Accuracy and impact of the MTBDR*plus* v2 and MTBDR*sl* v2 line probe assays for the detection of first-line and second-line drug resistant tuberculosis

by

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Declaration

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March 2023

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To my daughter Samiya…

“I was taught that the way of progress was never swift nor easy.” - Marie Curie

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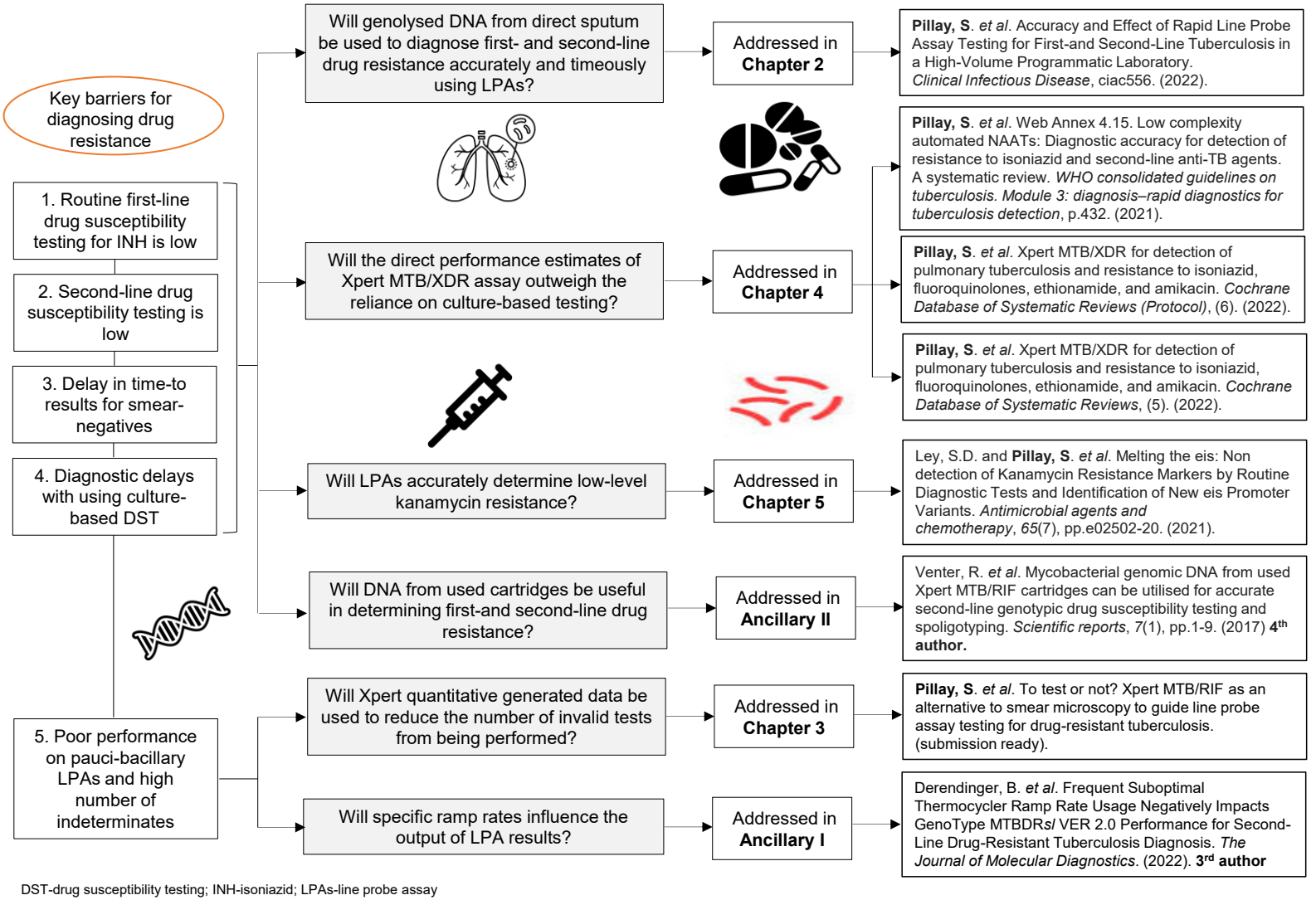
Graphical map shows the key challenges associated with detecting TB drug resistance and how this thesis aims to address these gaps.



DST-drug susceptibility testing; INH-isoniazid; LPAs-line probe assay

Summary

Combating drug-resistant tuberculosis (DR-TB) remains a challenge globally. Treatment success rates are often derailed by under-diagnosis and under-reporting of disease. Patients remain contagious for prolonged periods prior to initiation of appropriate treatment which is further exacerbated by the amplification of drug resistance and poor treatment outcomes. Using current and new diagnostic tools effectively is key to rapid diagnosis of tuberculosis and early detection of drug resistance.

Firstly, (chapter 2) line probe assays (LPAs) frequent inability to generate a resistance call in paucibacillary specimens is problematic. We showed that while MTBDR*plus* and MTBDR*sl* tests work well on smear-negative specimens for detecting drug resistance, failure rates remained high. We demonstrated with the use of routine key programmatic data how time-to-reporting of results improved with the use of molecular assays and provided evidence on how standard-of-care can be improved in a programmatic context.

Secondly, (chapter 3) LPA testing on smear-negative specimens is not always performed causing diagnostic delays and hindering their role as a direct front-line diagnostic tests. Thus, by using Xpert-generated data we determined the ratio of actionable-to-non-actionable results and the number of missed resistant cases at varying thresholds. We demonstrated that Xpert semiquantitation category is superior to informing reflex LPA testing than smear status. In short, this method provides a framework by which laboratories that currently do not test smear-negative specimens to expand testing.

Thirdly (chapter 4) current pathways using Xpert MTB/RIF or Xpert Ultra as frontline tests for diagnosing TB and rifampicin resistance lack further treatment guidance. We did a systematic review and assessed the performance of Xpert MTB/XDR for the detection of pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin. Participants consisted of 1228 for pulmonary tuberculosis detection and 1141 for drug resistance. We found Xpert MTB/XDR is unlikely to test positive as a follow-up test for the detection of *Mycobacterium tuberculosis* in samples

that test Xpert Ultra “trace positive” initially. We found accurate sensitivity and specificity estimates for the detection of resistance to isoniazid and fluoroquinolones. We showed that this assay is best used as a rule-out test for ethionamide drug resistance.

Lastly (chapter 5), second-line injectable drugs are still of value when the all-new oral TB regimen is unavailable. We showed that MTBDR*sl* can have false-positive and false-negative results associated with *eis* promotor mutations. Furthermore, detection of kanamycin resistance is complex, requiring a composite reference standard to determine true drug resistance.

In summary, these results show that current diagnostic molecular tools can improve the programmatic standard-of-care and thereby aid in the selection of TB regimens, however, TB detection failure hampers further drug susceptibility testing. We showed that with the use of defined cut-off thresholds, failure rates in downstream LPA testing can be detected earlier. We identified cases that were missed for detecting kanamycin resistance when routinely tested, potentially leading to ineffective treatment regimens. Furthermore, we showed Xpert MTB/XDR has added value for the robust diagnosis of key TB drugs, including fluoroquinolones, an integral component in preserving the integrity of new and repurposed drugs, and this informed a policy decision.

Opsomming

Die bekamping van dwelmweerstandige tuberkulose (DR-TB) bly 'n uitdaging wêreldwyd. Behandelingsukseskoerse word dikwels ontspoor deur onderdiagnose en onderrapportering van siektes. Pasiënte bly vir lang tydperke aansteeklik voor die aanvang van toepaslike behandeling, wat verder vererger word deur die versterking van middelweerstandigheid en swak behandelingsuitkomste. Die doeltreffende gebruik van huidige en nuwe diagnostiese instrumente is die sleutel tot vinnige diagnose van TB en vroeë opsporing van middelweerstand.

Eerstens is (hoofstuk 2) lynsondetoetse (LPA's) gereelde onvermoë om 'n weerstandsoproep in paucibacillêre monsters te genereer problematies. Ons het gewys terwyl MTBDRplus- en MTBDRsl-toetse goed werk op smeer-negatiewe monsters om middelweerstandigheid op te spoor, het mislukkingskoerse hoog gebly. Ons het gewys hoe tyd-tot-rapportering van resultate met die gebruik van molekulêre toetse verbeter het met behulp van roetine-sleutel-programmatiese data en het bewys gelewer oor hoe standaard-van-sorg verbeter kan word in 'n programmatiese konteks.

