for data to be collected at the time of trial discontinuation and follow-up.

6.2. Lost to Follow Up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the trial site.

The following actions must be taken if a participant fails to return to the clinic for a required trial visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the trial.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant. These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable after 3 attempts at different time points, he/she will be considered to have withdrawn from the trial.

7. Trial Procedures

- Trial procedures and their timing are summarized in the SOA. Protocol waivers or exemptions are not allowed.
- Adherence to the trial design requirements, including those specified in the SOA, is essential and required for trial conduct.
- Eligibility criteria must be completed and reviewed to confirm that potential participants meet the requirements. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (e.g. chest X-ray) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SOA.

7.1. Trial Workflow

Following initial screening, individuals will be referred to trial personnel for additional information and to review eligibility (if initial assessment was done by non-trial clinicians). If appropriate, samples will be obtained following the informed consent process. Information on signs/symptoms (duration of cough, fever, haemoptysis, night sweats and weight loss) and TB history (previous TB episodes, dates of treatment, treatment outcome) will be obtained. Information on TB history will be critical given the potential for molecular tests to detect DNA from nonviable, non-intact bacilli in patients with recent history of TB¹⁰.

7.2. Efficacy Assessments

See section 10.4 Efficacy Analyses.

7.3. Safety Assessments

See Section 9, Safety and Incident Reporting.

7.4. Health Economics

The data collected may be used to conduct exploratory economic analyses and a specific study protocol will be prepared.

8. Specimen Collection, Handling, Transport and Storage

Participants will be asked to provide a total of four sputum samples at enrolment and over 2 days. The intent is for all samples to be collected before the participant starts any form of TB treatment, unless the participant is part of the Drug Resistant TB group.

Each specimen should be of 2 ml or greater in volume. On Day 1, each participant will be asked to submit two spot sputa (S1 and S2, approximately 30-60min apart) after enrolment. Participants will be given a labelled sputum pot and instructions for use, and instructed to collect a second sputum (S3) the next morning (Day 2) before going to the clinic. At the clinic participants will be asked to provide a third spot sputum (S4). In the event that a participant fails to return on Day 2, S3 and S4 may be collected a maximum of 7 days after enrolment, provided that no TB treatment has been initiated (the Case Detection group).

HIV testing will be offered to all participants as part of the trial procedures unless any one or more of the following are present:

- written results of a positive HIV antibody test;
- written results of a positive HIV viral load;
- documentation in the medical record of positive HIV status by a treating clinician;
- immediate/verifiable documentation of HIV negativity within the preceding one month.

HIV testing can be performed using any test method approved by local health authorities. Depending on the test method used, this test will require approximately 1 ml of saliva (rapid oral test), or up to approximately 5 ml of blood. Participants refusing to undergo HIV testing will not be excluded from the trial.

8.1. Reference Standard Test and Index Test Procedures

At each site, as per laboratory flow (Figure 2), standard diagnostic algorithms will be followed according to national guidelines and policies of each participating country. If national guidelines cannot be fulfilled using study-specific samples and test results, then an additional spot sputum should be collected.

