CONFIDENTIAL

STATISTICAL ANALYSIS PLAN

Trial Title:

Phase II: Multicentre Clinical Study to Assess the Performance of the Xpert MTB/XDR Assay for INH- and Second-line Resistance Detection

Short title:
Xpert MTB/XDR Clinical Evaluation

Version: 1.0

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Sponsor Name: FIND

Disease Programme: TB

Protocol number: 1.1

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CONFIDENTIALITY STATEMENT:

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1 Introduction

This document describes the statistical analysis plan for the following project: “Phase II: Multicentre Clinical Study to Assess the Performance of the Xpert MTB/XDR Assay for INH- and Second-line Resistance Detection”, Version 1.1, date: February 5th 2019.

1.1 Description of the study

In recent years, tuberculosis (TB) control efforts have been complicated by the rise and spread of MDR-TB, or TB that is resistant to the first-line drugs isoniazid (INH) and rifampicin (RIF), and XDR-TB, or MDR-TB that has developed additional resistance to a fluoroquinolone and any of the injectable compounds [amikacin (AMK), kanamycin (KAN) and/or capreomycin (CAP)]. The rapid diagnosis and appropriate treatment of M/XDR-TB is essential to prevent significant morbidity, mortality and further transmission of disease. For treatment of uncomplicated MDR-TB, the World Health Organization (WHO) recently endorsed a 6-9 month treatment regimen, thereby replacing conventional 18-24 month regimens. The fluoroquinolones and second-line injectables are key components of the 6-9 month regimen, and so it is necessary to rule-out resistance to these compounds prior to treating patients with the shorter regimen.
The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) is an integrated, automated, cartridge-based system for MDR-TB diagnosis that uses the GeneXpert instrument platform. WHO confirmed evidence to support the widespread use of the Xpert MTB/RIF assay in 2010 and the assay has since been widely used in TB programs, but it is only capable of identifying Mycobacterium tuberculosis (Mtb) and detecting RIF resistance, and so it does not provide the comprehensive resistance profile necessary to determine if the shorter TB treatment regimen is appropriate for certain patients. Conventional diagnosis of TB drug resistance relies upon the slow growth of Mtb in solid or liquid media, only then followed by resistance testing to determine phenotypic drug resistance. These conventional drug susceptibility testing (DST) methods can take several weeks to yield results, require significant laboratory infrastructure and training, and are potentially biohazardous. In view of the inadequacy of these conventional tests for resistance detection, the development of rapid tests for INH and second-line resistance detection has become a research and implementation priority.
Rapid diagnostics for second-line drug resistance detection identify mutations in the gyrA and gyrB genes, associated with fluoroquinolone resistance, and rrs and eis gene regions, associated with second-line injectable resistance (with eis promoter mutations exclusively associated with KAN resistance).

Recently, a novel GeneXpert cartridge was developed to detect mutations occurring in these genes, as well as in the katG and inhA gene regions, associated with INH resistance. A Research Use Only (RUO) version of the Xpert MTB/XDR cartridge showed promising performance for INH and second-line resistance detection in a clinical evaluation study (sensitivity 92.7-98.1%; specificity 94.3-99.6%). The assay

chemistry has since been improved and additional gene targets have been added to the assay to improve the detection of INH resistance (i.e. fabG1 and ahpC). Evidence of assay accuracy from an external laboratory validation using a well-characterized set of Mtb strains as well as from large-scale, multicentre clinical studies is needed to confirm the validity of the most recent Xpert MTB/XDR assay for INH and second-line resistance detection, and to recommend its use in diverse clinical settings. The focus of this trial is the multicentre clinical evaluation.

The primary objectives of the trial are the following:
1.1 Estimate the diagnostic accuracy of the Xpert MTB/XDR assay for INH and ETH resistance detection
1.2 Estimate the diagnostic accuracy of the Xpert MTB/XDR assay for fluoroquinolone (FQ) resistance detection
1.3 Estimate the diagnostic accuracy of the Xpert MTB/XDR assay for second-line injectable resistance detection
1.4 Assess Xpert MTB/XDR technical performance, including non-determinant rates, ease of use and other systems operational characteristics.

