Study Protocol

Two-site laboratory-based diagnostic accuracy and feasibility study of the Xpert MTB/XDR assay for detection of isoniazid, fluoroquinolone, ethionamide and second-line injectable anti-tuberculosis drug resistance

Short title: TB-CAPT MTB/XDR Study

Protocol number: TB042

Registration number:

Study sponsor: Foundation for Innovative New Diagnostics (FIND)

Study coordinator: University of Cape Town (UCT)

Protocol version: 1.1

Date: 16 September 2020

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Confidentiality statement

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We, the undersigned, have developed, reviewed and approved this protocol, including appendices. We will supervise and coordinate the clinical trial according to the principles outlined in the Declaration of Helsinki and Good Clinical Practice and in compliance with applicable regulatory requirements. The signatures below confirm that the signatories have reviewed and approved this document which shall govern the conduct of the specified research study.

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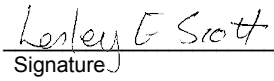
Lesley Scott		25020221
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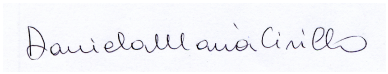
Daniela Maria Cirillo		25 Feb 2021
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Table of contents

Protocol history / amendment summary

Version number	Release date	Comments
1.0	9 July 2020	Initial version
1.1	16 September 2020	Includes institutional review committee suggested edits in red on pages 23 and 27

List of abbreviations and acronyms

CRF: Case report form
FIND: Foundation for Innovative New Diagnostics
IT: Index test
ITT: Intended to test
IUO: Investigational use only
IVD: In vitro diagnostic
LMU: Ludwig-Maximilians-Universitaet Muenchen
LPA: Line probe assay
MGIT: Mycobacterial Growth Indicator Tube (Bactec liquid culture)
Mtb: *Mycobacterium tuberculosis*
MDR-TB: Multidrug-resistant tuberculosis
NHLS: National Health Laboratory Service
OSR: Ospedale San Raffaele SRL
PP: Per protocol
RS: Reference standard
SR: Sample Reagent (Xpert MTB/RIF Ultra)
SOC: Standard of care
UCT: University of Cape Town
WHC: Wits Health Consortium (Pty) Ltd
WHO: World Health Organization

Protocol synopsis

Title	Two-site laboratory-based diagnostic accuracy and feasibility study of the Xpert MTB/XDR assay for detection of isoniazid, fluoroquinolone, ethionamide and second-line injectable anti-tuberculosis drug resistance
Short title	TB-CAPT MTB/XDR Study
Code	TB042
Protocol version and date	Version 1.1, 16 September 2020
Background and rationale	The WHO-endorsed Cepheid Xpert MTB/RIF and Xpert MTB/RIF Ultra cartridge-based molecular assays have been rolled out widely in South Africa and elsewhere, providing rapid TB diagnosis as well as rifampicin susceptibility testing. However, knowledge of susceptibility to isoniazid and second-line anti-tuberculosis drugs is critical to selecting the most effective treatment regimen for rifampicin-resistant and multidrug-resistant tuberculosis (MDR-TB), and for preventing generation of further resistance. With high burdens of rifampicin-resistant- and MDR-TB in South Africa and other countries, new second-line susceptibility tests that are rapid, scalable and affordable need to be evaluated and considered for implementation. The Cepheid Xpert MTB/XDR cartridge, which runs on the same platform as Xpert MTB/RIF Ultra, has been developed to detect additional resistance to isoniazid, fluoroquinolones and second-line injectable anti-tuberculosis drugs and provides results within 2 hours and on primary samples. An evaluation of the the Xpert MTB/XDR assay is currently underway in clinical settings in South Africa, India and Moldova. The TB-CAPT MTB/XDR Study will add further diagnostic accuracy and feasibility data to the evidence base for the Xpert MTB/XDR assay.
Study design	Two-site laboratory-based diagnostic accuracy and feasibility study
Index test	The Cepheid Xpert MTB/XDR assay performed directly on routine patient respiratory samples which have tested positive for rifampicin-resistant *Mycobacterium tuberculosis* (*Mtb*) by Xpert MTB/RIF Ultra
Reference standard	The primary analysis will be based on a composite reference standard comprising phenotypic susceptibility testing and whole genome sequencing performed on cultured isolates. For the composite reference standard, samples will be defined as “resistant” if they are resistant by either phenotypic susceptibility testing *or* whole genome sequencing, and “susceptible” if they tested susceptible by *both* methods. Isolates will be obtained from the second or follow-up routine specimen
Comparator	Secondary analyses will compare the accuracy of Xpert MTB/XDR to that of standard of care (SOC) susceptibility testing (comprising the Hain MTBDRplus and MTBDRsl line probe assays and selected phenotypic susceptibility testing as per the South African national diagnostic algorithm)
Primary objective(s)	To estimate the diagnostic accuracy of Xpert MTB/XDR for detection of isoniazid, fluoroquinolone, ethionamide and second-line injectable drug resistance on patient samples that test positive for rifampicin-resistant *Mtb* on Xpert MTB/RIF Ultra using a composite reference standard

Secondary objective(s)	1. To estimate the diagnostic accuracy of Xpert MTB/XDR for detection of isoniazid, fluoroquinolone and second-line injectable drug resistance compared to phenotypic susceptibility testing and whole genome sequencing separately 2. To estimate the diagnostic accuracy of Xpert MTB/XDR for detection of each resistance-conferring mutation as identified by whole genome sequencing 3. To compare the diagnostic accuracy of Xpert MTB/XDR to the accuracy of the SOC comparator for the detection of isoniazid, fluoroquinolone and second-line injectable drug resistance against the composite reference standard 4. To compare the proportion of participants for whom valid susceptibility results become available based on Xpert MTB/XDR to that of SOC 5. To determine time-to-result for isoniazid, fluoroquinolone and second-line injectable drug susceptibility testing, comparing Xpert MTB/XDR to SOC line probe assays 6. To evaluate the feasibility of performing Xpert MTB/XDR on residual specimen previously processed for Xpert MTB/RIF Ultra (the "Sample Reagent [SR]-sputum mix") under routine conditions
Primary endpoints (outcomes)	Sensitivity and specificity of Xpert MTB/XDR vs. the composite reference standard for detection of: • Isoniazid • Fluoroquinolone • Ethionamide • Second-line injectable drug resistance
Secondary endpoints (outcomes)	1. Sensitivity and specificity of Xpert MTB/XDR compared to phenotypic susceptibility testing and whole genome sequencing separately 2. Sensitivity and specificity of Xpert MTB/XDR for detection of individual resistance-conferring mutations 3. Sensitivity and specificity of Xpert MTB/XDR compared to that of SOC vs. the composite reference standard for detection of: o Isoniazid o Fluoroquinolone o Second-line injectable drug resistance 4. Proportion of participants for whom valid susceptibility testing results become available based on Xpert MTB/XDR assay or based on SOC testing 5. Median time-to-result comparing Xpert MTB/XDR to SOC (line probe assays) 6. Feasibility endpoints: o Proportion of participants with sufficient residual SR-sputum mix (≥2 ml) available to perform Xpert MTB/XDR o Median period between preparation of SR-sputum mix and start of Xpert MTB/XDR test o Proportion of specimens with non-determinate Xpert MTB/XDR results, separately for each drug class and combined for all, and comparing SR-sputum mix stored according to manufacturer recommendations (at 2-8ºC for ≤4 hours or 25-35ºC for ≤2.5 hours) and those exceeding manufacturer limits for operational reasons
Study sites	• UCT: National Health Laboratory Service (NHLS) Green Point TB Laboratory, Cape Town, South Africa (≥500 Xpert MTB/RIF Ultra tests performed daily) • WHC: NHLS Port Elizabeth Hospital Complex TB Laboratory, Port Elizabeth, South Africa (≥400 Xpert MTB/RIF Ultra tests performed daily)