Tweedens, (hoofstuk 3) is herhaal LPA's-toetsing op paucibacillêre monsters algemeen, wat diagnostiese vertragings veroorsaak en hul rol as 'n direkte frontlinie-diagnostiese toets belemmer. Dus, ons het gewys deur gebruik te maak van roetine Xpert-gegenereerde data, kan ons 'n proporsie toetse kwantifiseer wat van toetsing uitgesluit kan word deur 'n gedefinieerde drempel te gebruik, aangesien dit waarskynlik nie-uitvoerbare resultate op kliniese monsters lewer. Kortom, hierdie metode kan onnodige LPA-toetsing bespaar en bied 'n raamwerk waarvolgens laboratoriums wat tans nie smeer-negatiewe monsters met MTBDR*plus* of MTBDR*sl* toets nie, toetsing kan uitbrei.

Derdens (hoofstuk 4) het huidige weë wat Xpert MTB/RIF of Ultra gebruik as voorlyntoetse vir die diagnosering van TB en rifampisienweerstand, kortkom verdere behandelingsleiding. Ons het 'n sistematiese oorsig gedoen en die prestasie van Xpert MTB/XDR vir die opsporing van pulmonale tuberkulose en weerstand teen isoniazied, fluorokinolone, etionamied en amikasien beoordeel.

opsporing en 1141 vir middelweerstand. Ons het getoon dat dit onwaarskynlik is dat Xpert MTB/XDR positief sal toets as opvolgtoets vir die opsporing van Mtb in monsters wat aanvanklik Xpert Ultra-spoor positief toets. Ons het akkurate sensitiwiteit- en spesifisiteitskattings gevind vir die opsporing van weerstand teen isoniasied en fluorokinolon. Ons het gewys dat hierdie toets die beste gebruik word as 'n reël-uit toets vir etionamiedmiddelweerstand.

Laastens (hoofstuk 5) met vinnig opkomende middelweerstand, is tweedelyn inspuitbare middels steeds van waarde wanneer alle nuwe orale TB-regimen nie beskikbaar is nie. Ons het getoon dat MTBDRsl nie daarin geslaag het om ~<1% eis promotormutasies op te spoor nie en amikasien pDST is oneffektief om kanamisienweerstand alleen op te spoor. Verder is opsporing van kanamisienweerstand kompleks en vereis 'n saamgestelde verwysingstandaard om ware geneesmiddelweerstand te bepaal.

Samevattend, hierdie resultate toon dat huidige diagnostiese molekulêre instrumente haalbaar is om TB-regimes te rig, maar TB-opsporingsmislukking belemmer verdere geneesmiddelvatbaarheidstoetsing. Ons het gewys dat met die gebruik van gedefinieerde afsnydrempels mislukkingskoerse in stroomafwaartse LPA-toetsing vroeër opgespoor kan word. Ons het gevalle geïdentifiseer wat gemis is vir opsporing van kanamisienweerstand wanneer dit gereeld getoets is, wat moontlik lei tot ondoeltreffende behandelingsregimes. Verder het ons gewys dat Xpert MTB/XDR waarde toegevoeg het vir robuuste diagnose van sleutel-TB-middels, insluitend fluoroquinolones, 'n integrale komponent in die behoud van die integriteit van nuwe en herdoelde middels.

List of Abbreviations:

AMK	Amikacin
CAP	Capreomycin
CE	Cartridge extract
C_{Tmin}	Minimum cycle threshold
CFU	Colony forming units
DNA	Deoxyribonucleic acid
DR-TB	Drug resistant tuberculosis
DS-TB	Drug susceptible tuberculosis
DST	Drug susceptibility testing
ETH	Ethionamide
FQs	Fluoroquinolones
INH	Isoniazid
Kan	Kanamycin
LPA	Line Probe Assay
LOD	Limit of detection
MDR-TB	Multi-drug resistant Tuberculosis
MGIT	Mycobacteria growth indicator tube
MTB	*Mycobacterium tuberculosis*
MTBC	*Mycobacterium tuberculosis* complex
MUT	Mutation
NAAT	Nucleic acid amplification test

NALC	N-acetyl-L-cysteine
NRF	National Research Foundation
PCR	Polymerase chain reaction
pDST	Phenotypic drug susceptibility testing
RIF	Rifampicin
RR	Rifampicin resistance
RR-TB	Rifampicin-resistant tuberculosis
ROC	Receiver operating characteristic
SLIDs	Second-line injectables drugs
SR	Sample reagent
TB	Tuberculosis
Ultra	Xpert Ultra
WHO	World Health Organization
WT	Wild Type
Xpert	Xpert MTB/RIF
Xpert Ultra	Xpert MTB/RIF Ultra
Xpert XDR	Xpert MTB/XDR
XDR	Extensively drug-resistant

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Table 1. Current WHO approved diagnostic tools available for 2022. (Adapted from WHO Global TB Report, 2022)

Chapter 1

Diagnostic tools for tuberculosis

Introduction

South Africa has been ranked as one of the top ten countries with highest tuberculosis (TB) incidence (513/100 000) in the world in 2021(World Health Organization, 2022a). In 2019, 206 030 people were detected and notified of multi-drug resistant/rifampicin-resistant tuberculosis (MDR/RR-TB) of which 28 931 failed to register for treatment, hence treatment success rates were poor with only ~57% of the population being successfully treated in endemic settings (World Health Organization, 2015, Pietersen et al., 2014, World Health Organization, 2020b) (**Figure 1**).

Diagnosis of drug resistance to first-line drugs are frequently prioritised for rifampicin resistance (RR) alone. As a consequence fewer patients receive upfront testing for isoniazid (INH) resistance, despite mono-resistance being frequent with 1.1 million cases globally (World Health Organization, 2020a). Detection of drug susceptibility to fluoroquinolone (FQ) is an essential criteria needed for inclusion of the new short all-oral MDR regimens (Alagna et al., 2021). Furthermore, resistance to drugs is an increasing problem where only 50% of the MDR-TB population received testing to FQs, a critical drug in the drug-resistant TB regimens (**Figure 1**) (World Health Organization, 2022a). In addition, ~20% of new cases with MDR-TB (resistance to first-line drugs rifampicin (RIF) and INH) in South Africa are estimated to have resistance to FQ (ofloxacin, moxifloxacin, and levofloxacin) (World Health Organization, 2021a).

Drug-resistant tuberculosis (DR-TB) treatment is significantly more complicated than drug-susceptible because the duration is longer (6-18 months), the medications are more toxic, and there is a limited supply of pharmaceuticals (World Health Organization, 2022b). Hence, early diagnosis of drug resistance is key curbing transmission and improving patient treatment outcomes.

1. Effective treatment initiation are key to preventing acquisition of resistance

A large gap still remains with undiagnosed and underreporting of TB disease globally (World Health Organization, 2020b). Improved rates of diagnosis of DR-TB are important for reducing transmission. In South Africa, 80% of MDR-TB is thought to be spread from person-to-person transmission

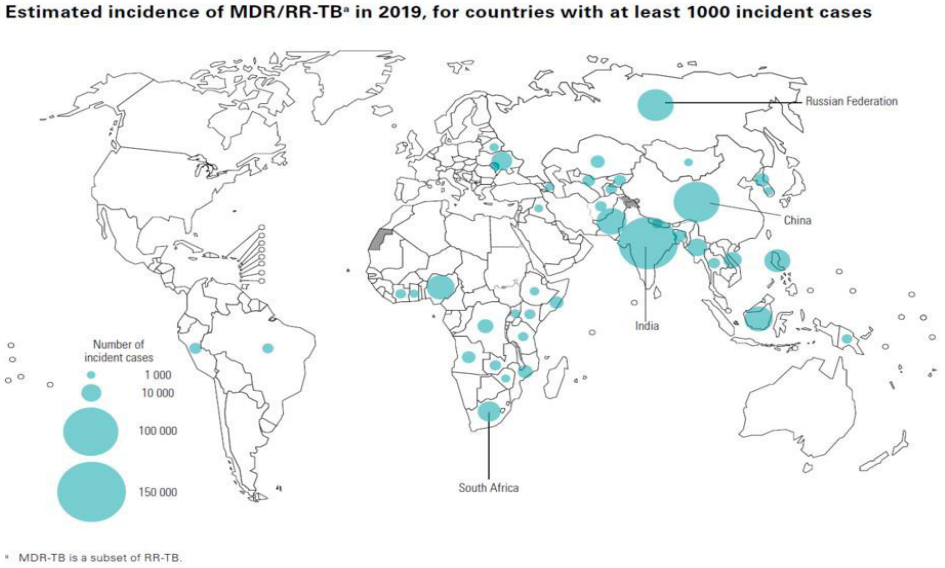
(Streicher et al., 2011, Dheda et al., 2017). Modelling studies (Basu et al., 2007, Basu et al., 2009, Dowdy et al., 2008) have shown that, through the improvement of capacity to rapidly diagnose DR-TB, patient cure rates can be improved through the earlier initiation of appropriate and effective TB treatment.