The secondary objectives of the trial are the following:
2.1 Assess additional Xpert MTB/XDR performance characteristics, including direct performance versus performance on cultured samples, performance between sites, by smear result, by gene target and compared to Hain MTBDRplus and MTBDRsl

This is a multicentre, cross-sectional diagnostic accuracy tral, performed at clinical sites with high rates of drug-resistant TB in India, Moldova and South Africa.
The expected duration of the trial is 10 weeks for IVD testing, with an additional 15 weeks to complete culture, DST, and NGS.

1.2 Timing of the analysis

The final analysis will be conducted once all results are available (i.e. patient enrolment is complete and all phenotypic DST and genotypic testing results have been returned). All objectives will be analysed together at this time.

2 Statistical hypotheses and methods

2.1 Primary outcome(s)

The primary outcomes of the study are the following:

1.1. Sensitivity and specificity estimates for INH and ETH resistance detection
1.2. Sensitivity and specificity estimates for fluoroquinolone resistance detection
1.3. Sensitivity and specificity estimates for second-line injectable resistance detection
1.4. Assessment of Xpert MTB/XDR operational aspects and ease of use

2.2 Secondary outcomes

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

2.1. Assessment of additional Xpert MTB/XDR performance characteristics

3 Trial population and analysis datasets

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

Patients meeting the following criteria will be screened by Xpert MTB/RIF or Xpert MTB/RIF Ultra:

- Age 18 years or above;
- Symptoms suggesting pulmonary TB, i.e. persistent cough (generally ≥3 weeks or as per local definition of TB suspect), **and** at least one of the following:
    - Previously received >1 month of treatment for a prior TB episode **or**
    - Failing TB treatment with positive sputum smear or culture after ≥3 months of a standard TB treatment **or**
    - Had close contact with a known drug-resistant TB case **or**
    - Newly diagnosed with MDR-TB within the last 30 days **or**
    - Previously diagnosed with MDR-TB and failed TB treatment with positive sputum smear or culture after ≥3 months of a standard MDR-TB treatment regimen

Patients meeting the above criteria will be screened by Xpert MTB/RIF or Xpert MTB/RIF Ultra. TB patients meeting the following criteria will be included in the study**:**

- A clear Mtb-positive and RIF-resistant or RIF-sensitive result by Xpert MTB/RIF or Xpert MTB/RIF Ultra
- Provision of informed consent;
- Production of an adequate quantity (≥3mL) of sputum

Participants will be excluded from the study if informed consent is not provided. For additional details, see section 4 of the trial protocol.

3.2 Analysis datasets

Describe the datasets that will be used in the analysis, like the examples below.

- 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, but not all (e.g. composite reference missing)
- PP (Per-Protocol) all subjects in ITT for whom results for all tests (Xpert MDR/XDR and composite reference standards) are available (complying with the protocol)
- QUEST (Questionnaire evaluation) all personnel who provided valid responses to the Xpert MTB/XDR Clinical Study User Appraisal Questionnaire

Samples meeting any of the following criteria in the course of the study will be excluded from the primary analyses of Xpert MTB/XDR accuracy:

- no valid result for NGS for relevant gene region or Xpert MTB/XDR,
- smear-positive/culture negative,
- single positive culture with ≤20 colonies (LJ) or time to positivity >28 days (MGIT),
- culture positive but no MTB complex identification available,
- specimens with growth of mycobacteria other than MTB complex only

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)

Table 1Classification metrics

4.2 Analysis of the primary outcome(s)

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

Outcome 1.1

Estimates of sensitivity and specificity of Xpert MTB/XDR will be calculated, for INH and ETH resistance detection with phenotypic DST and katG, fabG1, ahpC and inhA sequencing results as a composite reference standard (see Appendix). This outcome will be evaluated on the PP population.

Outcome 1.2
Estimates of sensitivity and specificity of Xpert MTB/XDR will be calculated, for fluoroquinolone resistance detection, with phenotypic DST and gyrA and gyrB sequencing results as a composite reference standard (see Appendix). This outcome will be evaluated on the PP population.