Study population	Respiratory specimens testing rifampicin-resistant *Mtb* positive on Xpert MTB/RIF Ultra
Eligibility criteria	Inclusion: • Ultra-positive, rifampicin-resistant respiratory specimen (expectorated sputum, induced sputum, tracheal aspirates and bronchoalveolar lavage) Exclusion for all endpoints: • Residual SR-sputum mix not retained • Patient specimen previously included in the study Exclusion for diagnostic accuracy and time-to-result endpoints: • Volume of residual SR-sputum mix for Xpert MTB/XDR is <2 ml • Xpert MTB/XDR unsuccessful • No second / follow-up specimen received • Second or follow-up specimen culture-negative, contaminated or not available for reference standard testing • Reference standard uninterpretable
Biostatistics	Analysis populations • Intended to test (ITT): all samples intended to be tested • Per protocol (PP): all samples with valid test results for the index test and reference standard Analysis of primary endpoint: Sensitivity and specificity will estimated together with their 2-sided 95%-Wilson-Score-intervals.
Sample size	For diagnostic accuracy and time-to-result study: N=220 UCT N=100 WHC Total N=320 For feasibility study: N=373 UCT N=380 WHC Total N=753
Study duration	38 weeks (estimated 2 rifampicin-resistant specimens/site/day)
Time schedule	July 2020: Submit for departmental and ethical review July 2020: Submit to NHLS, Department of Health (local and provincial) for approval October 2020: First patient in July 2021: Last patient In September 2021: Reference standard results available for all participants
Study flow	See Figure 2

Background

The WHO-endorsed Cepheid Xpert MTB/RIF and Xpert MTB/RIF Ultra cartridge-based molecular assays have been rolled out widely in South Africa and elsewhere,[1] providing rapid tuberculosis diagnosis as well as rifampicin susceptibility testing. However, knowledge of susceptibility to isoniazid and second-line anti-tuberculosis drugs is critical to selecting the most effective treatment regimen for rifampicin-resistant and multidrug-resistant tuberculosis (MDR-TB), and for preventing generation of further resistance.[2]

The Cepheid Xpert MTB/XDR cartridge has been developed to detect additional resistance to isoniazid, fluoroquinolones and second-line injectable anti-tuberculosis drugs. This rapid, cartridge-based real-time PCR assay, which runs on the same platform as Xpert MTB/RIF and Xpert MTB/RIF Ultra, provides results within 2 hours and on primary samples,[3,4] thereby having clear advantage over the WHO-endorsed Hain Lifescience Genotype MTBDRplus and Genotype MTBDRsl assays which are technically complex and perform better on cultured isolates especially in smear-negative cases. Other strategies like phenotypic testing are hampered by the long delay to get results, the need for specialised testing centers, technical demands and reliability. Next generation sequencing is currently expensive, too complex to be implemented widely and requires DNA from cultured isolates. With high burdens of rifampicin-resistant- and MDR-TB in South Africa and other countries,[5,6] new second-line susceptibility tests that are rapid, scalable and affordable need to be evaluated and considered for implementation.

In a feasibility study, the prototype of the Xpert MTB/XDR assay showed moderate sensitivity against phenotypic susceptibility testing to detect isoniazid resistance (83%), although this result may have been biased by the reference method utilised (critical concentration) and sensitivity was higher when compared to sequencing (98%).[3] The sensitivity of MTBDRplus for detection of phenotypic isoniazid resistance is estimated to be 90%.[7] Sensitivity of Xpert MTB/XDR for the detection of fluoroquinolone resistance was 94-97%,[3] comparing favourably with the sensitivity of MTBDRsl (93%).[8] An evaluation of the final version of the Xpert MTB/XDR assay is currently underway in clinical settings in South Africa, India and Moldova.[9]

The TB-CAPT MTB/XDR Study will add further diagnostic accuracy and feasibility data to the evidence base for the Xpert MTB/XDR assay.

Aims and objectives

The aim of the study is to evaluate the Xpert MTB/XDR cartridge for diagnostic accuracy, and to evaluate the feasibility of performing Xpert MTB/XDR on residual respiratory specimens previously processed for Xpert MTB/RIF Ultra (the "Sample Reagent [SR]-sputum mix") under routine conditions

The primary objective is to estimate the diagnostic accuracy of the Xpert MTB/XDR assay for the detection of resistance to:

a. Isoniazid
b. Fluoroquinolones
c. Ethionamide
d. Second-line injectable anti-tuberculosis drugs

in respiratory specimens positive for rifampicin-resistant *Mycobacterium tuberculosis* (*Mtb*) by Xpert MTB/RIF Ultra using a composite reference standard.

The composite reference standard comprises phenotypic susceptibility testing and whole genome sequencing (WGS) performed on culture isolates. For the composite reference standard, samples will be defined as "resistant" if they are resistant by either phenotypic susceptibility testing *or* WGS and "susceptible" if they tested susceptible by *both* methods.