Importantly, the infectiousness of patients with drug sensitive tuberculosis (DS-TB) declines within one to two weeks of treatment (Menzies, 1997). The exact "infectiousness period" for drug-resistant TB remains unclear; however, early treatment initiation, which depends in part on rapid diagnosis, may help curtail the spread of drug-resistant TB by reducing infectiousness and disrupting person-to-person transmission. Thus, there is an urgent need for rapid tests which allow the early detection of drug resistance and the selection of appropriate drugs.

1.1 Key knowledge gaps in diagnosis and treatment of tuberculosis

- The number of patients notified of TB and reported.
- The number of patients who require bacteriological confirmation of tuberculosis, as well as the limited availability for routine drug susceptibility testing (DST) of FQ and INH.
- Fewer registration of patients for treatment initiation and deviation from diagnostic pathways, defaulters and poor adherence to treatment (Namukwaya et al., 2011).
- Poor uptake of WHO-recommended diagnostic tests.

A



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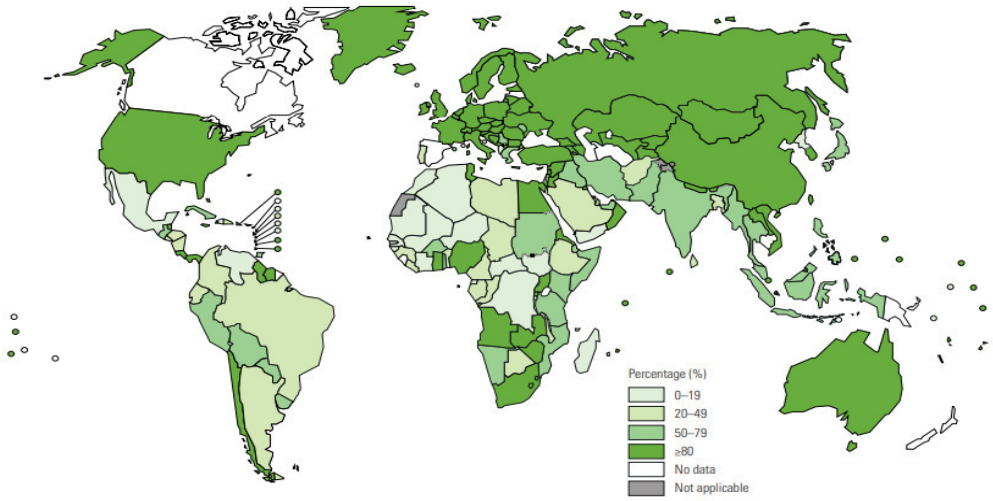

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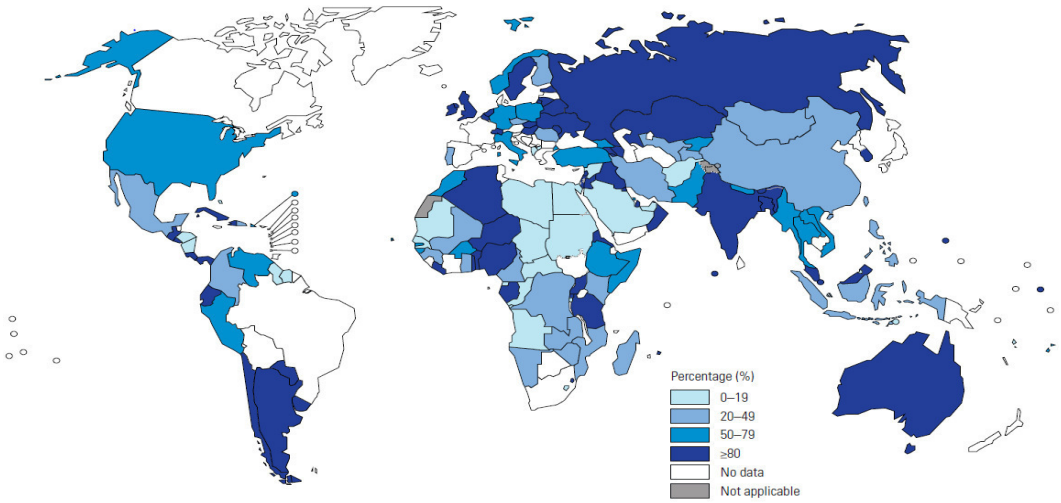

Figure 1. Panel **A.** Estimated incidence of MDR/RR-TB in 2019 (WHO Global TB Report 2020). (Adapted from WHO Global Tuberculosis Report 2021). **B**. In 2019, 2.2 million (61%) of the 3.6 million bacteriologically proven pulmonary tuberculosis cases reported

worldwide were tested for RR. **C**. Global estimates of population with MDR/RR-TB in 2019 show that only 71% of cases were tested for FQ drug resistance. (Panels **B** and **C**, Adapted from WHO Global TB Report 2020).

2. Drug susceptibility methods

2.1 Phenotypic testing (culture)

Culture-based pDST remains the gold standard for the detection of drug resistance. At peripheral testing laboratories within South Africa, both liquid and solid media are used for pDST. As a result, a verified MTB specimen is further cultured in solid or liquid media containing the necessary concentration of an anti-TB drug. MTB growth suggests resistance to a particular drug while the absence of MTB growth indicates susceptibility. The minimum inhibitory concentration (MIC), which is the lowest concentration of an anti-TB drug can stop the growth of an MTB strain in culture. Solid media testing is performed using 7H10, 7H11 or Lowenstein Jensen (LJ) media. LJ slants is the most widely used medium, MTB growth is observed by rough colonies and cording formations. The indirect proportion method uses a standardized and two 10-fold diluted dilutions of the inoculum with the anti-TB drug. Drug resistance is shown when at least 1% of growth is observed at the drug MIC when compared to growth without drug. Bactec Mycobacteria Growth Indicator tube (MGIT) 960 BD is an automated liquid system used for the detection of growth of MTB (Chihota et al., 2010). In the presence of a specific anti-TB drug concentration, a specimen is added to the MGIT. Because MTB consumes oxygen, bacterial growth is instantly identified by fluorescence, proving that MTB is present and resistant to the drug. For first-line agents (INH/RIF) and some second-line anti-TB drugs [KAN, AMK, ofloxacin, levofloxacin], phenotypic DST is generally consistent, reproducible, and widely used, except for pyrazinamide (PZA), which requires technical expertise to avoid false positive results. All critical repurposed drugs such as Clofazimine, Linezolid, Bedaquiline and Delamanid are important drugs recommended for use by WHO as their use is essential in the new 6-9-month MDR treatment regimens (World Health Organization, 2022b).

2.2 Limitations of phenotypic drug susceptibility testing

Some of the challenges faced with phenotypic drug susceptibility testing include with solid bacterial growth is firth needed before pDST can be performed leading to significant time delays in reporting of results. Hence, liquid media (MGIT) is more rapid in reporting these results 14 days compared to 42 days. In addition, contamination rates also contribute to diagnostic delays and procurement of media is not always efficient with supply chains.

2.3 Genotypic testing (molecular)

Molecular diagnostic tests have altered the paradigm of delivery of results from months to days by offering rapid, robust turn-around times. Nucleic acid amplification tests (NAATs) offer vast advantages such as differentiating between MTB species and nontuberculosis mycobacteria (NTMs), and detection of specific mutations associated with resistance to TB drugs. NAATs such as the LPA has an advantage in that once extraction of DNA is completed in a strict biosafety level 3 lab, DNA is then rendered non-infectious and safe to work with. Tests can be performed directly as well as indirectly on specimens included in the genotypic tests listed below.

2.3.1 Limitations of Molecular Drug Susceptibility Testing

Molecular diagnostic assays consist of a combination of semi-quantitative tests e.g., Xpert, Xpert Ultra, Xpert XDR and non-quantitative tests. Only hotspot regions are targeted in the genome and therefore sensitivity estimates may be reduced for drugs where resistance mechanisms are not well characterized. Molecular assays are however prone to false negative results due the presence of inhibitors (haemoglobin, metabolites), silent mutations (if the assay cannot distinguish between natural occurring polymorphisms and resistance conferring mutations), failure to amplify a targeted region (when large scale deletions occurred) or if the limit of detection (LOD) for the identification of heteroresistance is low.

Some NAATs cannot be used for treatment monitoring and most cannot distinguish between viable and non-viable MTB (Wang et al., 2020). Most diagnostic NAATS cannot be implemented in

undeveloped rural clinics and hospitals due to the laboratory infrastructure and platform requirements, steady water supply, uninterrupted power supply and highly skilled laboratory personal (Walzl et al., 2018).