Outcome 1.3
Estimates of sensitivity and specificity of Xpert MTB/XDR will be calculated, for second-line injectable resistance detection, with phenotypic DST and rrs and eis sequencing results as a composite reference standard (see Appendix). This outcome will be evaluated on the PP population.

Outcome 1.4
Assess Xpert MTB/XDR operational aspects and ease of use, including Xpert MTB/XDR non-determinant rates.

4.3 Analysis of the secondary outcome(s)

Outcome 2.1
Direct performance versus performance on cultured samples:
- Estimates of diagnostic performance for each drug split by specimen type used for Xpert MTB/XDR (sputum or culture)

Performance between sites:
- Estimates of diagnostic performance for each individual site (India-NITRD, India-Hinduja, Moldova, and South Africa)

Performance by smear result:
- Estimates of diagnostic performance by smear gradation (smear negative, scanty, +1, +2, +3)

Performance by gene target
- Count of the number of times resistance was detected by the assay when a mutation was present in the gene region (NGS) for each gene target (katG, inhA, ahpC, fabG1, gyrA, gyrB, rrs, eis)

Performance compared to Hain MTBDR*plus/sl*
- Estimates of diagnostic performance for INH by Xpert MTB/XDR vs. Hain MTBDRplus
- Estimates of diagnostic performance for ETH by Xpert MTB/XDR vs. Hain MTBDRplus
- Estimates of diagnostic performance for FQ by Xpert MTB/XDR vs. Hain MTBDRsl
- Estimates of diagnostic performance for AMK/KAN/CAP by Xpert MTB/XDR vs. Hain MTBDRsl

Performance by patient HIV status:

- Estimates of diagnostic performance by HIV status (positive and negative)

Performance by patient pre-treatment status:

- Estimates of diagnostic performance by patient report of previous treatment for TB (yes and not)

Performance as reflex test to Ultra or MTB/RIF assay

- Estimates of diagnostic performance for each drug split by which test was used upfront (Ultra or MTB/RIF)

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, and age 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

Once 200 clinical samples have been tested by Xpert MTB/XDR both directly on the clinical sample and on the culture isolate, and have returned NGS results for all drugs tested, an interim analysis will be conducted. The diagnostic sensitivity and specificity of the Xpert MTB/XDR assay will be estimated against NGS for each drug evaluated, including for important sub-groups as detailed in Table 2.
The results will serve as an early indicator of Xpert MTB/XDR accuracy in the clinical trial. If performance estimates fall outside of the presented confidence intervals for resistance detection (Table 2), the Data Monitoring Committee (DMC) will review all data for accuracy and consistency. Additionally, the sponsor may conduct a monitoring visit to one or more sites to ensure adherence to SOPs and effective data management.

7 Additional sub-group analyses

Subgroups analyses, and definitions of subgroups, are described in section 4.3. No additional subgroup analyses will be performed.

8 Multiple comparisons/multiplicity adjustments

No multiple testing of statistical hypotheses is going to be performed.

9 Exploratory analyses

No exploratory analyses will be performed.

10 Sample size

To achieve the targeted precision for accuracy estimates, it is estimated that 760 samples will need to be tested for this study, with a final 284 MTB-positive and an additional 316 RIF-resistant samples tested by Xpert MTB/XDR and all reference standards and comparators, i.e. a total of 600 specimen results available for analysis. The desired precision for the accuracy estimates was chosen to achieve high confidence in the estimates for Xpert MTB/XDR resistance detection when considering evidence from both the 'external laboratory validation' (phase I) and the clinical trial (phase II). The sample size is detailed below in Table 2.

		n	Point estimate (95%CI) *	Total width of CI *
INH	Sensitivity	395	90% (86, 93)	7%
	Specificity	205	98% (95, 99)	4%
FQs	Sensitivity	93	90% (82, 95)	13%
	Specificity	507	98% (96, 99)	3%
AMK	Sensitivity	34	90% (74, 97)	23%
	Specificity	566	98% (96, 99)	3%
KAN	Sensitivity	71	90% (80, 96)	16%
	Specificity	529	98% (96, 99)	3%
CAP	Sensitivity	34	90% (74, 97)	23%
	Specificity	566	98% (96, 99)	3%

Table 2 Sample size

* 95%CI based on Wilson's score method (as recommended by Newcombe et al. as well as CLSI/FDA) using continuity correction.