The secondary objectives are to:

1) Estimate the diagnostic accuracy of Xpert MTB/XDR for detection of resistance to isoniazid, fluoroquinolones and second-line injectable anti-tuberculosis drugs compared to:
    a. Phenotypic susceptibility testing alone
    b. WGS alone
2) Estimate the diagnostic accuracy of Xpert MTB/XDR for detection of individual resistance-conferring mutations as determined by WGS
3) Compare the diagnostic accuracy of Xpert MTB/XDR to the accuracy of standard of care (SOC) susceptibility testing (comprising Hain MTBDRplus and MTBDRsl line probe assays [LPA]s and selected phenotypic testing according to the national diagnostic algorithm) against the composite reference standard
4) Compare the proportion of participants for whom valid[1] susceptibility testing results become available based on Xpert MTB/XDR to the proportion of participants for whom valid susceptibility testing results become available based on SOC testing
5) Determine the time-to-result of Xpert MTB/XDR and compare to that of LPAs performed as SOC
6) Evaluate the feasibility of performing Xpert MTB/XDR on residual SR-sputum mix under routine conditions:
    a. Determine the proportion of participants with sufficient residual SR-sputum mix (≥2 ml) available to perform Xpert MTB/XDR
    b. Determine the median period between processing for Xpert MTB/RIF (preparation of SR-sputum mix) and the start of the Xpert MTB/XDR test
    c. Determine the non-determinate rate of Xpert MTB/XDR:
        i. For each drug separately and combined for all
        ii. Comparing specimens stored according to manufacturer recommendations (at 2-8ºC for ≤4 hours or 25-35ºC for ≤2.5 hours) and those exceeding those limits for operational reasons
        iii. Correlated with semi-quantitation of bacterial load on Xpert MTB/RIF Ultra

[1] Valid result defined as a non-indeterminate result for all three anti-tuberculosis drug classes tested

iv. By sub-group (sufficient specimen, smear status, age group)

7) Exploratory analyses:
   a. Diagnostic accuracy by sub-group
   b. Diagnostic accuracy by rifampicin susceptibility result concordance between Xpert MTB/RIF Ultra and LPA
   c. Detection of heteroresistance for each anti-tuberculosis drug class, comparing Xpert MTB/XDR to WGS
   d. Description of discordance between phenotypic testing and WGS
   e. Description of discordance between testing methodologies by presence of heteroresistance

Endpoints

Primary endpoint

Sensitivity and specificity of Xpert MTB/XDR assay vs. the composite reference standard for detection of resistance to:

1. Isoniazid
2. Fluoroquinolones
3. Ethionamide
4. Second-line injectable anti-tuberculosis drugs

Secondary endpoints

1) Sensitivity and specificity of Xpert MTB/XDR for detection of resistance to:
    a. Isoniazid
    b. Fluoroquinolones
    c. Second-line injectable anti-tuberculosis drugs

    compared to phenotypic anti-tuberculosis drug susceptibility testing and WGS separately

2) Sensitivity and specificity of Xpert MTB/XDR vs. WGS for detection of mutations in the following gene targets:
    a. *inhA* promoter
    b. *katG*
    c. *gyrA*
    d. *gyrB*
    e. *eis*
    f. *rrs*

3) Sensitivity and specificity of Xpert MTB/XDR compared to that of SOC testing against the composite reference standard for detection of resistance to:
    a. Isoniazid
    b. Fluoroquinolones
    c. Second-line injectable anti-tuberculosis drugs

4) Proportion of participants for whom valid test results become available based on:
    a. Xpert MTB/XDR
    b. SOC testing:
        i. LPAs performed directly on specimen (“direct”)
        ii. LPAs performed on culture isolates (“indirect”)

5) Median time-to-result:
    a. Separately for each drug class, and comparing:
        i. Xpert MTB/XDR
        ii. SOC testing:
            1. Direct LPAs
            2. Indirect LPAs

6) Feasibility endpoints:
    a. Proportion of participants with sufficient residual SR-sputum mix (≥2 ml) available to perform Xpert MTB/XDR

b. Median period between preparation of SR-sputum mix and start of Xpert MTB/XDR test
c. Proportion of specimens with non-determinate MTB/XDR results:
    i. Combined for all
    ii. Separately for each drug class
    iii. Separately for each target gene
    iv. Comparing specimens stored according to manufacturer recommendations (at 2-8ºC for ≤4 hours or 25-35ºC for ≤2.5 hours) and those exceeding those limits for operational reasons
    v. By subgroup:
        1. Sufficient specimen (yes/no)
        2. Smear status
        3. Age group

7) Exploratory endpoints:
    a. Sensitivity and specificity of Xpert MTB/XDR assay for detection of resistance to each anti-tuberculosis drug class by subgroup:
        i. Sufficient specimen (yes/no)
        ii. Smear status
        iii. Age group
    b. Sensitivity and specificity of Xpert MTB/XDR assay for detection of resistance to each anti-tuberculosis drug class by rifampicin susceptibility result concordance between Xpert MTB/RIF Ultra and LPA
    c. Agreement between Xpert MTB/XDR and WGS for the detection of heteroresistance for each anti-tuberculosis drug class
    d. Description of discordance between phenotypic testing and WGS
    e. Description of discordance between testing methodologies by presence of heteroresistance

Study design

Study description

This is a 2-site laboratory-based diagnostic accuracy and feasibility study. Residual SR-sputum mix remaining from samples which have tested positive with rifampicin-resistant *Mtb* on Xpert MTB/RIF Ultra will be submitted for Xpert MTB/XDR (the index test). A second or follow-up specimen, submitted routinely either together with the first specimen (Cape Town) or after the rifampicin-resistant result (Port Elizabeth), will undergo Bactec MGIT culture routinely. Should the MGIT culture be positive, it will be used for the reference standard tests.

Reference standard

The composite reference standard comprises phenotypic susceptibility testing and WGS performed on culture isolates from the second or follow-up specimen. For the composite reference standard, samples will be defined as "resistant" if they are resistant by either phenotypic susceptibility testing *or* WGS, and "susceptible" if they tested susceptible by *both* methods. This reference standard has been chosen as it is the same standard used in the previous diagnostic accuracy study.

Standard of care comparator

The SOC comparator will comprise susceptibility testing for isoniazid, fluoroquinolones and second-line injectable anti-tuberculosis drugs using MTBDRplus and MTBDRsl LPAs either directly on specimens, or if unsuccessful, on culture isolates, and phenotypic testing, according to the South African national algorithm (Figure 3).

Time-to-result study

Time-to-result (in hours) will be defined as per Table 1 and compared for each drug class separately.

Table 1. Definition of time-to-result endpoints in the TB-CAPT MTB/XDR Study

	Start point	End point	
		SOC	Xpert MTB/XDR
Registration-to-first second-line anti-tuberculosis drug susceptibility result	Time at which first specimen is registered in the laboratory	Time at which first LPA (direct or indirect) susceptibility result is entered on the laboratory information system	Time at which Xpert MTB/XDR resulted on the GeneXpert / or entered on the laboratory information system if feasible
Rifampicin-resistant result-to-first second-line anti-tuberculosis drug susceptibility result	Time at which the rifampicin-resistant result is reported on the laboratory information system		

Study sites

UCT: Green Point TB Laboratory, Cape Town

Green Point laboratory is a centralised National Health Laboratory Service (NHLS) tuberculosis diagnostic laboratory located in Green Point, Cape Town, Western Cape, South Africa. The laboratory receives specimens from about 420 facilities in the Western Cape, including secondary hospitals and primary health

clinics. The laboratory performs >500 Ultra tests daily. It has facilities for MGIT culture, Hain MTBDRplus and MTBDRsl LPAS performed directly or indirectly on culture isolates. The laboratory is SANAS-accredited and has ongoing collaboration with UCT and Stellenbosch University. There is a dedicated microbiologist on site.