3. Tests studied in thesis for the detection of drug resistance

At the time of the study, we focused on the promising new diagnostic second-line assay Genotype MTBDR*sl* v2. However, for the purpose of this literature review we have included the most recently available rapid molecular tools that has come through the pipeline.

3.1 Xpert MTB/ RIF assay

The Xpert assay (Xpert, Cepheid, Sunnyvale, United States of America) is a rapid, fully automated nucleic acid assay recommended for usage by the WHO in 2010 for diagnosing suspect MDR-TB and human immune deficiency virus (HIV) associated with MTB (World Health Organization, 2019). This test is based on real-time polymerase chain reaction (PCR) and uses five amplified target regions of the *rpoB* gene for the detection of MTB and resistance to RIF and is meant to be used as the initial diagnostic test instead of smear microscopy (Denkinger et al., 2014). Limitations of the test include suboptimal sensitivity and specificity in pauci-bacillary specimen, annual calibration of modules, external quality control performed quarterly and inability to use for patient treatment monitoring.

3.2 Xpert Ultra

Xpert Ultra which was developed by Cepheid Inc, Sunnyvale, CA, USA as a new and improved next-generation assay for the detection of MTB and resistance conferring mutation to rifampicin. This sophisticated test was launched in the year 2017 with substantial upgrades from the Xpert MTB/RIF promising much higher sensitivity which is closely sensitive to liquid culture. The assay incorporates two different multicopy amplification targets (IS6110 and IS1081), incorporates fully nested nucleic acid amplification, more rapid thermal cycling, and improved fluidics and enzymes. An addition

quantitation used to identify the amount of MTB detected in a specimen using Xpert Ultra is referred to as “Trace call” which detects the bare minimum of MTB present.

3.3 Line Probe Assay Technology

LPA is a rapid test, where amplified sequences are hybridized to a membrane strip (with immobilized probes for both wild type and mutant sequences) for the detection of *Mycobacterium tuberculosis complex* (MTBC) and resistance to RIF, INH, FQs and SLIDs (**Figure 2**). The LPA strip contains an internal control amplification band (AC) which must be present to ensure correct interpretation of wildtype (WT) and mutation (MUT) probes and the conjugation band (CC) to ensure that valid conjugate binding and substrate reaction occurred. The TUB band is used to detect the presence of MTBC DNA. For each target included on the strip, gene locus probes are used as controls for the amplification of the specific target. LPAs can be performed either directly using clinical specimens (NALC-decontaminated sputum) or indirectly on cultured isolate. LPAs require a three-step procedure before visual assessment of results. Limitations of this assay include high biosafety standards; due to the open-tube analysis the test is prone to inter sample and amplicon contamination.

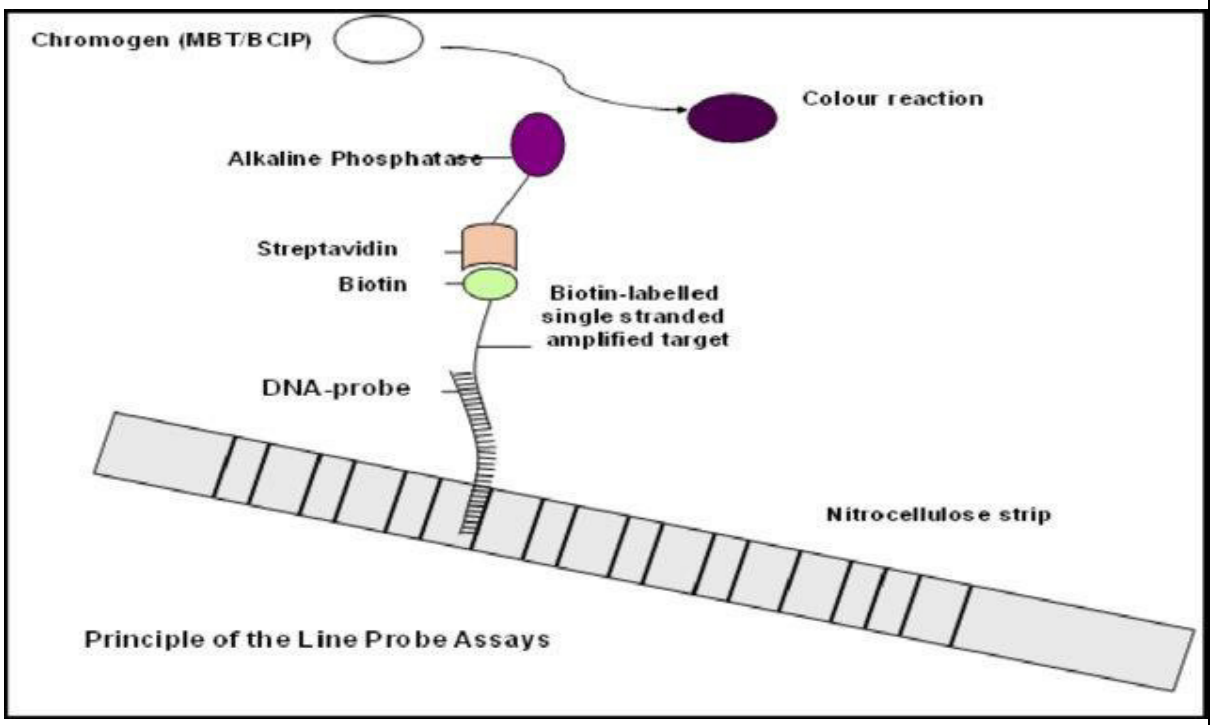

Figure 2. Principle of line probe assay involves the binding of amplicons to specific probes coated on nitrocellulose strips which target the most common resistance associated mutations to first and second-line drugs. (Adapted from Barnard 2012.B)

3.3.1 Genotype MTBDRplus

The line probe assay, Hain MTBDR*plus* version 1, was first endorsed by WHO in 2008 (World Health Organization, 2008) for the detection of first-line drugs. In 2011 version 2, of the assay was developed and endorsed by WHO in 2016 along with the Nipro NTM-MDRTB detection kit (World Health Organization, 2016a). MTBDR*plus* targets the identification of mutations associated with RIF and INH resistance. For RIF resistance, the assay targets the RR determining region (RRDR) in *rpoB,* while the identification of high- and low-level INH resistance is identified through *katG* (codon 315) and the *inhA* promoter region (-16 to -8 nucleotide upstream), respectively. MTBDR*plus* is recommended to be performed on smear-positive specimens directly or on cultured isolates indirectly.

