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STATISTICAL ANALYSIS PLAN

Study Title:

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

Short title:
Truenat Evaluation

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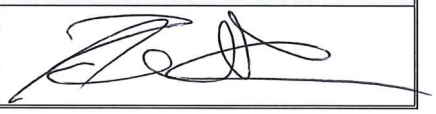
Adam Penn-Nicholson – Trial Manager	12/MAR/2019	

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1 Introduction

This document describes the statistical analysis plan for the following project: "Prospective, Multicentre Trial to Assess the Diagnostic Accuracy of the Truenat Assays at Intended Settings of Use", Version 1.0, date: 07.09.2018.

1.1 Description of the study

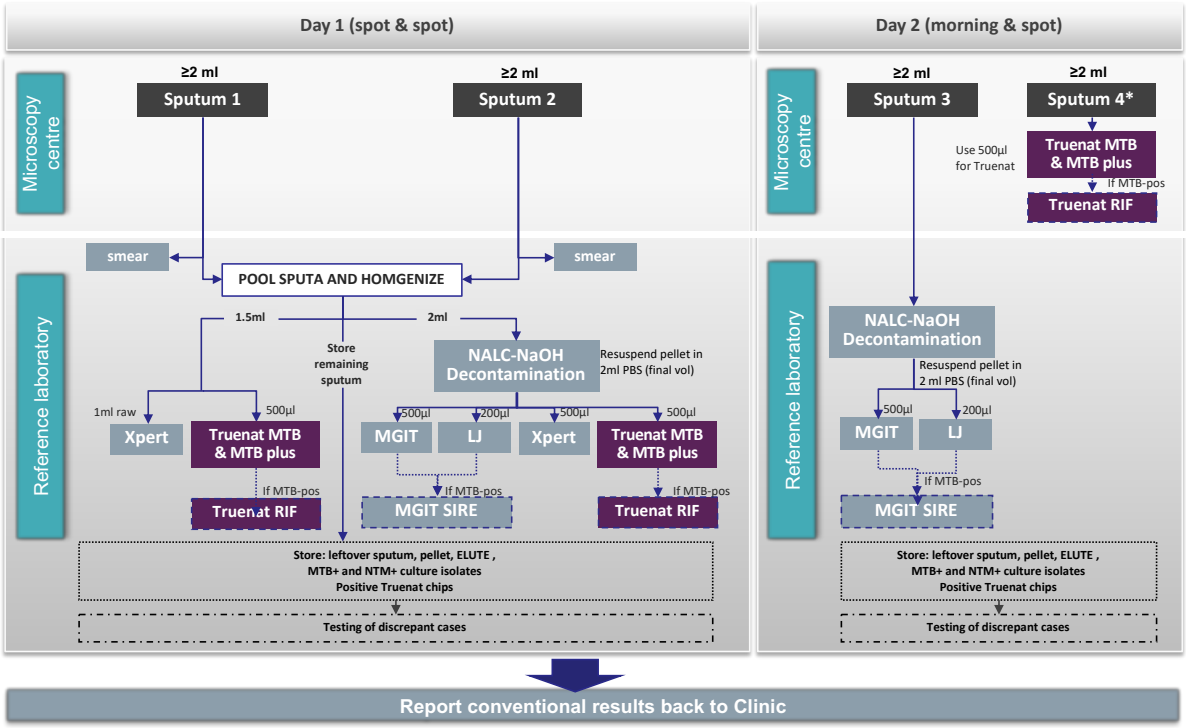
The Truenat MTB (including both MTB and MTB plus) and the MTB-RIF Dx reflex assays (Molbio Diagnostics; Bangalore, India) utilize chip-based real-time micro PCR for detection of tuberculosis (TB) and rifampicin (RIF) resistance from DNA extracted from sputum samples in about 25 minutes.
A pilot trial 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.
The goal of this trial is 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).

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.

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.

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.

At each site, as per the laboratory flow (Figure 1), 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.