Sites: All

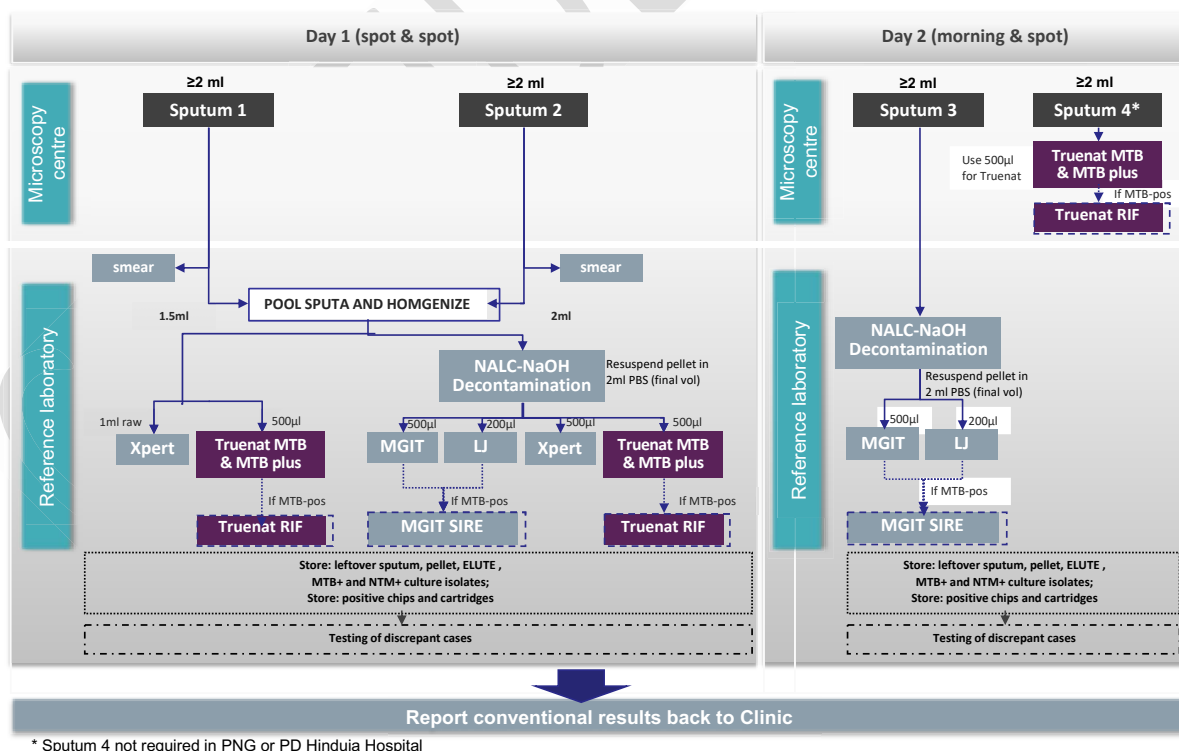


Figure 2. Laboratory workflow

Study spot Sputum 1 (S1) will be collected on Day 1. Approximately 30-60 minutes later, a second spot sputum (S2) will be collected and sent directly to the reference laboratory.

Participants will be given a labelled sputum pot and instructions on how to use it to collect an early-morning sputum (S3), and asked to bring this back to the clinic the next day (Day 2), and provide another spot sputum (S4).

On Day 1, smear microscopy will be performed on Sputum S1 and S2 in the reference laboratory or, if required, in the microscopy centre. In the reference lab, S1 and S2 will be pooled together and homogenised using glass beads and a vortex. Homogenised sputa will be further split: 1.5ml will be used for analysis on raw sputa, and at least 2ml used for NALC-NaOH decontamination.

Briefly, DNA will be extracted independently from i) raw sputum and ii) decontaminated pellet, by the Trueprep Auto device and tested by both the Truenat MTB and the MTB Plus chips, both of which are read by the Truelab UnoDx real-time PCR analyser. All DNA samples testing positive by the MTB assay will be subsequently tested by the Truenat MTB-RIF Dx assay (reflex) which is also read by Truelab UnoDx analyser.

Xpert assays will be performed on the same raw and decontaminated samples.

MGIT and LJ culture will be performed on only on the decontaminated sample.

On Day 2, morning sputum S3 will be returned to the clinic in a labelled sputum pot and the participant will be asked to provide spot sputum S4. S3 will be sent to the reference laboratory and a second round of MGIT and LJ culture will be performed on decontaminated sample. As for S1 and S2, each positive culture will be identified for MTB complex using MPT64 identification test and/or Line Probe Assay. MGIT SIRE will be used to determine the phenotypic DST for RIF. All samples that exhibit RIF resistance, as determined by either phenotypic DST or Truenat RIF, will be sent for DNA sequencing to determine the mutations responsible for drug resistance. A subset of drug sensitive samples, of approximately matching sample size, will be randomly selected for sequencing, to act as a comparator sequence.

Spot sputum S4 will be processed in the microscopy centre: 500ul raw sputum will be used for DNA extraction by Trueprep Auto and MTB detection by the Truenat assays. Any MTB-positive samples will be subsequently tested by the Truenat MTB-RIF Dx assay (reflex).

The intended objective is to test the Truenat assay in the setting of use (i.e. microscopy centre). If the microscopy centre has an Xpert machine (or if the

microscopy centre and the reference lab are the same), then the Truenat assay need only be performed once along side Xpert (on Day 1).

All leftover pellet, eluate and culture isolates will be stored as per the Manual of Procedures for further testing in case of discordant results e.g. result of Truenat RIF differs from that of phenotypic DST or Xpert MTB/RIF.

Importantly, laboratory technicians performing smear microscopy will be blinded to the results of Truenat MTB assays and other conventional test results. Moreover, the results of the Truenat MTB assays and Truenat RIF will not be reported back to the clinic i.e. will not be used for patient management.

8.2. Trial supplies and incoming quality check

- A list of materials for Truenat MTB and RIF testing is provided in Appendix 1.
- Income Quality Check (IQC) procedures are described in the Manual of Procedures. This will be conducted upon arrival of each new shipment of assays. New lots may only be used after this quality check is successfully passed.

8.3. Other Trial Procedures

8.3.1. Assessment of patient important outcomes

The following will be assessed as patient important outcomes using the Truenat MTB assays and RIF assay compared to routine tests

- Time to detection of TB and susceptibility to RIF.
- Yield over smear microscopy and Xpert MTB/RIF.
- Upfront diagnosis of RIF (reflex on all Truenat MTB or MTB Plus positive versus current standard of smear-positive reflexed to Xpert MTB/RIF based on risk factors).

See Patient important outcomes under Statistical analysis plan.

8.3.2. Participant follow-up

A follow-up visit at Day 56 (+/- 14 days) post-enrolment will be conducted to a subset of participants in order to collect additional information on the TB status.