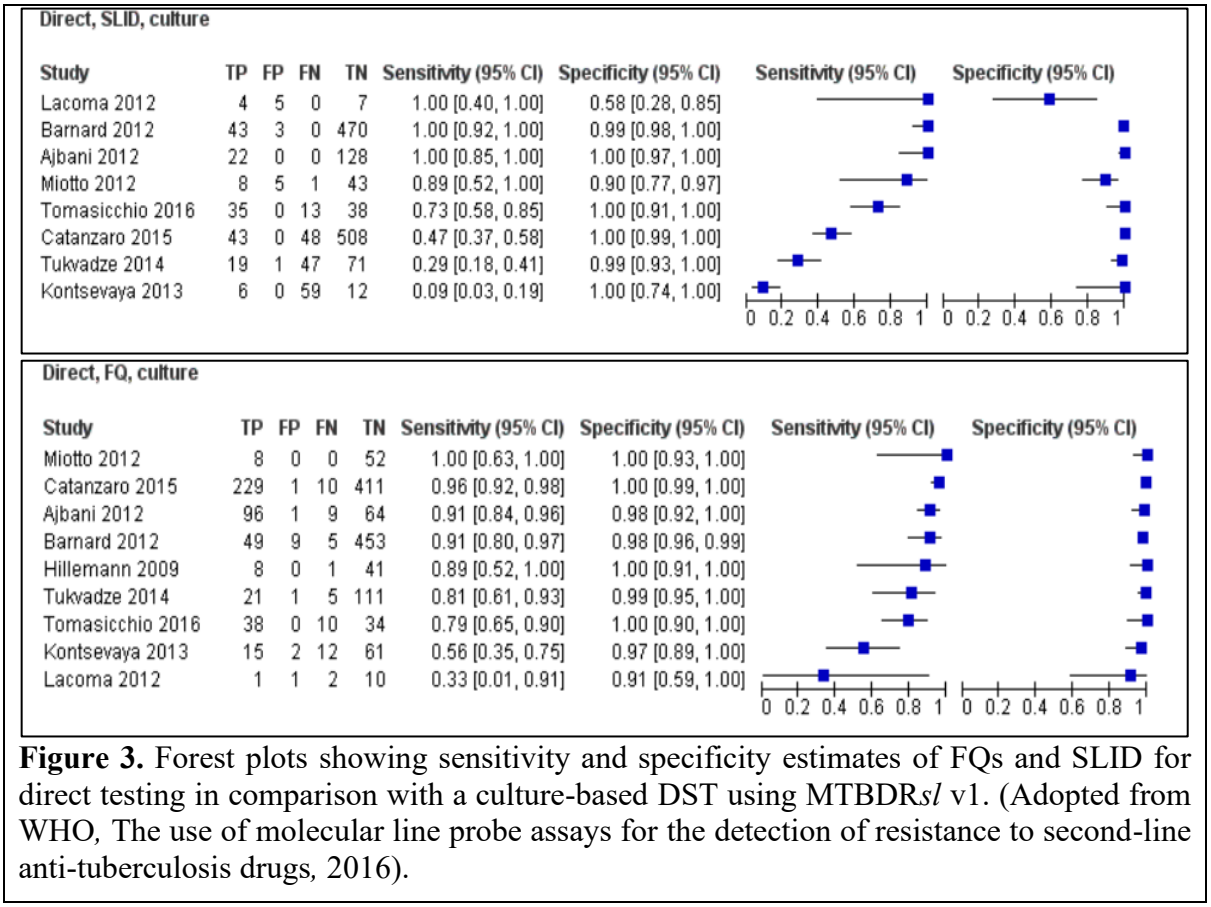

Figure 3. Forest plots showing sensitivity and specificity estimates of FQs and SLID for direct testing in comparison with a culture-based DST using MTBDR*sl* v1. (Adopted from WHO, The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs, 2016).

3.3.2 Genotype MTBDRsl

In 2016, MTBDR*sl* version 2 was WHO endorsed and recommended for use on confirmed RR-TB or MDR-TB as the initial test to detect resistance to FQ and second-line injectables instead of pDST irrespective of smear status (World Health Organization, 2016b). Limited data is available on the performance of MTBDR*sl* version 2 using direct testing (**Figure 3**). Version 1 targeted regions included *gyrA* (for the fluoroquinolones: ofloxacin and moxifloxacin), *rrs* (for the aminoglycosides/ cyclic peptides: CAP, viomycin, KAN, AMK) and *embB* (for ethambutol). Similar to version 1, MTBDR*sl* v2 includes the quinolone resistance determining region (QRDR) *gyrA* (codon 85-96), *gyrB* (codon 536-541) for FQ resistance; and *rrs* region (codon 1401,1402 and 1484), with the addition of the *eis* promoter region (-37 to -2 upstream) for second-line injectable drug resistance; and the exclusion of *embB* (Global Laboratory Initiative and World Health Organization, 2018) (**Figure 4**).

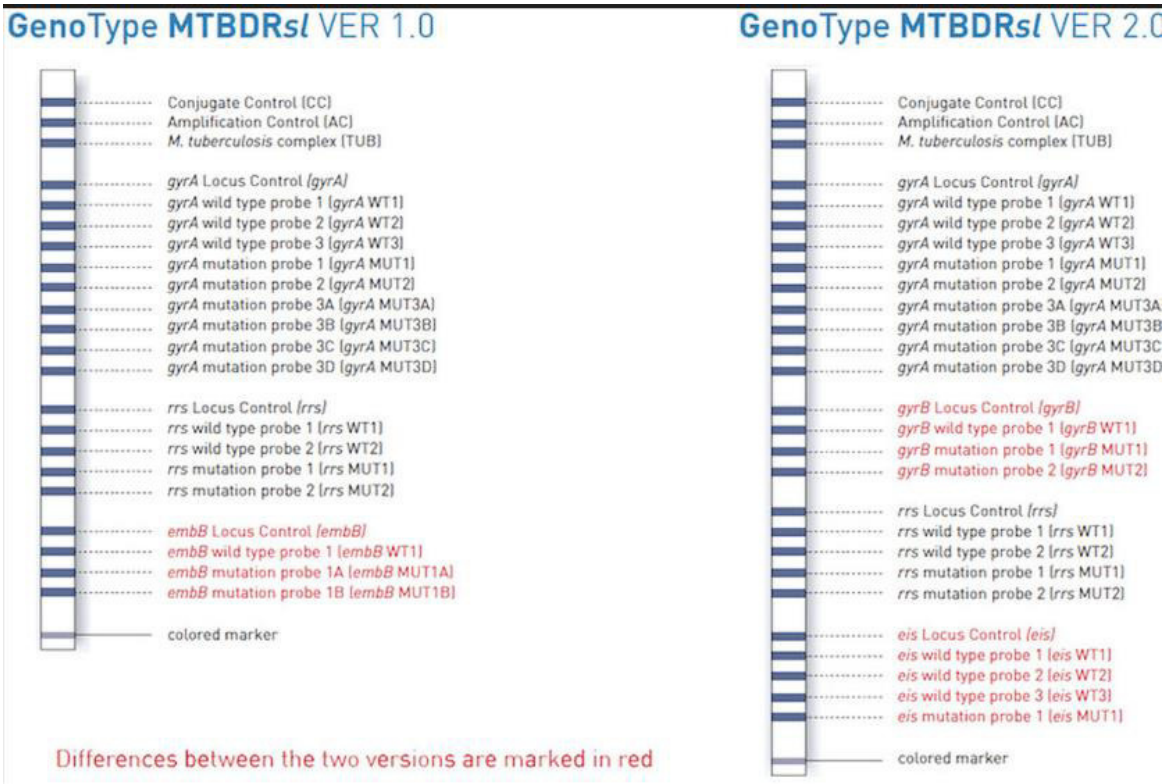

Figure 4. Genotype MTBDR*sl* v2 is an updated version which includes additional target regions *gyrB* which detects resistance to moxifloxacin and *eis* region which can detect low-level kanamycin resistance. (Adapted from Hain Lifesciences)

3.4 Xpert MTB/XDR

This is reflex NAAT which detects MTB complex as well mutations associated with resistance to INH, FQ, ETH and second-line line injectables (AMK, CAP and KAN). It can be easily implemented at peripheral laboratories as it uses the same platform as Xpert or Xpert Ultra but follows multiplexing. An additional advantage includes same sample reagent (SR) buffered treated sample can be used for testing both Xpert Ultra and Xpert XDR if the initial volume sputum sample processed is enough. However, due to the Xpert XDR assay detecting additional targets, it requires a 10 colour-technology which is more expensive than 6 colour-technology (utilized by Xpert/ Xpert Ultra), (World Health Organization, 2019). A limitation of this assay is the lowered LOD compared to Ultra (as MTB detection is not done with a single copy target) and therefore does not include "trace" as a semi-quantification result. In addition to the high instrument cost, the cost per cartridge test is also quite high at US $19.80 (FIND, 2021).

3.5. Knowledge gaps of diagnostic tools at the time of the study

- LPA performance when used in diagnostic algorithms in programmatic settings.
- The cost-effectiveness and cost-benefit of line probe assays when reducing the number of tests with invalid results in programmatic settings.
- The role of line probe assays when direct testing is performed compared to indirect testing using conventional culture in smear-negative specimens.
- Methods to optimize LPAs' use, especially from specimens with pauci-bacillary specimens, with the goal to reduce the number non-actionable test results.

4. New technology that has emerged since study completion

The diagnostic assays discussed in this section were unavailable at the time of the study, hence we will briefly detail what recent advances have been made (according to WHO consolidated guidelines for TB, 2021) and how these latest products work (**Table 1**). NAATs in this section include Xpert MTB/RIF Ultra, Truenat MTB, MTB PLUS and MTB-RIF Dx, FluoroType MTB and MTBDR version 2, Cobas MTB and MTB-RIF/INH, Abbott RealTime MTB and MTB-RIF/INH, BD MAX MDR-TB and next-generation sequencing (no recommendation for usage) (World Health Organization, 2021b).

Table 1. Current WHO approved diagnostic tools available for 2022. Of all the diagnostic tools intended for use, only two assays target second-line drugs.