* Sputum 4 not required in PNG or PD Hinduja Hospital

Figure 1. Laboratory workflow

1.2 Timing of the analysis

As part of the assessment/risk mitigation plan, a preliminary analysis will be conducted approximately one month after enrolment, with available data (which will exclude culture results, as the time to obtain these would not be compatible with the timing of the analysis). The objective of this analysis is to evaluate the frequency of positive Truenat samples compared with positive Xpert. From prior studies, a false positive rate between 3 and 5% is expected. If the proportion of Molbio positive results after one month is 8% (or more) higher than the proportion of Xpert results, a root cause analysis will be conducted to limit potential contamination, without implications on the continuation of the study. As daily swab testing is required before testing of any clinical sampled, such contamination events should not go undetected, and therefore no study pause is intended during root cause analysis steps.

The analysis of the primary objectives should be conducted within one month of the final database lock, while the analysis of the secondary objectives should be completed after the resolution of any query on discordant samples.

2 Statistical hypotheses and methods

2.1 Primary outcome(s)

The primary outcomes of the study are the estimates following:

1.1. Sensitivity of the Truenat MTB assays (including analysis by smear-status and by sample, with the primary Truenat evaluation based on the S4 sample, the sputum processed in the microscopy lab)

1.2. Specificity of the Truenat MTB assays (including analysis by sample)
1.3. Sensitivity of the Truenat MTB-RIF Dx assay
1.4. Specificity of the Truenat MTB-RIF Dx assay

The secondary outcomes of the study are the estimates of the following:

2.1. Sensitivity of the Truenat MTB assays and MTB-RIF Dx assay compared to Xpert MTB/RIF
2.2. Specificity of the Truenat MTB assays and MTB-RIF Dx assay compared to Xpert MTB/RIF
2.3. 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 and Xpert MTB/RIF.
    - The number of TB (culture-positive) cases detected by the Truenat MTB assays compared to smear or Xpert MTB/RIF
    - The number of RIF-resistant cases detected using the Truenat RIF assay compared to those detected following the current standard of care.

3 Trial population and analysis datasets

3.1 Criteria for eligibility, recruitment, withdrawal and follow-up

3.1.1 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)

3.1.2 Exclusion Criteria

Participants are excluded from the trial in case of:
Receipt of any dose of TB treatment within 60 days prior to enrolment (even if within last two days only).

3.2 Analysis datasets

- ITT (Intention-To-Test) all subjects successfully enrolled in the study
- MITT (Modified-Intention-To-Test) all subjects in ITT for whom at least one test result is available
- PP (Per-Protocol) all subjects in ITT for whom results for all tests are available (complying with the protocol)
- MTB_POP (MTB Population) all subjects with uncontaminated culture results and without indeterminate test results, without any of the following:
    - no valid Truenat result for both Truenat MTB and no valid result for Truenat MTB Plus
    - no valid culture result
    - 2 contaminated cultures unless other criteria for culture-positivity/negativity are met,
    - smear-positive-culture negative,
    - single positive culture with ≤20 colonies (LJ) or >28 days time to positivity (MGIT)
    - culture positive but no MTB complex identification available
    - specimens with growth of mycobacteria other than MTB complex only
- RIF_POP (RIF Population) all subjects with uncontaminated culture results and without indeterminate test results, without all of the above criteria for MTB_POP, and without:
    - For RIF detection, a valid phenotypic DST result for RIF

3.3 Case definitions

The reference standard for TB classification is the result of the culture: a sample is defined as TB positive if any of the culture results is positive; a sample is defined as negative if all the culture results are negative.
In addition to this, the following case definitions will be used for the analyses of MTB and RIF detection. For MTB detection, the main analyses will be based on the three categories with TB defined based on microbiological tests; case definitions additionally using clinical information will be used in sensitivity analyses.
For RIF detection, the main analyses will be based on phenotypic test results; genotypic test results will be used for sensitivity analyses.