WHC: Port Elizabeth Hospital Complex, Port Elizabeth

The Port Elizabeth National Health Services Laboratory is a high-throughput tuberculosis diagnostic laboratory based in Port Elizabeth, Eastern Cape, South Africa. It services approximately 69 primary health care facilities and hospitals in the Eastern Cape. The laboratory performs 400-500 Ultra tests daily. The laboratory also performs MGIT culture, first- and second-line line probe assays performed directly from specimens and from culture isolates and first and second-line phenotypic drug susceptibility testing. The laboratory is SANAS accredited with a dedicated pathologist on site.

Study population and setting

The study population will comprise patients from any of the laboratory's referral facilities who test positive for rifampicin-resistant *Mtb* by Xpert MTB/RIF Ultra testing of respiratory specimens. Respiratory specimens are expectorated sputum, induced sputum, tracheal aspirates and bronchoalveolar lavage, but exclude gastric washings and nasopharyngeal aspirates.

For patients with two or more respiratory specimens, only the first will be utilised.

Eligibility criteria

Inclusion:
- *Mtb*-positive, rifampicin-resistant respiratory specimen identified on Xpert MTB/RIF Ultra

Exclusion:
- Residual SR-sputum mix not retained or not found
- Patient previously included in the study

Exclusion for diagnostic accuracy and time-to-result endpoints:
- Insufficient residual SR-sputum mix remaining for Xpert MTB/XDR (<2 ml)
- Xpert MTB/XDR unsuccessful
- No second / follow-up specimen received
- Second / follow-up specimen culture-negative, contaminated or not available
- Reference standard uninterpretable (phenotyping or WGS)
    - Where phenotypic susceptibility testing results are uninterpretable, specimens will still be included in WGS comparison
    - Where WGS results are uninterpretable, specimens will still be included in phenotypic susceptibility testing comparison

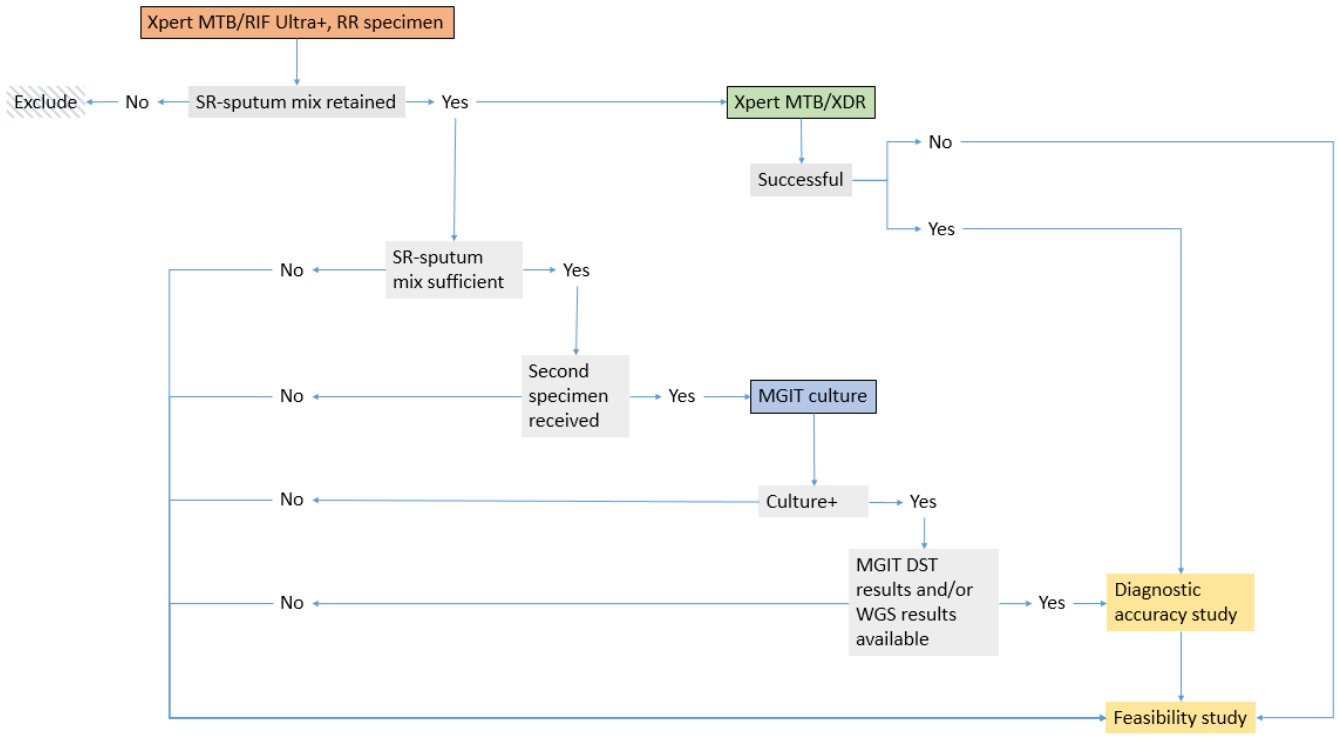

Figure 1. Eligibility flow in the TB-CAPT MTB/XDR Study. RR = rifampicin-resistant, SR = Xpert MTB/RIF Ultra Sample Reagent, MGIT = Mycobacterial Growth Indicator Tube, DST = drug-susceptibility testing, WGS = whole genome sequencing

Sample size

320 specimens will be included in the diagnostic accuracy study. The sample size is dictated by the resources allocated to the project and it is expected that the data generated will be combined in meta-analyses with other studies currently underway. The sample size calculation regards the uncertainty of estimation of sensitivity among reference standard (RS)-positives and specificity among RS-negatives, respectively, expressed by the width of the 2-sided 95% confidence intervals. For technical reasons, the Pearson-Clopper-CI have been used in sample size considerations. The differences compared to the Wilson-Score-CI planned to be used in statistical analysis are negligible. Within sample size considerations, the widths of 95%-Pearson-Clopper-intervals for 70%, 80% and 90% sensitivity (or specificity) were dependent on prevalence of resistance to a specific anti-tuberculosis drug (11-50%) and sample sizes of 320, 500 and 750. The width ranges from more 30% (70% sensitivity at a prevalence (for D+) of 11% among 320 patients) down to 4.7% (90% spec at a prevalence (for D-) of 89% among 750 patients.

Table 2 shows expected prevalence[6] of resistance to anti-tuberculosis drug class and the related number of resistant and susceptible specimens among 320, 500 and 750 participants together with confidence intervals for proportions of 70%, 80% and 90%.