- **Culture-negative, Truenat MTB (and/or MTB plus) and Xpert discordant cases.** During the prospective assessment, Truenat results will not be provided to clinicians or participants or used for

decision-making. Thus participants who are Xpert-negative but Truenat-positive at enrolment (“discordant”) will not be treated on the basis of Truenat results. All culture negative cases with discrepant Truenat and Xpert results will undergo a follow-up visit performed at Day 56 (+/-14) post-enrolment to assess:

- Interval medication history, including TB treatment and clinical evolution.
- An additional spot sputum will be obtained for smear microscopy and culture (LJ and MGIT) provided the patient was not started on therapy and is able to provide a spontaneously produced sputum sample.

This will aid in the identification of patients who would be diagnosed (in the absence of Truenat MTB assays being available for decision making) on clinical grounds.

- **Cases Detection group with negative results.** The first 267 patients who are negative on all tests (approximately 20%) will be followed at Day 56 (+/-14) post-enrolment to assess:
 - Interval medication history, including TB treatment and clinical evolution
 - An additional spot sputum will be obtained for smear microscopy and culture (LJ and MGIT) provided the pulmonary symptoms persist and the patient was not started on therapy

The purpose of this follow-up visit is to identify the participants in this subset who are diagnosed/initiated on treatment on clinical grounds and those that are missed completely.

A follow-up visit is not required for participants who are started on treatment (based on Xpert or culture results).

8.3.3. Analysis of discordant results

Given that the sensitivity and specificity of culture and culture-based DST for Rifampicin is not perfect, misclassification of samples by these reference standards may introduce bias into the sensitivity and specificity estimates for the Truenat assays and underestimate their diagnostic performance.

In order to address this bias, cases with discordant results (and a subset of cases with concordant results) will be further assessed following a Manual of Procedures and described separately in the final trial report. The same number of non-discordant cases will be tested to avoid introduction of additional bias.

8.4. Genetics

Besides sequencing of *M. tuberculosis* to identify mutations recognized to be associated with drug resistance, no other genetic tests are evaluated in this trial.

8.5. Biomarkers

Biomarkers are not evaluated in this trial.

9. Safety and Incident Reporting

Given the nature of this trial i.e. diagnostic accuracy and comparison of diagnostic solutions, the probability of an AE and SAE to be associated with the investigational products is exceedingly rare.

Safety reporting is therefore limited in scope to events associated with the collection of samples and those that occur at participating laboratories using the investigational medical device i.e. TruePrep Auto and Truenat UnoDx. For the purposes of this trial only fatal SAEs and medical device incidents fulfilling the definition of AE or SAE (see section 9.2 Medical Device Incidents) will be reported.

The definitions can be found in Appendices 2 and 3.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up all AEs that are serious, considered related to the trial intervention or trial procedures, or that caused the participant to discontinue the trial. If the AE meets the definition of a SAE it must be reported to FIND regardless if associated with and of the investigational diagnostic devices.

9.1. Time Period for Collecting SAE Information

Relevant AE or SAEs will be collected from the signing of the informed consent form (ICF) until the follow-up visit.

Fatal SAEs will be recorded and reported to FIND or designee within 24 hours, as indicated in Appendix 2. The investigator will submit any updated SAE data to FIND within 24 hours of it being available.

Investigators are not obligated to actively seek SAEs after conclusion of the trial participation.

The method of recording, evaluating, and assessing causality of the SAE and the procedures for completing and transmitting SAE reports are provided in Appendix 2.

9.1.1. Reporting and Follow up of SAEs

Further information on follow-up procedures is given in Appendix 2.

9.1.2. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to FIND of a fatal SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and investigational medical device operators are met.
- FIND has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a trial intervention under clinical investigation. FIND will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- An investigator who receives an investigator safety report describing a fatal SAE or other specific safety information (e.g. summary or listing of SAEs) from FIND will review and then file it in the ISF and will notify the IRB/IEC, if appropriate according to local requirements.

9.2. Medical Device Incidents (including Malfunctions)

Medical devices are being provided for use in this trial for the purpose of evaluating diagnosis of TB and detecting RIF resistance. In order to fulfil regulatory reporting obligations worldwide, the investigator is responsible for the detection and documentation of events meeting the definitions of incident or malfunction that occur during the trial with such devices.

The definition of a Medical Device Incident can be found in Appendix 3.

NOTE: Incidents fulfilling the definition of an AE/SAE (e.g. any event that occurs as a result of insufficient or inadequate instructions for use, installation, or operation, or any malfunction of the investigational device, as well as any event resulting from operator error(s), intentional misuse of the investigational medical device or protocol violation) will follow the processes outlined above and in Appendix 3 of the protocol.

9.2.1. Time Period for Detecting Medical Device Incidents

Medical device incidents or malfunctions of the device that result in an incident will be detected, documented, and reported during all periods of the trial in which the medical device is used.

Medical device incidents meeting the definition of AE or SAE will be reported to FIND or designee within 24 hours, as indicated in Appendix 3. The investigator will submit any updated SAE data to FIND within 24 hours of it being available.

If the investigator learns of any incident at any time after a participant has been discharged from the trial, and such incident is considered reasonably related to a medical device provided for the trial, the investigator will promptly notify FIND.

The method of documenting Medical Device Incidents is provided in Appendix 3.