WHO-approved NAAT for detection of MTB and drug resistance	**DST**	**First (√) or Second- line (√√) drugs**	**Complexity**
Cepheid Xpert MTB/RIF or Xpert Ultra	RIF	√	Low
Molbio Truenat MTB/ MTB Plus/ MTB-RIF	RIF	√	Low
Abbott Real-T*ime* MTB-RIF/INH	RIF/INH	√	Moderate
BD MAX (MDR-TB)	RIF/INH	√	Moderate
Hain Fluorotype MTB/ MTBDR	RIF/INH	√	Moderate
Roche cobas MTB/ MTB RIF/INH	RIF/INH	√	Moderate
Hain-Bruker MTBDR*plus*	RIF/INH	√	High
Hain-Bruker MTBDR*sl*	FQ, SLID	√√	High
Cepheid Xpert MTB/XDR	INH, FQ, AMK, ETH	√√	Low

TB diagnostic assays (adapted from WHO Global TB Report, 2022)
Abbreviations: WHO-World Health Organization, NAAT-nucleic acid amplification test, MTB-*Mycobacterium Tuberculosis*, DST-drug susceptibility testing, RIF-rifampicin, INH-isoniazid, FQ-fluoroquinolone, SLID-second-line injectable drug, AMK-amikacin, ETH-ethambutol, MDR-TB-multi-drug resistant tuberculosis, Xpert MTB/RIF-Xpert, Xpert Ultra-Xpert MTB/RIF Ultr

5. Drug susceptibility testing in the Western Cape Province, South Africa

Xpert (Cepheid, California, USA) was endorsed by the World Health Organization (WHO) for the diagnosis of pulmonary drug DS-TB and DR-TB in 2010 (World Health Organization, 2011). Xpert is an automated molecular test for the detection of *Mycobacterium tuberculosis* (MTB) complex deoxyribonucleic acid (DNA) and RR (Boehme et al., 2010). Due to its high accuracy, the WHO endorsed Xpert as the initial test in patients with suspected TB (Boehme et al., 2010, Boehme et al., 2011).

The diagnostic algorithm currently used "at the time of the study" in June 2016-September 2019 in the Western Cape Province, South Africa, included the collection of two sputum specimens from all patients under the suspicion of having TB (**Figure 5**). The first sputum specimen was tested with Xpert. If positive for MTB and RR, the patient is referred for MDR-TB treatment initiation (Boehme et al., 2011, Coordination, 2014).

A pooled meta-analysis suggested that MDR-TB patients require a minimum of four effective drugs to which their infecting strain is susceptible to, to improve the likelihood of a positive treatment response (Ahuja et al., 2012). The published Preserving Effecting TB Treatment study showed that MDR-TB patients with baseline resistance to two or more second-line drugs were at increased risk for the acquisition of additional resistance during treatment (Cegielski et al., 2014).

Concurrently, a second sputum specimen is used for smear microscopy and for the confirmation of MDR-TB using the MTBDR*plus* assay and this is performed irrespective of smear result (Hain LifeScience GmbH, Germany). In cases where invalid results and drug susceptibility was detected to either first-line drugs, repeat testing was performed on MTBDR*plus* using a cultured isolate and in addition phenotypic DST for INH (0.1-0.4mg/L) would follow. MTBDR*plus* is a qualitative test that utilises DNA-strip technology for the identification of MTB as well as detecting mutations associated with resistance to both RR determination region (RRDR) codon 505-533 (within the *rpoB* gene) and INH (within the *inhA* promoter and *katG* gene) (Hain Lifescience, 2012).

Using the same DNA extract molecular second-line drug testing was performed using MTBDR*sl* assay. This test works on the same principle as MTBDR*plus* and is one of the few commercially available rapid molecular tests for second-line anti-TB drugs. The test is used for the detection of resistance to FQ (*gyrA, gyrB* genes) and SLID (*rrs, eis* genes) (Hain Lifescience, 2015). Since kanamycin formed part of the second-line injectable drugs for the MDR treatment regimen, MTBDR*sl* v2.0 was optimised to include the *eis* promoter mutation in the targeted region (-37 to -2 upstream) for the detection of low-level kanamycin resistance.

Furthermore, phenotypic drug susceptibility testing (pDST) for a FQ (moxifloxacin at 0.25 mg/L and 1.0 mg/L) and amikacin (AMK) (1.0mg/L) were performed on all specimens which tested Xpert rifampicin resistant (World Health Organization, 2018). However, AMK was also used as the surrogate reference drug for kanamycin drug resistance based on the assumption of complete cross resistance. Thus, this limitation may have led to ineffective treatment regimens.

Currently the diagnostic algorithm followed in the Western Cape remains largely unchanged with two sputum specimens still processed for TB, Xpert Ultra is used instead of Xpert. Although there have been advancements in frontline TB diagnostic assays LPAs still form part of routine testing.

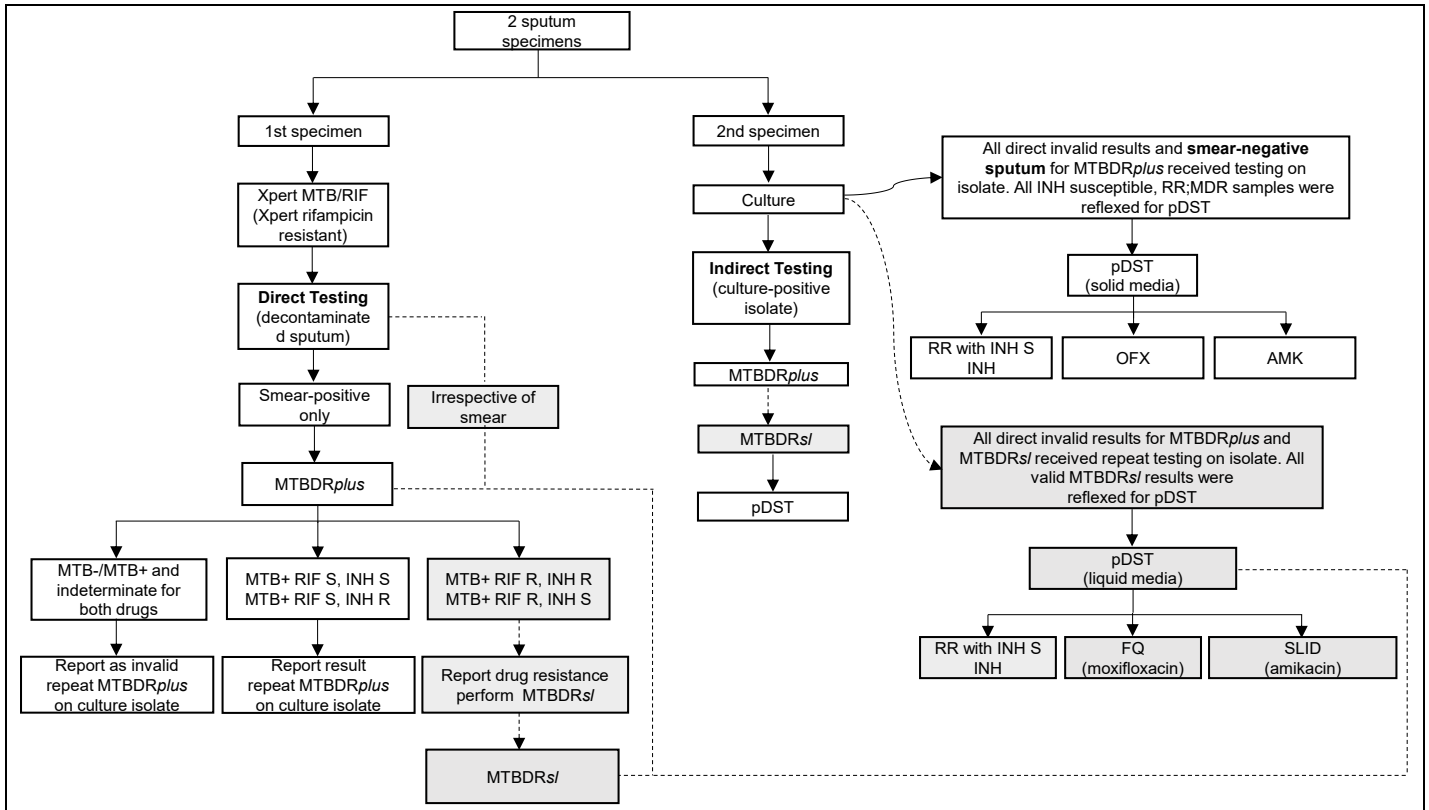

Figure 5. The algorithm followed at the time of the study, allowed for low bacillary load specimens to be tested (irrespective of smear), second-line susceptibility results (MTBDR*sl*) were reported prior to culture-based pDST. Algorithm followed prior to study (white blocks), algorithm at the time of study (grey blocks).

Abbreviations: 1st-first, MTB-/+-*Mycobacterium Tuberculosis* negative/positive, S-sensitive, R-resistant, RIF-rifampicin, INH-isoniazid, 2nd-second, pDST-phenotypic drug susceptibility testing, RR-rifampicin resistance, OFX-ofloxacin, AMK-amikacin, FQ-fluoroquinolone, SLID-second-line injectable drug.