Table 2. Widths of 95%-Pearson Clopper confidence intervals dependent on prevalence of resistance and diagnostic accuracy for a total sample size of 320

Type of resistance	Resistant	Prev.	Measure	D+ or D- among								
				320			500			750		
				N	lCL	uCL	N	lCL	uCL	N	lCL	uCL
Isoniazid resistance	yes	61%	n (Prev.)	196			305			458		
			70% Sens	137	63.0%	76.3%	214	64.5%	75.1%	321	65.6%	74.2%
			80% Sens	157	73.7%	85.4%	244	75.1%	84.3%	366	76.0%	83.6%
			90% Sens	176	84.9%	93.8%	275	86.1%	93.1%	412	86.9%	92.6%
	no	39%	n (1-Prev.).	125			195			293		
			70% Spec	88	61.2%	77.9%	137	63.0%	76.3%	205	64.4%	75.2%
			80% Spec	100	71.9%	86.6%	156	73.7%	85.4%	234	75.0%	84.4%
			90% Spec	113	83.4%	94.6%	176	84.9%	93.8%	264	86.0%	93.2%
Second-line injectable resistance	yes	15%	n (Prev.)	48			75			113		
			70% Sens	34	54.9%	82.5%	53	58.3%	80.0%	79	60.6%	78.3%
			80% Sens	38	65.9%	90.2%	60	69.2%	88.4%	90	71.4%	86.9%
			90% Sens	43	77.6%	96.9%	68	80.9%	95.7%	102	82.9%	94.9%
	no	85%	n (1-Prev.)	272			425			638		
			70% Spec	190	64.2%	75.4%	298	65.4%	74.3%	447	66.3%	73.5%
			80% Spec	218	74.7%	84.6%	340	75.9%	83.7%	510	76.7%	83.0%
			90% Spec	245	85.8%	93.3%	383	86.7%	92.7%	574	87.4%	92.2%
Fluoroquinolone resistance	yes	11%	n (Prev.)	36			55			83		
			70% Sens	25	52.3%	84.2%	39	56.1%	81.6%	58	58.9%	79.6%
			80% Sens	29	63.2%	91.5%	44	67.0%	89.6%	66	69.8%	88.0%
			90% Sens	32	75.2%	97.5%	50	78.9%	96.4%	75	81.3%	95.6%
	no	89%	n (1-Prev.).	285			445			668		
			70% Spec	200	64.3%	75.3%	312	65.5%	74.2%	468	66.4%	73.5%
			80% Spec	228	74.9%	84.5%	356	76.0%	83.6%	534	76.8%	83.0%
			90% Spec	257	85.9%	93.2%	401	86.8%	92.6%	601	87.5%	92.2%
Ethionamide resistance	yes	50%	n (Prev.)	160			250			375		
			70% Sens	112	62.3%	77.0%	175	63.9%	75.6%	263	65.1%	74.6%
			80% Sens	128	73.0%	85.9%	200	74.5%	84.8%	300	75.6%	83.9%
			90% Sens	144	84.3%	94.2%	225	85.6%	93.4%	338	86.5%	92.8%
	no	50%	n (1-Prev.)	160			250			375		
			70% Spec	112	62.3%	77.0%	175	63.9%	75.6%	263	65.1%	74.6%
			80% Spec	128	73.0%	85.9%	200	74.5%	84.8%	300	75.6%	83.9%
			90% Spec	144	84.3%	94.2%	225	85.6%	93.4%	338	86.5%	92.8%

D+ =presence of resistance, D- =absence of resistance, Prev=prevalence, y=yes, n=no, lCL=lower confidence level, uCL=upper confidence level
Red and green: widest and narrowest interval
Due to rounding errors, deviations of +/-1 case could occur for total N.

It is expected that a proportion of specimens will not be available for the diagnostic accuracy study because of an invalid Xpert MTB/XDR result (~5%), unavailability of a second or follow-up specimen and negative or contaminated culture. An additional 433 specimens will be required giving a final total of N=753 (Table 3).

Table 3. Number of specimens required to undergo Xpert MTB/XDR to achieve final sample size of N=320 for diagnostic accuracy study, and anticipated enrolment period

	UCT		WHC		Total
	%	N	%	N	N
Total specimens required		373		380	753
Available for feasibility study	100	373	100	380	753
Not available for diagnostic accuracy and time-to-result study	41	153	71	280	433
Insufficient specimen remaining for Xpert MTB/XDR	1	4	1	4	
Xpert MTB/XDR indeterminate	5	19	5	19	
No second / follow-up specimen	10	37	40	152	
Culture-negative or contaminated	20	75	20	76	
MGIT not available	5	19	5	19	
Available for diagnostic accuracy and time-to-result study	59	220	29	100	320
Enrolment period (@ 2 rifampicin-resistant specimens/day)		37 weeks		38 weeks	

Minimisation of error and bias

Consecutive, unselected samples with rifampicin-resistant MTB detected on Xpert Ultra testing in the routine laboratory will be used. These will include samples from a wide variety of facility types, geographical areas and patient/clinical backgrounds. Patients will only be excluded if they have been included previously, and not for any patient related factors. The study population will therefore be directly representative of the future target population and context of the Xpert MTB/XDR test.

Reference standard phenotyping, WGS and downstream bioinformatics analysis will be performed blinded to the index test result, which will have been performed in a different laboratory several weeks or months earlier.

Study intervention

Study Intervention is defined as any investigational intervention(s), marketed product(s), or medical device(s) intended to be used with a study participant according to the study protocol.

In vitro diagnostics

The IVD manufactured for FIND use in this study is the Xpert MTB/XDR assay. The Investigational Use Only (IUO) product is intended for the detection of *Mtb* and mutations associated with isoniazid, ethionamide, fluoroquinolone and second-line injectable drug resistance. Likewise, the 10-color GeneXpert system provided for this study should only be used for testing IUO cartridges and study samples. The investigational product and 10-color system will be strictly accounted for, including receipt and inventory, storage, use during the trial, and return or disposal, as detailed in the Study Manual for this study.

Any IVD incidents, including those resulting from malfunctions of the IVD, must be detected, documented and reported by the investigator throughout the study

Study procedures

Respiratory specimens submitted routinely from healthcare facilities in the laboratory's referral area will be used for study procedures (Figure 2). Residual SR-sputum mix from the first specimen will be used for Xpert MTB/XDR, and the second or follow-up specimen for the reference standard and SOC comparator.