DIAGNOSIS	DESCRIPTION
Smear-positive, culture-positive pulmonary TB	Patient with ≥ 1 positive smear (inclusive of scanty positive smears) and any positive culture result as per definitions of test results
Smear-negative, culture-positive pulmonary TB	Patient with all negative smears and any positive culture result as per definitions of test results
Microbiologically non-TB case	Smear- and culture-negative case as per definitions of test results
Non-TB case	Smear-negative, Xpert-negative and culture-negative and not started on TB treatment on the basis of clinical criteria. For Truenat-positive/Culture-discordant cases, a follow-up with repeated clinical and bacteriological work-up will be required to exclude TB with the highest possible likelihood. Only if the bacteriological work-up remains negative, the participant is called Non-TB.
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 and possibly other diagnostic tests such as chest-X-ray.
NTM	Culture-positive with NTM on rapid speciation test AND no other culture positive for MTB
Phenotypic RIF resistant	Culture-positive and growth for Rif in conventional DST testing.
Phenotypic RIF sensitive	Culture-positive and no growth for Rif in conventional DST testing
Genotypic RIF resistant	Sequencing identifies mutations recognized to be associated with resistance (defined based on consultation with WHO prior to analysis)
Genotypic RIF sensitive	Sequencing identifies no mutations recognized to be associated with resistance (defined based on consultation with WHO prior to analysis)
Composite reference standard RIF resistant	If phenotypic DST shows sensitivity but sequencing identifies mutations recognized to be associated with resistance, the composite reference standard will be considered RIF-resistant. 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).
Composite reference standard RIF sensitive	If phenotypic DST shows sensitivity and sequencing shows either no mutations or only mutations that are not associated with resistance.

4 Description of statistical methods

4.1 General approach

Point estimates and 95% confidence intervals (based on Wilson's score method) of sensitivity and specificity will be derived on based on the following definitions:

	Reference standard classification			
Case prediction		Positive	Negative	Total
	Predicted positive	a	b	(a + b)
	Predicted negative	c	d	(c + d)
	Total	(a + c)	(b + d)	(a + b + c + d)

a = True Positives,
b = False Positives
c = False Negatives
d = True Negatives

Sensitivity = a / (a + c)

Specificity = d / (b + d)

4.2 Analysis of the primary outcome(s)

The primary objectives 1.1, 1.2, 1.3 and 1.4 will be analysed with the methodology described in section 4.1.

Outcomes 1.1 and 1.2

Estimates of sensitivity and specificity of the Truenat MTB assays will be calculated on the MTB_POP population (both smear positive and smear negative), using as reference standard of ≥ 1 culture positive sample.

The analysis will also be stratified by smear-status:

1) Smear-positive, culture-positive pulmonary TB
2) Smear-negative, culture-positive pulmonary TB

and by sample (raw sputum vs. decontaminated sputum from Day 1) and setting (Reference lab vs. microscopy centre, comparing Day 1 raw sputum to Day 2 raw sputum).

Outcomes 1.3 and 1.4

Estimates of sensitivity and specificity of the Truenat MTB-RIF assays will be calculated on the RIF_POP population, using as reference standard the results of the MGIT DST testing.

4.3 Analysis of the secondary outcome(s)

Outcomes 2.1 and 2.2
Estimates of sensitivity and specificity of the Truenat MTB assays and MTB-RIF Dx assay will be calculated on the MTB_POP population (both smear positive and smear negative), based on the methodology described in section 4.1, and the results will be compared with Xpert MTB/RIF, using culture as a reference standard. Comparisons will be determined based on identical sample types, i.e. comparing Truenat assays and Xpert MTB/RIF on raw sputum from Day 1, and similarly comparing Truenat assays and Xpert MTB/RIF on decontaminated sputum from Day 1, using culture from Day 1 or Day 2 as a reference standard.
In order to evaluate the difference in performance between the two tests, the difference of the proportions will be also reported, together with 95% confidence intervals based on Tango's score.