9.2.2. Follow-up of Medical Device Incidents

- All medical device incidents involving an AE will be followed and reported in the same manner as other AEs (see Appendix 2). This applies to all participants, including those who discontinue trial intervention as well as medical device operators.
- The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the incident.
- New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

9.2.3. Reporting of Medical Device Incidents to Sponsor

- Medical device incidents will be reported to FIND within 24 hours after the investigator determines that the event meets the protocol definition of a medical device incident.
- The Medical Device Incident Report Form will be sent to FIND by email.
- The same individual will be the contact for the receipt of medical device reports and SAE.

9.2.4. Regulatory Reporting Requirements for Medical Device Incidents

- The investigator will promptly report all incidents occurring with any medical device provided for use in the trial in order for FIND to fulfil the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.
- The investigator, or responsible person according to local requirements (e.g. the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of incidents to the IRB/IEC.

10. Statistical Considerations

This trial is designed to assess diagnostic accuracy of the index test compared to culture reference standard, and to assess the diagnostic accuracy of RIF resistance detection compared to a reference standard of phenotypic drug susceptibility testing. Further this trial will compare diagnostic accuracy and RIF detection of the index tests to that of Xpert MTB/RIF. As such, the trial has been designed to capture a sufficient number of smear negative TB cases to be able to provide estimates of sensitivity with a confidence interval width of approximately 20%.

This section provides an overview of the data analysis strategy and methodology. A detailed Statistical Analysis Plan (SAP) will be written before the start of recruitment.

10.1. Statistical Hypotheses

Primary endpoint 1.1 is to assess the diagnostic accuracy of the Truenat MTB assays for MTB detection among individuals undergoing evaluation for pulmonary TB, overall and per sample, separately for smear-positive and smear-negative TB samples using a culture reference standard.

Primary endpoint 1.2 will estimate diagnostic accuracy of the Truenat RIF assay for RIF resistance detection among individuals undergoing evaluation for pulmonary TB and DR TB, using a reference standard of phenotypic/genotypic DST i.e. MGIT SIRE and sequencing for RIF resistance.

Secondary endpoints will compare the diagnostic accuracy of the Truenat MTB assays and RIF assay to that of Xpert MTB/RIF, using a reference standard of culture for TB diagnosis and MGIT SIRE and sequencing for detection of RIF

resistance. Additionally, the trial will assess patient important outcomes, including: i) time to detection of TB and RIF resistance, ii) yield over smear microscopy and Xpert MTB/RIF and iii) upfront diagnosis of RIF (reflex testing on all Truenat MTB or MTB Plus positive versus current standard of smear-positive reflexed to Xpert MTB/RIF based on risk factors).

10.2. Sample Size Determination

The sample size was chosen to achieve high confidence in the accuracy estimates for MTB-detection and RIF resistance detection for the overall multi-country trial for which the Indian trial is one of the countries.

Based on an expected sensitivity of Truenat MTB plus for detection of TB among smear-negative/culture-positive cases of 67% (based on preliminary data), 80 smear-negative/culture-positive cases would be required to achieve a total width of the 95% confidence interval of 20% (95%CI: 57 to 77). Assuming a TB prevalence of 20% and a prevalence of smear-negative/culture-positive cases among TB cases of 30%, the total number of subjects to be enrolled would be 1,333. To account for losses, this is inflated by 20%, yielding a final sample size of 1,666 participants under investigation for TB overall.

In India, two thirds of these trial participants will be recruited i.e. $n = 1,110$ thus 67 smear-negative/culture-positive cases and a 95% confidence interval of 21% could be achieved (95%CI 55 to 79).

The other one-third of enrolled participants ($n = 556$) will be recruited in three other countries in order to provide geographic variation.

The secondary objective of determining diagnostic accuracy of RIF resistance by Truenat MTB is based upon an expected Truenat RIF sensitivity of 95% with a confidence interval of 10% (90-100%), requiring $n=37$ RIF-resistant participants detected. Assuming a prevalence of 20% culture-positive TB cases detected across all presumed TB cases, and 2.8% RIF resistance amongst all culture-positive TB cases and 12% prevalence of RIF resistance amongst TB retreatment cases, we predict 1,542 re-treatment patients would thus need to be enrolled. While the prevalence of culture-positive TB cases may be higher if enrolment is conducted at a drug-resistance TB referral clinic, we conservatively accept that this may not be the case. As such, we may not reach sufficient sample size to allow analysis of secondary objectives in this trial. Detection of RIF resistance will be continually monitored throughout the trial, and any possible shortfall will be supplemented with a future sub-study, if needed, using confirmed RIF-resistant sputum samples from the FIND specimen bank of cryopreserved samples. We will place an enrolment cap of 200 enrolled participants in the drug resistance group for the entire trial, in order to not undermine the primary objective of enrolment of smear-negative culture

positive TB cases through inadvertent over-enrolment of DR cases who are more likely to be smear-positive culture-positive.