Xpert MTB/XDR

Respiratory specimens routinely received by the laboratory for Xpert MTB/RIF Ultra will be processed as per routine (non-study) standard operating procedures:

1. Add SR at a ratio of 2:1 SR:sputum to unprocessed sputum
2. Vortex for 10 s
3. Incubate at room temperature for 10 m
4. Vortex for 10 s
5. Incubate at room temperature for 5m
6. Transfer 2 ml of SR-sputum mix to the Xpert MTB/RIF Ultra cartridge
7. Load Xpert MTB/RIF Ultra cartridge on GeneXpert instrument

Under routine non-study conditions, the residual SR-sputum mix is discarded after it has been used for Xpert MTB/RIF Ultra. In the CAPT-TB MTB/XDR Study, these samples will be not be discarded immediately, but set aside for possible Xpert MTB/XDR later:

1. After transferring SR-sputum mix to Xpert MTB/RIF Ultra cartridge, set residual SR-sputum mix aside at room temperature
2. Review Xpert MTB/RIF Ultra results on the GeneXpert after 2 hours and note laboratory numbers of rifampicin-resistant samples
3. Remove rifampicin-resistant SR-sputum samples from the batch that was set aside in (1)
4. Transfer 2 ml of SR-sputum mix to the Xpert MTB/XDR cartridge
5. Load Xpert MTB/XDR cartridge on GeneXpert instrument
6. Discard remaining SR-sputum mix samples
7. If operational factors preclude performing (4) and (5) immediately after the rifampicin-resistant result is reported on the GeneXpert instrument:
    a. Place rifampicin-resistant SR-sputum samples in a refrigerator (4℃)
    b. The next morning, rifampicin-resistant specimens will be removed from the refrigerator and brought to room temperature
    c. Continue (4) to (6)

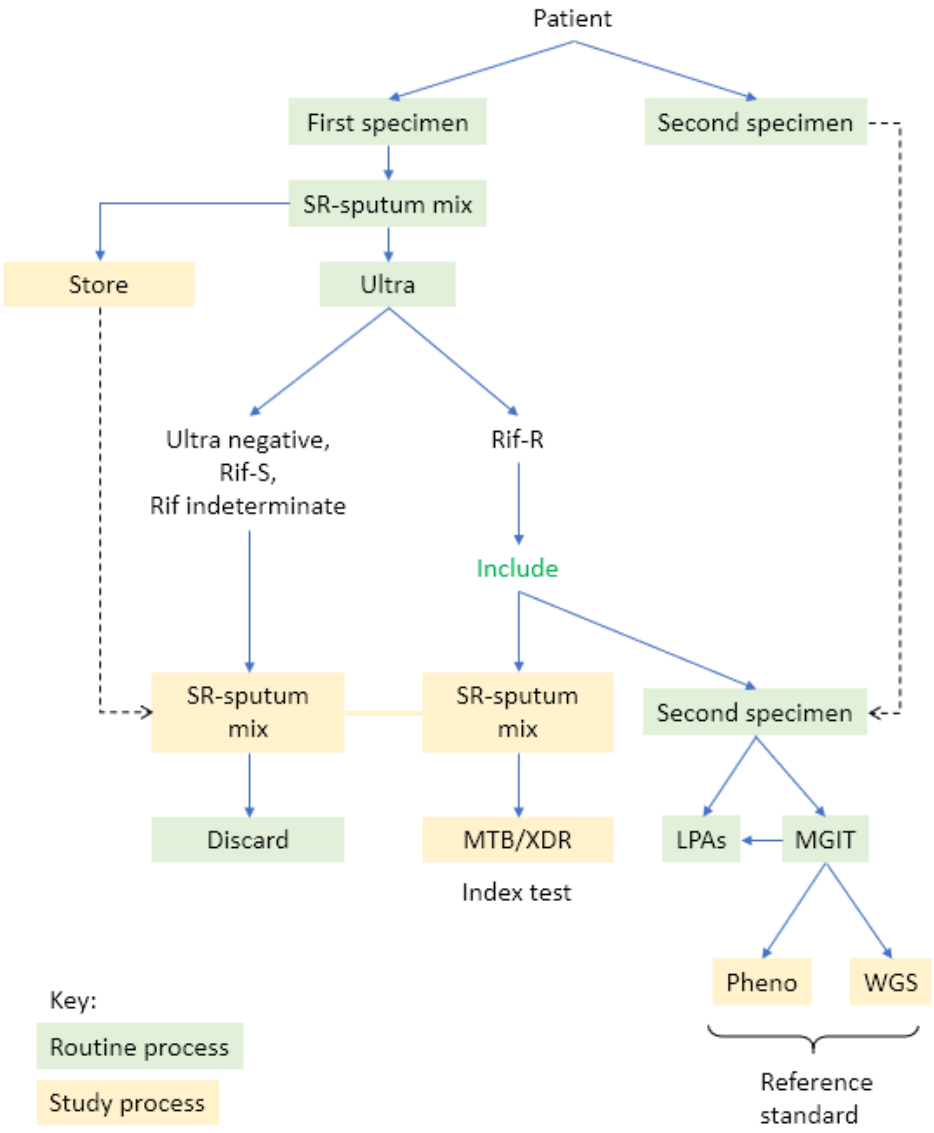

Figure 2. Specimen flow in the TB-CAPT MTB/XDR Study with both routine (green) and study (yellow) processes included. SR = Sample Reagent, Rif-S = rifampicin-susceptible, Rif-R = rifampicin-resistant, LPA = line probe assay, MGIT= Mycobacterial Growth Indicator Tube, Pheno = phenotypic drug susceptibility testing, WGS = whole genome sequencing

SOC comparator

A second or follow-up specimen is sent routinely according to national guidelines for any rifampicin-resistant case of tuberculosis. In Cape Town, the second specimen is "front-loaded" / sent at the same time as the first specimen. In PE, the follow-up specimen will be sent routinely when the patient is brought back for the rifampicin-resistant result. The second or follow-up specimen undergoes routine MTBDRplus and MTBDRsl LPA testing, directly on the specimen ("direct"), and, if direct testing is unsuccessful, on the BACTEC MGIT culture isolate ("indirect"), with or without supplementary phenotypic testing as required (Figure 3). The finalised NHLS result will count as the SOC comparator.