11 Minimization of error and bias

11.1 Enrolment and randomization procedures

Describe how enrolment will be carried out (from the clinical protocol) and add details on the randomization procedure (if applicable)

12 Case definitions

Table 3 describes the categorization of results based on test results.

TEST RESULT	DESCRIPTION
Phenotypic Drug*-resistant	Culture-positive and growth for Drug* in conventional DST testing.
Phenotypic Drug*-sensitive	Culture-positive and no growth for Drug* in conventional DST testing
Genotypic Drug*-resistant	NGS identifies mutations recognized to be associated with resistance (defined based on consultation with WHO prior to analysis)
Genotypic Drug*-sensitive	NGS identifies no mutations recognized to be associated with resistance (defined based on consultation with WHO prior to analysis)
Composite reference standard Drug*-resistant	If Phenotypic Drug*-sensitive but NGS identifies mutations recognized to be associated with Drug* resistance for the respective gene regions, the composite reference standard will be considered Drug*-resistant. If Phenotypic Drug*-resistant but NGS does not identify mutations recognized to be associated with Drug* resistance for the respective gene regions, the composite reference standard will be considered Drug*-resistant (as mutations will be assumed outside of the region sequenced).
Composite reference standard Drug*-sensitive	If Phenotypic Drug*-sensitive and NGS shows either no mutations or only mutations that are not associated with Drug* resistance for the respective gene regions.

* Drug: INH, ETH, FQ (moxifloxacin and levofloxacin for phenotypic DST), AMK, KAN or CAP

Table 3 Case definitions

13 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 or higher).

14 References

Add required references.

15 Document history

Version	Notes / Changes
1.0	Initial version

16 Appendix

The definitions of composite reference standards are given in table 4.

DRUG RESISTANCE	COMPOSITE REFERENCE STANDARD
INH-resistant	NGS of *katG, fabG1, ahpC* and *inhA* detects ≥1 mutation associated with INH resistance and MGIT culture is INH drug-resistant at the critical concentration.
ETH-resistant	NGS of *inhA* detects ≥1 mutation associated with ETH resistance and MGIT culture is ETH drug-resistant at the critical concentration.
FQ-resistant	NGS of *gyrA* and *gyrB* detects ≥1 mutation associated with MFX resistance and MGIT culture is MFX drug-resistant at the critical concentration.
AMK-resistant	NGS of *rrs* and *eis* detects ≥1 mutation associated with AMK resistance and MGIT culture is AMK drug-resistant at the critical concentration.
KAN-resistant	NGS of *rrs* and *eis* detects ≥1 mutation associated with KAN resistance and MGIT culture is KAN drug-resistant at the critical concentration.
CAP-resistant	NGS of *rrs* detects ≥1 mutation associated with CAP resistance and MGIT culture is CAP drug-resistant at the critical concentration.
INH-susceptible	NGS of *katG, fabG1, ahpC* and *inhA* does NOT detect any mutation associated with INH resistance and MGIT culture is INH-susceptible at the critical concentration.
ETH-susceptible	NGS of *inhA* does NOT detect any mutation associated with ETH resistance and MGIT culture is ETH-susceptible at the critical concentration.
FQ-susceptible	NGS of *gyrA* and *gyrB* does NOT detect any mutation associated with MFX resistance and MGIT culture is MFX-susceptible at the critical concentration.
AMK-susceptible	NGS of *rrs* and *eis* does NOT detect any mutation associated with AMK resistance and MGIT culture is AMK-susceptible at the critical concentration.
KAN-susceptible	NGS of *rrs* and *eis* does NOT detect any mutation associated with KAN resistance and MGIT culture is KAN-susceptible at the critical concentration.
CAP-susceptible	NGS of *rrs* does NOT detect any mutation associated with CAP resistance and MGIT culture is CAP-susceptible at the critical concentration.

Table 4 Definition of composite reference standards