Outcomes 2.3
Outcome 2.3.1
The time from sputum 4 sample collection to detection of TB in the microscopy centre using the Truenat MTB assays will be estimated and compared with the standard of care.
Time-to-event data will be modelled using a survival modelling approach based on the Kaplan Meier estimator: the median survival will be reported, with estimate of the variance based on Greenwood's formula.
The Kaplan-Meier curves for the different tests will be compared among each other by a log-rank test, with level of significance alpha set at 0.05.
The same methodology will be used to estimate the median time to detection of RIF resistance using the Truenat RIF assay, and compared with that of phenotypic DST.
Outcome 2.3.2
The number of TB (culture-positive) cases detected by the Truenat MTB assay, smear (any positive grade) and Xpert MTB/RIF will be reported in a table, and the percentage change of Truenat versus the other methods will be reported.
Outcome 2.3.3
The number of RIF-resistant cases detected using the Truenat RIF assay will be reported together with the number of RIF-resistant cases detected following the current standard of care. This will allow us to evaluate how many RIF resistant cases are missed by using smear and Xpert instead of the Truenat test.

5 Baseline 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 mean, median, standard deviation, minimum, maximum and quartiles.

6 Planned interim analyses

A preliminary analysis will be conducted approximately one month after enrolment, with available data (which will exclude culture results, as the time to obtain these would not be compatible with the timing of the analysis). The objective of this analysis is to evaluate the frequency of positive Truenat samples compared with positive Xpert. The study will neither be interrupted nor stopped as a result of this analysis.

7 Additional sub-group analyses

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

- Sensitivity for MTB detection:
    - Truenat MTB assays on Day 1 sample against culture status (based on cultures from Day 1 and Day 2 (sputum 3) samples)
    - Per-sample processing method: Truenat MTB assays on raw sputum from Day 1 sample against culture result on Day 1 sample and Truenat MTB assays on decontaminated sputum on Day 1 sample against culture result on Day 1 sample (same specimen)
    - By smear status (any grade)
    - By HIV status (HIV positive versus negative)
    - On unprocessed (Day 1 raw sample) vs decontaminated specimen (Day 1 decontaminated sample)
    - After repetition (if result invalid)
    - Compared with Xpert MTB/RIF on Day 1 sample (based on culture from Day 1 sample)
    - By gender
- Specificity for MTB detection:
    - Comparing Xpert and Truenat assays for detection of MTB on unprocessed (Day 1 raw sample) and comparing Xpert and Truenat assays for detection of MTB on decontaminated specimen (Day 1 decontaminated sample)
    - By TB history (no previous episode of TB versus at least one)
    - By prior TB treatment (> 60 days ago vs less than 60)
- Intermediate/invalid/erroneous results
    - For MTB detection
    - For RIF resistance detection

8 Multiple comparisons/multiplicity adjustments

No statistical tests will be performed for any of the primary objectives, therefore no adjustments will be made. Given the limited number of tests foreseen for outcome 2.3.1, adjustments are not considered necessary.

9 Exploratory analyses

The time from sputum 4 sample collection to detection of TB in the microscopy centre using the Truenat MTB assays will be compared to that of time to detection for smear, Xpert MTB/RIF, liquid and solid cultures as per standard laboratory technique (i.e. not relative to procedures conducted within the context of this trial).

10 Sample size

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.
A numerical simulation based on an expected sensitivity of Truenat MTB plus and Xpert of 67% among smear-negative/culture-positive samples, with a correlation of 0.5 between the two tests, indicates that with the planned sample size, a 95% confidence interval of +/- 11% should be expected on the estimate of the percentage difference between the two tests

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 at least n = 37 RIF-resistant participants detected. Assuming a prevalence of 20% culture-positive TB cases detected across all presumed TB cases, 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, it might be possible that the sufficient sample size to allow analysis of secondary objectives in this trial will not be reached. 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. An enrolment cap of 200 enrolled participants in the drug resistance group will be placed 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.

11 Minimization of error and bias

11.1 Enrolment and randomization procedures

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.
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.

12 Statistical software

The analysis will be performed using the R statistical language (version 3.4.0 or higher) on OsX, and Microsoft Excel 2018 (version 16.16.5 or higher).

13 References

None

14 Document history

Version	Notes / Changes
1.0	Initial version