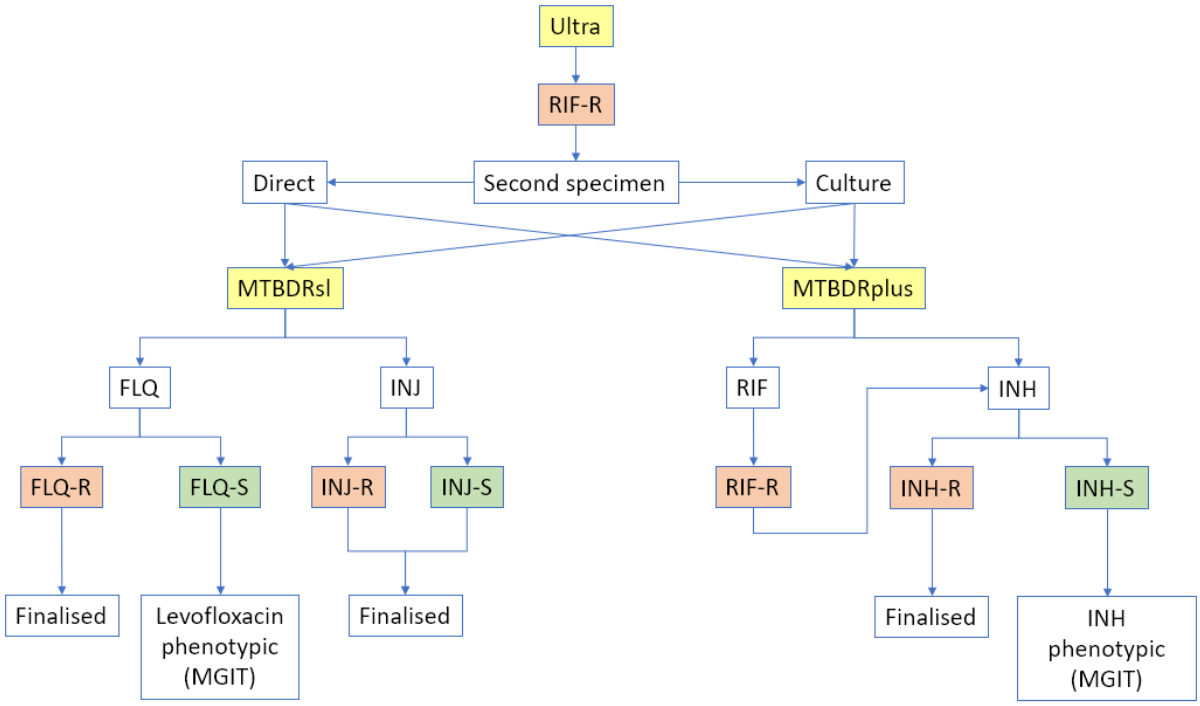

Figure 3. Routine NHLS workflow for second / follow-up specimen received from patients with rifampicin-resistant results on Xpert MTB/RIF Ultra testing of the first specimen. Testing for rifampicin, fluoroquinolones and second-line injectable anti-tuberculosis drugs are included in the algorithm. Testing for bedaquiline and linezolid, and others, are also performed routinely in the NHLS. Ultra = Xpert MTB/RIF Ultra, FLQ = fluoroquinolone, INJ = injectables, RIF = rifampicin, INH = isoniazid, MGIT = Mycobacterial Growth Indicator Tube, R = resistant, S = susceptible

Reference standard

The primary analysis will be based on a composite reference standard comprising phenotypic susceptibility testing and WGS performed on culture isolates. Cultured isolates will be obtained from routine MGITs performed on the second or follow-up specimen. For the composite reference standard, samples will be defined as "resistant" if they are resistant by either phenotypic susceptibility testing *or* WGS and "susceptible" if they tested susceptible by *both* methods.

The reference standard will be performed on the second / follow-up specimen received for participants with rifampicin-resistant *Mtb* result on Xpert MTB/RIF Ultra testing of the first specimen (Figure 4).

Phenotypic susceptibility testing will be performed on the second / follow-up specimen, if culture positive, using Bactec MGIT and testing the following anti-tuberculosis drugs at the stated concentrations:

- Isoniazid @ 0.1 µg/ml
- Levofloxacin @ 1.0 µg/ml
- Kanamycin @ 2.5 µg/ml

A proportion of phenotypic testing will be done routinely by the NHLS laboratory according to the national testing algorithm. Where phenotypic testing is not indicated according to the national algorithm, testing will be performed at a local research laboratory with appropriate expertise (Figure 5). In summary, non-SOC phenotypic testin will be performed in the these scenarios: MDR-TB (isoniazid), levofloxaxin-resistant MDR-TB (levofloxacin) and all isolates (kanamycin). The Bactec MGIT platform will be utilised at all sites and testing will therefore be standardised. Non-SOC testing will not be reported to the clinical service.

Isolates of M*tb* from MGIT culture of the second / follow-up specimen will be inactivated and shipped to Ospedale San Raffaele SRL (OSR) in Milan, Italy for WGS to detect resistance-conferring mutations. Appropriate bioinformatic pipelines and anti-tuberculosis drug resistance databases will be utilised.

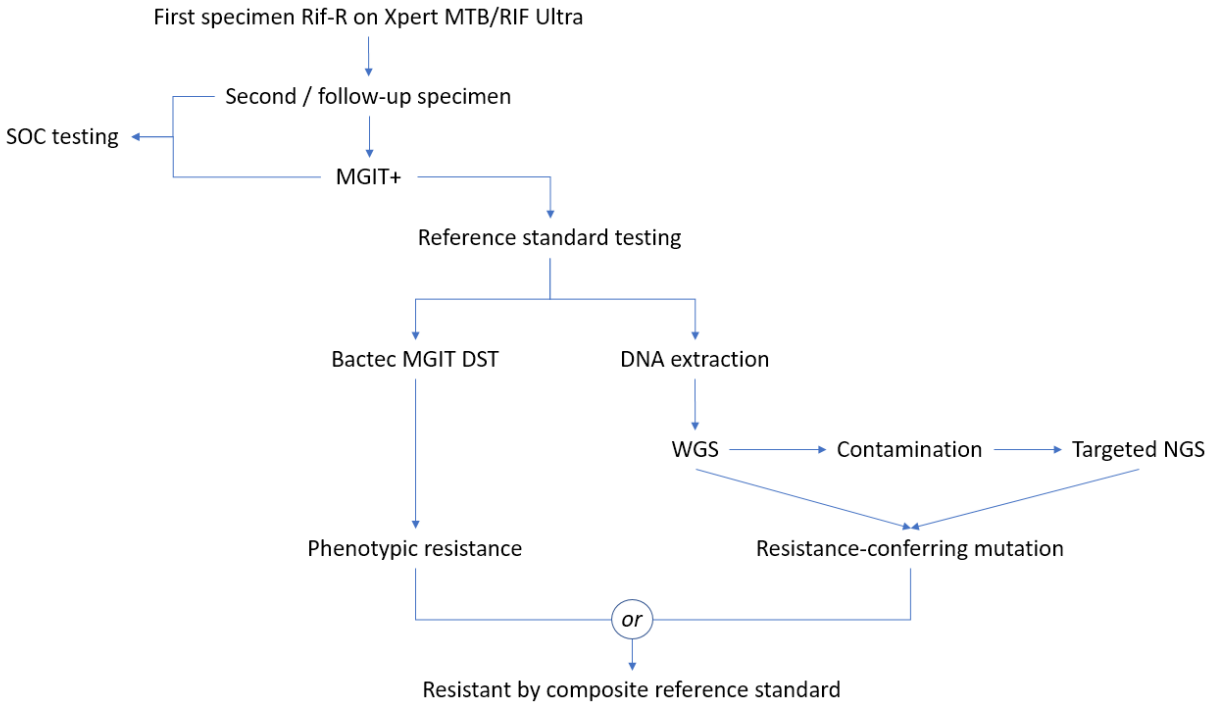

Figure 4. Reference standard workflow in the TB-CAPT MDR/XDR Study. Rif-R = rifampicin-resistant, SOC = standard-of-care, MGIT = Mycobacterial Growth Indicator Tube, DST = drug susceptibility testing, WGS = whole genome sequencing, NGS = next generation sequencing