5.1 Key challenges with diagnostic algorithm "At the time of study"

- In the Western Cape two sputum specimens are required upfront as part of diagnostic TB algorithm. Hence, patients who are unable to expectorate a second sputum specimen, only receive Xpert testing and no further additional DST.
- A limitation of this algorithm is that currently patients diagnosed as Xpert RR receive the new standardised MDR-TB drug combination without knowledge of the baseline resistance for the other drugs included in the regimen (World Health Organization, 2020c).
- Patients who test Xpert-RIF susceptible, only receive smear microscopy and no additional testing to confirm for INH susceptibility. According to a survey performed in South Africa between 2012-2014, the level INH mono-resistance is greater than 5% and is predominant in countries such as Western Pacific and South East Asia (Ismail et al., 2018) (Yuen et al., 2015).
- Pauci-bacillary specimens are prone to false negative results due to the nature of the specimen. Thus, this subset of vulnerable population is placed at highest risk as they may be falsely identified as Xpert negative due to low sensitivity of the assay leading to further failure in downstream testing.

6. Study rationale

A large proportion of patients who are smear-negative do not receive direct second-line DST. There has been limited adoption for diagnosing resistance in patients with paucibacillary disease and increased reliance on culture-based DST, which is unacceptably slow and expensive. Performance data on MTBDR*sl* for direct testing is needed. The uptake of WHO approved diagnostic tests and reliance on smear microscopy to guide reflex testing is inefficient due to low sensitivity of smear. Xpert generated data is widely available and can be utilised as an alternative to guide diagnostic tests. MTBDR*sl* v2.0 has been optimised to include *eis* promoter mutation for detection of low-level

kanamycin resistance. Performance data on the number of missed mutations are lacking. Xpert MTB/XDR is a new rapid nucleic acid amplification test for detection of tuberculosis and drug resistance. Performance data on accuracy of Xpert MTB/XDR for detecting pulmonary tuberculosis and resistance to tuberculosis drugs (i.e., isoniazid, fluoroquinolones, ethionamide, and amikacin) is needed. With the inconsistency in DST amongst countries with high MDR cases, scaling up the use of current diagnostic tools can aid in diagnosing TB disease earlier and guiding treatment regimens for successful treatment outcomes.

7. Summary of knowledge gaps and aims

Aim 1: To evaluate the diagnostic accuracy of MTBDR*plus* (v2) and MTBDR*sl* (v2) using phenotypic DST as a reference standard for the detection of resistance to the first- and second- line drugs, respectively, in a routine diagnostic laboratory. (**Figure 6**)

Sub-aim 1a: To evaluate the sensitivity and specificity of MTBDR*plus* (v2) and MTBDR*sl* (v2) when performed (i) directly on sputum or (ii) indirectly on culture isolates.

Sub-aim 1b: To investigate discrepant samples, thereby identifying potential novel mechanisms of resistance that are missed by conventional tests but detected using new technologies like sequencing.

Sub-aim 1c: To compare time to diagnosis, time to treatment initiation and in the before period when phenotypic drug testing was performed only and after period of when MTBDR*plus* and MTBDR*sl* was implemented.

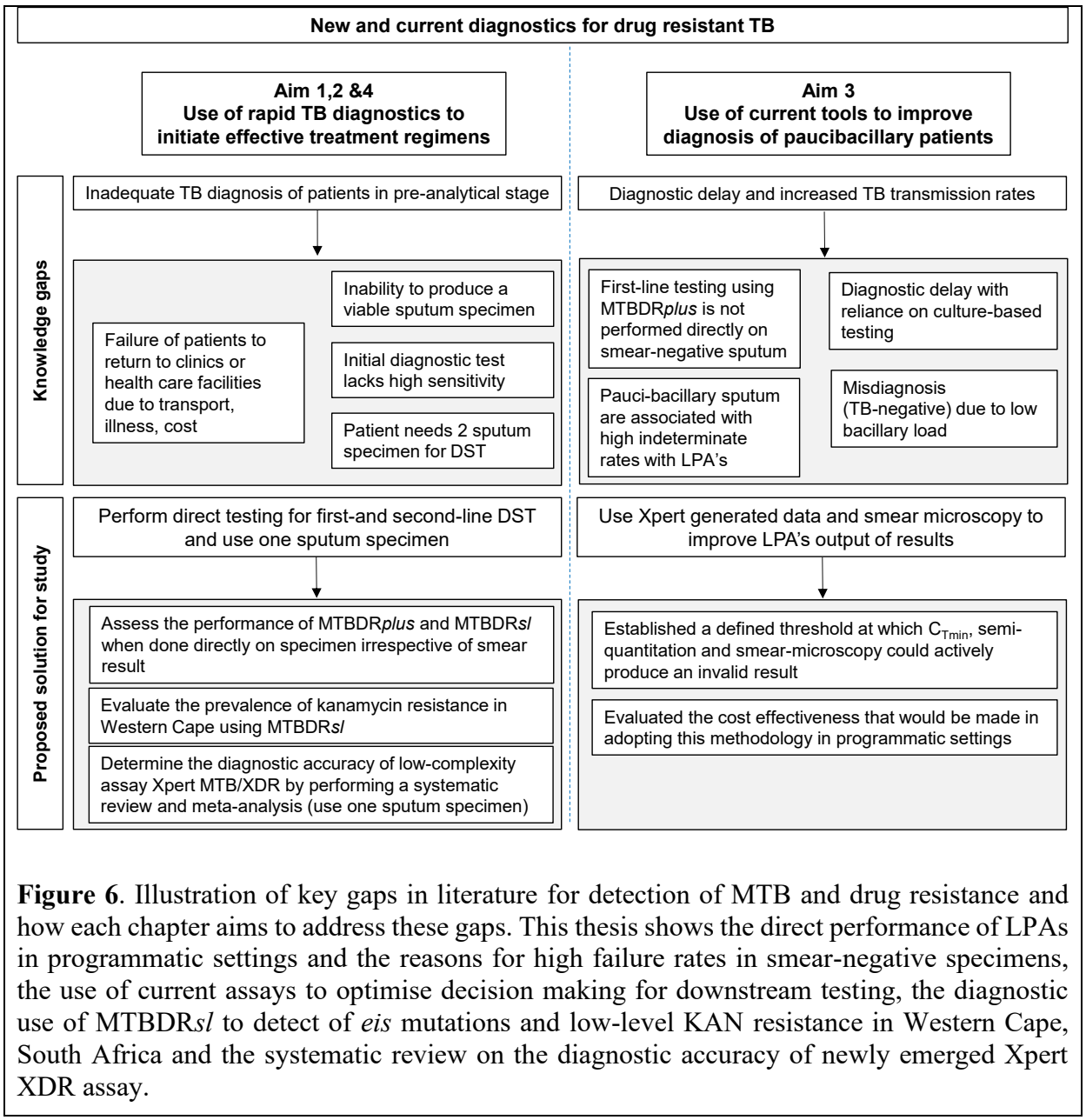

Figure 6. Illustration of key gaps in literature for detection of MTB and drug resistance and how each chapter aims to address these gaps. This thesis shows the direct performance of LPAs in programmatic settings and the reasons for high failure rates in smear-negative specimens, the use of current assays to optimise decision making for downstream testing, the diagnostic use of MTBDR*sl* to detect of *eis* mutations and low-level KAN resistance in Western Cape, South Africa and the systematic review on the diagnostic accuracy of newly emerged Xpert XDR assay.

Aim 2: To investigate type of *eis* promoter mutations that are circulating in the Western Cape.

Sub-aim 2a: To determine the cut-off minimum inhibitory concentration (MIC) for kanamycin resistance.

Sub-aim 2b: To assess the diagnostic accuracy of MTBDR*sl* in detecting low-level kanamycin resistance.

Aim 3: To evaluate using Xpert generated data to indicate the likelihood of an invalid LPA test in pauci-bacillary specimens prior to testing.

Sub-aim 3a: To measure the cost-effectiveness in programmatic settings when employing a novel method to current diagnostic algorithm.

Aim 4: To assess the diagnostic accuracy of Xpert XDR for pulmonary tuberculosis in people with signs and symptoms of pulmonary tuberculosis.

Sub-aim 4a: To assess the diagnostic accuracy of Xpert XDR for resistance to INH, FQ, ETH, and AMK in people with and without (irrespective of) RR and with RR.

Sub-aim 4b: To perform a systematic review and meta-analysis of Xpert XDR assay.