10.3. Populations for Analyses

For purposes of analysis, the following participant populations are defined:

Table 4. Populations for analyses

Population	Description
Enrolled	All participants who sign the ICF
Evaluable	All participants with a culture positive or culture negative results. Contaminated cultures will be excluded. Contamination is defined as below:
Smear-positive	≥ 1 positive smear (inclusive of scanty positive smears). Smear positives with only negative or contaminated cultures will be excluded from analysis.
Culture-positive	≥ 1 LJ and/or MGIT culture growth confirmed MTB complex. Cross-Contamination: A single LJ culture with ≤ 20 colonies or a single MGIT culture with MTB growth ≥28 days per patient will be excluded from analysis.
Culture-negative	At least 2 LJ or MGIT have no culture growth after >56 days and >42 days
Contaminated culture	LJ: Cultures completely overgrown by bacterial or fungal contaminations within 3 weeks (discarded). In case of mixed cultures, isolated MTB colonies transferred to new LJ tube (repeat culture). MGIT: Instrument positivity tests for confirmation of MTB complex negative. 2 contaminated cultures will lead to exclusion of this patient from analysis unless other criteria for culture-positivity/negativity are met
Xpert-positive	MTB positive on Xpert® MTB/RIF.
Xpert-negative	MTB negative on Xpert® MTB/RIF.
Xpert-indeterminate	Any indeterminate, error, or inability to produce a result from a single Xpert® MTB/RIF run.
Non-TB case	Smear-negative, Xpert-negative and culture-negative and not started on TB treatment on the basis of clinical criteria. NTM: Specimens with growth of mycobacteria other than MTB complex only.
Clinical TB case	Any participant who tests smear-negative, Xpert-negative, culture-negative but is started on TB treatment on the basis of clinical criteria.

Phenotypic RIF resistant	Culture-positive and growth for RIF on MGIT SIRE DST testing.
Phenotypic RIF sensitive	Culture-positive and no growth for RIF on MGIT SIRE DST testing
Genotypic RIF resistant	Sequencing identifies mutations recognized to be associated with RIF-resistance (defined based on consultation with WHO prior to analysis)
Genotypic RIF sensitive	Sequencing identifies no mutations recognized to be associated with RIF-resistance (defined based on consultation with WHO prior to analysis)
Composite reference standard RIF resistant	<p>If phenotypic DST shows sensitivity but sequencing identifies mutations recognized to be associated with resistance, the composite reference standard will be considered RIF-resistant.</p> <p>If phenotypic DST shows resistance but sequencing does not identify mutations to be associated with resistance, the composite reference standard will be considered RIF-resistant (as mutations will be assumed outside of the region sequenced).</p>
Safety	All participants assigned to trial intervention.

10.4. Statistical Analysis Plan

The statistical analysis plan (SAP) will be developed and finalized before database lock and will describe the participant populations to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a high-level summary of the planned statistical analyses of the primary and secondary endpoints.

10.5. Efficacy Analyses

Table 5. Statistical analysis methods

Endpoint	Statistical Analysis Methods
Primary	Sensitivity and specificity, and ANOVA comparison of Likelihood Ratio test.
Secondary	Difference in sensitivity and specificity between each index assay and Xpert with 95%CI around the difference using methods appropriate for paired data (e.g. Tango score CI)

10.5.1. General Approach

For each test, estimates of sensitivity and specificity will be derived together with 95% confidence intervals. Estimates will be calculated on the overall dataset and by subgroups defined below.

10.5.2. Analysis of Primary Outcomes

Analyses of the diagnostic accuracy of the index test will be done according to the case definitions. Table 6 summarizes the way diagnostic tests results are usually reported when comparing the index test results to a reference standard. Based on the definitions in the table, the following values are defined:

Sensitivity = $TP / (TP + FN)$

Specificity = $TN / (FP + TN)$

Table 6. Definition of classification metrics

Test result	Case definition			
		TB	Non-TB	Total
	Detected	TP	FP	TP + FP
	Not detected	FN	TN	FN + TN
	Total	TP + FP	FP + TN	All

Abbreviations: TB=true positive, FP=false positive, FN=false negative, TN=true negative

Only patients with uncontaminated culture results and without indeterminate test results will be included in the analysis of performance characteristics for MTB detection. Similarly, such patients will contribute to the analysis of the performance characteristics for RIF detection. For each point estimate, a 95% confidence interval will be derived based on Wilson score. Missing data and invalid results will be reported in the descriptive tables, and will not be imputed.

10.5.3. Analysis of Secondary Outcomes

- Accuracy of Truenat MTB assays and RIF assay compared to Xpert MTB/RIF.

The same parameters and sub-groups as for the analysis of primary outcomes will be determined for Xpert MTB/RIF and compared to those of Truenat MTB assays and RIF assay side by side.

- Patient-important outcomes
 - The median time to detection of TB using the Truenat MTB assays will be determined and compared to that of smear, Xpert MTB/RIF, liquid and solid cultures. Furthermore, the median time to detection of RIF resistance using the Truenat RIF assay will be determined and compared to that of phenotypic DST.
 - The number of additional TB (culture-positive) cases detected by the Truenat MTB assays compared to smear and Xpert MTB/RIF.
 - The number of RIF-resistant cases detected using the Truenat RIF assay compared to those detected following the current standard of smear-positive reflexed to Xpert MTB/RIF.