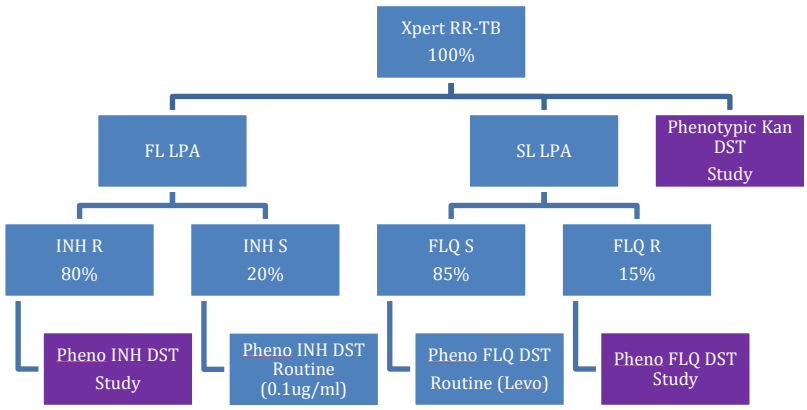

Figure 5. Phenotypic susceptibility testing in the TB-CAPT MTB/XDR study. Blue = routine procedure, purple = study procedure. RR = rifampicin-resistant, FL = first-line, SL = second-line, LPA = line probe assay, DST = drug susceptibility testing, FLQ = fluoroquinolones, pheno = phenotypic

Blinding procedure

The index test and reference standards will be performed independently and at different times on different samples by different operators in separate laboratories. The index test will be performed on the first sample in the routine laboratory and the reference standard on the second sample in either a different part of the routine laboratory or in a research laboratory. Each specimen will be assigned a separate laboratory number and the report issued separately.

Statistical analysis

A full Statistical Analysis Plan will be developed prior to conclusion of the study. A brief overview of planned statistical analyses is provided here.

Analysis populations

- Intended to test (ITT): all samples intended to be tested
- Per protocol (PP): all samples with valid test results for the index test and reference standard

Descriptive statistics

Continuously scaled variables are described by mean, standard deviation, median, minimum and maximum. Categorical scaled variables are described by absolute and relative frequencies.

Analysis of endpoints

This is a study to estimate diagnostic accuracies, thus hypotheses are not established.

Diagnostic accuracy study

Estimation of sensitivity and specificity

The following 2x2 table is analysed:

Table 4. Contingency table for analysis of diagnostic accuracy

		RS		
		Positive	Negative	Total
IT	Positive	TP = a	FP = b	a+b
	Negative	FN = c	TN = d	c+d
	Total	a+c	b+d	a+b+c+d

RS=reference standard, IT=index test, TP=true positive, FP=false positive, FN=false negative, TN=true negative

The diagnostic accuracy measures are calculated according the following formulas:

Table 5. Formulas and rules to calculate diagnostic accuracy measures

Measure	**Formula**	**Confidence interval**
Sensitivity	a/(a+c)	2-sided 95%-Wilson-Score-CI
Specificity	d/(b+d)	2-sided 95%-Wilson-Score-CI

Comparison of diagnostic accuracies

More details will be provided in the Statistical Analysis plan

Further endpoints / feasibility study

Other endpoints are evaluated by means of descriptive statistics; estimates are presented with 95%-confidence interval. Time-to-result analyses will include calculation of medians and Kaplan-Meier analyses.

More details will be provided in the Statistical Analysis plan.

Missing values

No imputation

Sample size

See page 21

Data management

Data will be entered into case report forms or obtained from the laboratory information system.

Data management will be co-ordinated by Ludwig-Maximilians-Universitaet Muenchen (LMU) and FIND.

Ethical considerations

This study will address an urgent need for rapid susceptibility testing in rifampicin-resistant and MDR-TB, which currently takes days, weeks or months to complete, and often results in long delays in the initiation of or change to appropriate treatment.[10] We are evaluating a test that could reduce that turnaround time to hours, with potential knock-on effects on more rapid treatment initiation and reduction in development / transmission of resistance. Therefore, the potential benefits of this study to patient care and society are substantial.

Because rifampicin resistance is relatively rare and difficult to predict, prospective enrolment of patients would take too long and be too resource intensive to undertake. Laboratory-based screening would enable identification of patients with drug resistance and therefore eligibility for second-line testing. However, the centralised tuberculosis laboratory receives patient samples from a geographically wide referral area – locating and recalling these patients for enrolment in the study would be impractical. Furthermore, the Xpert MTB/XDR test is currently an investigational assay and therefore not reportable or able to be used to impact patient care. The proposed study procedures would not affect the usual care of patients as we will utilise specimens that would ordinarily be discarded. Similarly, the reference standard tests (next generation sequencing) are non-routine tests not currently utilised for patient care in South Africa. These tests will be performed post hoc with patient identifiers removed.

For these reasons, we are proposing a waiver of informed consent for this diagnostic accuracy and feasibility study.

The study poses no risks or benefits to participants. Participants will still receive standard of care testing according to the national algorithm and only leftover samples will be utilised.

This study will be conducted according to the ethical principles set forth in the Declaration of Helsinki, ICH-GCP, and local regulatory requirements as applicable. All source documents will be available for review by relevant institutional review committees when requested.

Safety assessments

The study will comply with all biosafety precautions appropriate to the study procedures, including the preparation of primary specimens for Xpert MTB/XDR and handling of culture isolates for the reference standard. Study procedures will be performed in laboratories accredited for safe handling of these specimen types, and according to the laboratories' safety protocols. All testing will be performed by appropriately trained and accredited personnel. Study staff will be monitored for TB symptoms, including cough, fever, weight loss and night sweats.

Covid-19 considerations

Study procedures will comply with all UCT, WHC, NHLS and FIND Covid-19 safety policies in effect at the time of implementation. A Covid-19 Risk Management Plan will be submitted separately. No patient contact is required for the study.

End of study definition

Participation in this laboratory-based study will start on Xpert MTB/XDR testing of the first specimen and end with finalisation of the reference standard testing results from the second specimen. There are no specific stopping rules on an individual participant level. The study will end when the reference standard tests for the last sample included are finalised. The study will be stopped if any information becomes available with regard to any risk to participants or study personnel. Should Xpert MTB/XDR be approved and implemented nationally, patient results will be reported to the clinical service.

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