10.5.4. Descriptive statistics

Descriptive statistics tables will be generated to summarize the characteristics of the participants. The number of participants included and excluded will be reported. Among the included participants, the information will be broken down by site, gender, age group, HIV status, history of TB and enrolment group (Case Detection Group and Drug Resistant Group). Results will be reported either in absolute numbers (e.g. number of subjects in a group) or summarized by IQR, percentage.

10.5.5. Additional sub-group analyses

The trial outcomes defined will be also evaluated on the following subpopulations:

- Sensitivity for MTB detection:
 - Per-patient: Truenat MTB assays on S1 and S2 against culture status (based on cultures from S2 and S3)
 - Per-sample processing method: Truenat MTB assays on S1 against culture result on S2 and Truenat MTB assays on S2 against culture result on S2 (same specimen)
 - By smear status
 - By HIV status (where available)
 - On unprocessed (S1) vs decontaminated specimen (S2)
 - After repetition (if result invalid)
 - Compared to Xpert MTB/RIF on S2 (based on culture from S2 and S3)
- Specificity for MTB detection:
 - By TB history
 - By prior TB treatment (> 60 days ago)
 - Compared to Xpert MTB/RIF (based on cultures from S2 and S3)
- Sensitivity and specificity for rifampin resistance detection:
 - Against phenotypic DST
 - Compared to Xpert MTB/RIF on S2 (based on DST from S2 and S3)
- Intermediate/invalid/erroneous results
 - For MTB detection
 - For RIF resistance detection
- Quantitative ability (based on assay cycle threshold (Ct) values)
 - In comparison to culture (positivity and time to positivity, grade for solid culture)
 - In comparison to smear (positivity and grade)

Additional subgroups will be defined and specified in the final SAP.

10.5.6. Planned Interim Analyses

No interim analysis is planned. Continued data monitoring will be performed (see section 12 Data handling and record keeping).

10.6. Safety Analyses

Not applicable. Given the nature of this trial i.e. diagnostic accuracy and comparison of diagnostic solutions, the probability of an AE and SAE to be associated with the investigational products is exceedingly rare. Therefore, no safety analysis will be conducted. However, AE and SAE will be reported as per Section 9 Safety and incident reporting.

10.7. Independent Data Monitoring Committee (IDMC)

Not applicable. Based on FIND internal procedures, an IDMC will be established only for trials where participants are at greater than minimal risk e.g. invasive techniques for specimen collection or use of index test results to inform patient care.

11. Regulatory and Ethical Considerations

11.1. Regulatory and Ethics Approvals

This trial will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the trial is initiated.

- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the trial design, except for changes necessary to eliminate an immediate hazard to trial participants.
- The investigator will be responsible for the following:

- Providing written summaries of the status of the trial to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the trial at the site and adherence to requirements of ICH guidelines, the IRB/IEC, the WHO Good Clinical Laboratory Practice (GCLP), European regulation 536/2014 for clinical studies (if applicable), and with applicable national regulations.

11.2. Financial Disclosure

Investigators and sub-investigators will provide FIND with sufficient, accurate financial information as requested to allow FIND to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the trial and for 1 year after completion of the trial.

11.3. Informed Consent Process

- The investigator or his/her representative will explain the nature of the trial to the participant or his/her legally authorized representative and answer all questions regarding the trial.
- Prospective participants must be informed that their participation is voluntary and will be required to sign and date a statement of informed consent that meets the requirements of local regulations and/or ICH guidelines where applicable, and the IRB/IEC or trial centre.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the trial and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Illiterate patients must provide a thumbprint on the ICF and the ICF must be signed and dated by an impartial witness.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the trial.

11.4. Data Protection

- Participants will be assigned a unique identifier by FIND. Any participant records or datasets that are transferred to FIND will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal trial-related data will be used by FIND in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by FIND, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

11.5. Subject Confidentiality

The Principal Investigator will be responsible for the recording of all subject's personal details, screening number and unique trial number in the Subjects Identification List. To ensure confidentiality, this list must be kept in a place, with access restricted to authorized trial staff. All laboratory specimens, including stored specimens, as well as trial reports, data collection tools, and administrative forms will be identified by a using the patient's unique trial number. Names will not be used on any of these documents. All local databases will be secured with password-protected access systems. The Investigator must ensure anonymity of the patient and ensure that all documents are anonymised before being transmitted to FIND.

11.6. Other ethical considerations

11.6.1. Use of stored specimens:

Sputum volume after processing as well as Trueprep used chips will be stored temporarily in case further testing is deemed to be necessary. Samples will be labelled with a trial identifier; the specimens will be linked to results of other mycobacteriology tests, the final trial diagnosis (e.g. non-TB case, culture-positive TB case, clinical TB case, Indeterminate) of the participant. Specimens will be stored temporarily at the sites. Further testing may be required at a Reference Laboratory or specialized research laboratories. An export permit will be obtained accordingly if required. Moreover, after trial completion leftover well-characterised specimens could be used for future research of product development as long as trial participants have agreed to this during the initial visit.

11.6.2. Protocol deviations

Protocol deviations occur when there is non-adherence to the Protocol and includes Informed Consent, enrolment, and other occurrences of non-adherence to the Protocol. Protocol deviations should be sent to the local IRB/IEC per the local IRB/IEC guidelines. Any protocol deviation that meets reporting requirements of the local IRB/IEC will also be reported with the same timeliness to the FIND project manager.

11.6.3. Termination of the Trial

Given the nature of the trial, termination due to safety or other reasons is not anticipated. However, should the trial be terminated, participants will be referred to the appropriate local health service for TB evaluation and care. If positive for MTB, results of conventional microbiological tests (i.e., sputum smear microscopy and mycobacterial cultures) will be reported to appropriate local health authorities in accordance with local public health reporting regulations.

11.6.4. Site Performance and Data Submission

In the event that one site does not meet enrolment targets within the trial timelines, the trial will go forward at the other sites and the investigators will proceed with data submission as per trial plan.

12. Data Handling and Record Keeping

- All participant data relating to the trial will be recorded on printed or electronic CRF unless transmitted to FIND or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The investigator must permit trial-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- FIND or designee is responsible for the data management of this trial including quality checking of the data.

- Trial monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the trial is being conducted in accordance with the currently approved protocol and any other trial agreements, ICH GCP, and all applicable regulatory requirements.

12.1. Source Data and Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site and will include testing logs, registers, screening forms, laboratory forms, operating characteristics surveys, and data collected through direct patient interviews.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or reports if available.
- The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trials subjects. Source data should be attributable, legible, contemporaneous, original, accurate and complete. Changes to source data should be traceable, should not obscure the original entry and should be explained if necessary.

12.2. Data to be captured

Demographic data, treatment and testing records, clinical status, comparator and reference test results, index test results and selected operational and environmental data will be collected using paper and/or eCRF and transmitted electronically to FIND using the OpenClinica.

12.3. Data Management

FIND is responsible for the data management for the trial, including the setup of the database, programming, editing and range checking. Trial site staff will be responsible for entering trial data from a paper CRF into OpenClinica within 10 working days of a participant's visit or the availability of results. The site will be provided with individual password-protected accounts to access OpenClinica, following a training session given by FIND. Data entry training will be provided by FIND, either on site or remotely sharing screen through Skype

or any other similar system. OpenClinica provides an audit trail system recording all data entries/changes and queries between FIND and the site.

No information concerning the trial or the data generated from the trial will be released to any unauthorized third party without prior written approval of FIND and manufacturers.

12.4. Data Archiving

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.

13. Quality Management

Quality Control and Quality Assurance systems with written Standard Operating Procedures will be established to ensure that the trial is conducted and the data are generated, documented and reported in compliance with this protocol, good clinical practice (GCP), good clinical laboratory practice (GCLP) and applicable regulatory requirements. Data should be collected according to trial specific Data Management plan.

13.1. Quality Control

Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

Risk Based Monitoring will be done and the trial will be monitored remotely (centrally) as well as on-site. Further details on monitoring visits will be included in the Monitoring Plan.

13.2. Quality assurance

FIND will conduct clinical and laboratory assessments at the DMC, selected private laboratories and reference laboratory sites prior to trial start. On-site visits will include observation of routine procedures, assessment of quality management systems as well as review of historical data such as quality indicators (including but not limited to smear- and culture-positivity, smear-positive/culture-negative, Xpert-positive/culture-negative rates). Following the initial assessments and implementation of any recommendations, on-site training will be conducted by FIND (see section Training plan and piloting of laboratory and data collection procedures).

The FIND Clinical Trial Standard will be implemented to verify the clinical and laboratory quality prior to initiation of the trial and the data quality, applicable regulatory documentation, and subject safety throughout the trial.

The trial co-investigators are responsible for conducting routine quality assurance and quality control activities to internally monitor trial progress and protocol compliance in addition to procedures in the FIND Manual of Procedures. The co-investigators will ensure that all trial personnel are appropriately trained and applicable documentations are maintained on site.

13.3. Training plan and piloting of laboratory and data collection procedures

The principal investigators should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s) and their trial-related duties and functions.

The principal investigators are responsible for supervising any individual or party to whom the investigator delegates trial-related duties and functions conducted at the trial site. The principal investigators should ensure this individual or party is qualified to perform those trial-related duties and functions and should implement procedures to ensure the integrity of the trial-related duties and functions performed and any data generated. All key trial staff shall be trained and certified in GCP and certificates provided to FIND.

On-site training on all relevant trial procedures that are not part of routine care will be provided by FIND to the relevant staff, i.e. the trial coordinator at each site, the data entry personnel, the staff at sputum collection sites and the DMC and reference laboratory staff. The training will include clinical and laboratory aspects with focus on hands-on training on the Truenat MTB assays and RIF assay. Proficiency to perform the Truenat MTB assays and RIF assay will be assessed using a proficiency testing tool designed for this trial.

13.4. Trial and Site Closure

FIND designee reserves the right to close the trial site or terminate the trial at any time for any reason at the sole discretion of FIND. Trial sites will be closed upon trial completion. A trial site is considered closed when all required documents and trial supplies have been collected and a trial-site closure visit has been performed.

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 the early closure of a trial site by FIND or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, FIND's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further trial intervention development

14. Work plan & timeline

Trial preparation activities start with IRB approval. Trial initiation followed by enrolment will last for 32 weeks and details of the trial timeline are shown below.

Table 7. Trial timeline

Phase	Activity	Duration	Estimated timeline
Preparation	IRB approval	10 weeks	Q3-2018
	Training	3-4 days	Q4-2018
Implementation	Enrolment	32 weeks	Q4-2018 to Q2-2019
	Follow-up	8 weeks	Q3-2019
	Laboratory testing (enrolment, FU, discordant cases)	26 weeks	Q4-2019
Reporting	Data analysis	3 weeks	Q4-2019
	Report write up	4 weeks	Q4-2019

15. Publication Policy

- The results of this trial may be published or presented at scientific meetings. Details are provided in the Trial Agreement (e.g. Memorandum of Understanding, Collaboration Agreement).
- FIND will comply with the requirements for publication of trial results. In accordance with standard editorial and ethical practice.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

16. References

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17. Appendices

Appendix 1: Supply requirements for Truenat testing

Appendix 2: Safety Definitions and Reporting

Appendix 3: Incident Definitions and Reporting

Appendix 3: Protocol Amendment Summary

Appendix 1: Supply requirements for Truenat testing

Item
Trueprep Auto Sample Prep
Truelab Uno DxAnalyzer
Truenat MTB chip
Truenat MTB Plus chip
Truenat MTB-RIF Dx chip
Additional Trueprep Supplies
Trueprep Auto Sputum sample prep kit (cartridges, reagents)
Additional Truenat Supplies
Truenat Universal Control Kit
Precision micropipettes (fixed volume) including micropipette tips
Additional Truelab Supplies
Truelab AC adapter
Other Supplies <u>not provided</u>
- 70% isopropyl alcohol
- Gloves
- Paper towel
- Squash bottles
- Discard bin for waste (dry)
- Discard bin for waste (wet)
- 0.5% sodium hypochlorite solution
- Mask
- Gown
- Timer

All the Molbio instruments and reagents will be supplied including package inserts and user manuals

Appendix 2: Safety Definitions and Reporting

AE Definition

- An AE is any untoward medical occurrence in a patient or clinical trial participant, temporally associated with the use of trial intervention, whether or not considered related to the trial intervention.
- NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of trial intervention.

SAE Definition

a. Results in death

b. Is life-threatening

The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

SAE Reporting to FIND via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to FIND will be the electronic data collection tool.
- If the electronic system is unavailable for more than 24 hours, then the site will use the paper SAE data collection tool (see next section).
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the trial is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a trial participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the [X/medical monitor/SAE coordinator] by telephone.
- Contacts for SAE reporting can be found in the Manual of Procedures.

SAE Reporting to FIND via Paper CRF

- E-mail transmission of the SAE paper CRF is the preferred method to transmit this information to FIND.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in the Manual of Procedures.

Appendix 3: Incident Definition and Reporting

Medical Device Incident Definition

- A medical device incident is any malfunction or deterioration in the characteristics and/or performance of a device as well as any inadequacy in the labeling or the instructions for use which, directly or indirectly, might lead to or might have led to the death of a participant/user/other person or to a serious deterioration in his/her state of health.
- Not all incidents lead to death or serious deterioration in health. The nonoccurrence of such a result might have been due to other fortunate circumstances or to the intervention of health care personnel.

It is sufficient that:

- An **incident** associated with a device happened.

AND

- The **incident** was such that, if it occurred again, might lead to death or a serious deterioration in health.

A serious deterioration in state of health can include any of the following:

- Life-threatening illness
- Permanent impairment of body function or permanent damage to body structure
- Condition necessitating medical or surgical intervention to prevent one of the above
- Foetal distress, foetal death, or any congenital abnormality or birth defects

Medical Device Incident Documenting

- Any medical device incident occurring during the trial will be documented in the participant's medical records, in accordance with the investigator's normal clinical practice, and on the appropriate form of the CRF.
- For incidents fulfilling the definition of an AE or an SAE, the appropriate AE/SAE CRF page will be completed as described in Appendix 3.
- The CRF will be completed as thoroughly as possible and signed by the investigator before transmittal to FIND or designee.
- It is very important that the investigator provides his/her assessment of causality (relationship to the medical device provided by FIND) at the time of the initial SAE report and describes any corrective or remedial actions taken to prevent recurrence of the incident.
- A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of an incident. This includes any amendment to the device design to prevent recurrence.

Appendix 4: Protocol Amendment Summary

The Protocol Amendment Summary of Changes Table is located directly before the Table of Contents (TOC).

Amendment Number 1 (India only).

Overall Rationale for the Amendment

Since the first submission of the trial protocol to the Ethics Committee in India, updates were needed given that: i) new results on Truenat became available, ii) further assessment of trial risks and revision of mitigation strategies including enrolment targets to determine the performance of the Truenat MTB-RIF Dx assay, adoption of the Common Protocol Template (TransCelerate).