8. Originality of study

1. There have been limited studies up to date which investigated the direct performance of MTBDR*plus* and MTBDR*sl* (both v2) on a clinical specimen for first- and second-line drug resistance. Several studies have been performed using MTBDR*sl* v2 however most studies focused on the indirect performance of the assay by only including cultured isolates (Gardee et al., 2017, Gao et al., 2018, Chandak et al., 2019) and comparison of MTBDR*sl* v1 and v2 (Tagliani et al., 2015, Rufai et al., 2020). Novelty of this study includes first programmatic study to be performed in South Africa using a large sample size (n=1001), mainly consisting of smear-negative specimens and testing the performance of the assay on direct clinical specimen.
2. To the best of my knowledge there have been no studies published which looked at using Xpert generated data to identify cut-off thresholds in which specimens especially low bacillary load will likely have a non-actionable LPA result.

3. Currently there are no published systematic reviews and meta-analysis on the new Xpert XDR assay, furthermore we were the first to perform a systematic report on the Xpert XDR assay which contributed to the WHO policy recommendation in 2021.
4. There have been no published studies performed in South Africa that looked at how effective MTBDR*sl* v2 is at detecting low-level KAN resistance and the prevalence of *eis* mutations circulating in the Western Cape using routine surveillance data.

Chapter 2

Non-actionable Results, Accuracy, and Effect of First- and Second-line Line Probe Assays for Diagnosing Drug-Resistant Tuberculosis, Including on Smear-Negative Specimens, in a High-Volume Laboratory

Pillay, S., de Vos, M., Derendinger, B., Streicher, E., Dolby, T., Scott, L.A., Steinhobel, A.D., Warren, R.M. and Theron, G., 2022. Non-actionable results, accuracy and effect of the first-and second-line line probe assays for diagnosing drug resistant tuberculosis, including on smear-negative specimens, in a high-volume laboratory. *Clinical Infectious Diseases*.
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Key findings:

Pauci-bacillary specimens contributes to a high number of invalid results in MTBDR*sl*. Xpert detects RR more accurately than MTBDR*plus* than MTBDR*sl*. ~25% of Xpert RR specimens are INH-susceptible. With the current diagnostic algorithm followed in programmatic settings, time to results is quicker, the number of patients receiving second-line results prior to treatment has improved, and the reliance on an additional specimen for culture is reduced compared to previous algorithm used.

Candidate's role:

Assisted in conception and design of study; clinical data collection, performing and running of all tests molecular and phenotypic for study; collection of all genolyse DNA and culture isolates for sequencing; data interpretation, data analysis and preparation of manuscript.

Clinical Infectious Diseases

MAJOR ARTICLE

Non-actionable Results, Accuracy, and Effect of First- and Second-line Line Probe Assays for Diagnosing Drug-Resistant Tuberculosis, Including on Smear-Negative Specimens, in a High-Volume Laboratory

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Background. Rapid tuberculosis (TB) drug susceptibility testing (DST) is crucial. Genotype MTBDR*sl* is a widely deployed World Health Organization (WHO)–endorsed assay. Programmatic performance data, including non-actionable results from smear-negative sputum, are scarce.

Methods. Sputa from Xpert MTB/RIF individuals (n = 951) were routinely-tested using Genotype MTBDR*plus* and MTBDR*sl* (both version 2). Phenotypic DST was the second-line drug reference standard. Discrepant results underwent Sanger sequencing.

Findings. 89% (849 of 951) of individuals were culture-positive (56%, 476 of 849 smear-negative). MTBDR*plus* had at least 1 nonactionable result (control and/or TB-detection bands absent or invalid, precluding resistance reporting) in 19% (92 of 476) of smear-negatives; for MTBDR*sl*, 40% (171 of 427) were nonactionable (28%, 120 of 427 false-negative TB; 17%, 51 of 427 indeterminate). In smear-negatives, MTBDR*sl* sensitivity for fluoroquinolones was 84% (95% confidence interval, 67%–93), 81% (54%–95%) for second-line injectable drugs, and 57% (28%–82%) for both. Specificities were 93% (89%–98%), 88% (81%–93%), and 97% (91%–99%), respectively. Twenty-three percent (172 of 746) of Xpert rifampicin-resistant specimens were MTBDR*plus* isoniazid-susceptible. Days-to-second-line-susceptibility reporting with the programmatic advent of MTBDR*sl* improved (6 [5–7] vs 37 [35–46]; $P < .001$).

Conclusions. MTBDR*sl* did not generate a result in 4 of 10 smear-negatives, resulting in substantial missed resistance. However, if MTBDR*sl* generates an actionable result, that is accurate in ruling-in resistance. Isoniazid DST remains crucial. This study provides real-world, direct, second-line susceptibility testing performance data on non-actionable results (that, if unaccounted for, cause an overestimation of test utility), accuracy, and care cascade impact.

Keywords. Genotype MTBDR*plus*; Genotype MTBDR*sl*; smear-negative; TB; resistance.

Drug-resistant tuberculosis (DR-TB) is a leading cause of death. Globally, there were half a million rifampicin-resistant (RR) TB cases in 2019; 78% were estimated to be multidrug-resistant (MDR) [1]. Only 59% of RR-MDR individuals started on treatment in 2018 were treated successfully [2], partly due to the underdiagnosis of resistance to drugs other than rifampicin (RIF) such as isoniazid and the fluoroquinolones (FQs) [3, 4].

The Genotype MTBDR*plus* (Hain Lifesciences, Germany) and MTBDR*sl* (Hain Lifesciences, Germany) molecular line probe assays (LPAs) are globally used for rapid DR-TB detection. Both are World Health Organization (WHO)–endorsed and commercially available [5]. According to the Western Cape Province Department of Health TB guidelines [6], MTBDR*plus* is done after Xpert MTB/RIF (Xpert) to check for Xpert-detected false-positive rifampicin resistance and confirm MDR [7]. MTBDR*sl* is subsequently done to detect second-line resistance. One underappreciated yet important component of these workflows is that, even when an individual is confirmed as TB-positive using Xpert, the downstream reflex test must itself successfully amplify *Mycobacterium tuberculosis* complex (*Mtb*) DNA (LPAs *Mtb* detection is reported as TUB-band positivity). This applies to many reflex technologies and not just LPAs, including new drug susceptibility tests (DSTs) such as Xpert MTB/XDR [8, 9], which have yet to be available at scale.

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As frontline TB test performance improves, it can outstrip reflex tests' ability to detect TB and do DST (eg, Xpert MTB/RIF is almost always done before the LPAs, despite LPAs being an older technology) [10]. Both MTBDR*plus* and MTBDR*sl* can generate nonactionable results (indeterminate or invalid results) that are critical to report in order to quantify the overall number of drug-resistant cases missed (ie, not just due to imperfect sensitivity for resistance but also due to a failure of the test to detect TB). Such performance data that includes nonactionable results are scarce and a major limitation of the current literature. Despite increased demand for DST due to new oral regimens for RR-MDR TB (with the possibility of new FQ-based first-line regimens), MTBDR*sl* is 1 of only 2 WHO-endorsed rapid tests that can be used to confirm eligibility for these regimens.

The WHO recommends that MTBDR*plus* be used on smear-positive sputum (direct testing) and on culture isolates (indirect testing) for smear-negatives [11]. In contrast, MTBDR*sl* version 2 is recommended for direct smear-negative testing; however, evidence is of "low certainty" [5, 12], and meta-analyses have had insufficient data to create summary point estimates [13–16]. This uncertainty in performance is one reason why LPA uptake for the direct testing is suboptimal. In a global survey of 32 LPA-using laboratories, 66% and 50% tested smear-negative specimens with MTBDR*plus* and MTBDR*sl*, respectively [17], despite the positive WHO recommendation. Critically, more data are therefore needed.

Our overarching aim was to evaluate MTBDR*plus* (version 2) and MTBDR*sl* (version 2) performance, including in smear-negative specimens, and describe the nonactionable result rate. Importantly, we did this in a programmatic context that relies on affordable existing diagnostic tools to help guide therapeutic decisions. This approach enabled us to evaluate the association between the expansion of direct second-line DST and time to treatment and compare this to the period prior to the advent of direct second-line DST. Our intention was to provide data for laboratories and clinicians diagnosing and treating drug-resistant TB in resource-constrained settings where programmatic laboratory decisions and policies related to rapid diagnostic testing follow WHO guidance.

METHODS

Study Design

This study was performed in a programmatic context following the TB diagnostic algorithm in the Western Cape, South Africa (Figure 1). Direct testing was performed initially using MTBDR*plus* and MTBDR*sl* on sputum consecutively tested with no study-specific criteria between 1 June 2016 and 30 September 2019. MTBDR*plus* was performed on specimens of all smear status, defined below as the "after period." All valid results were reported

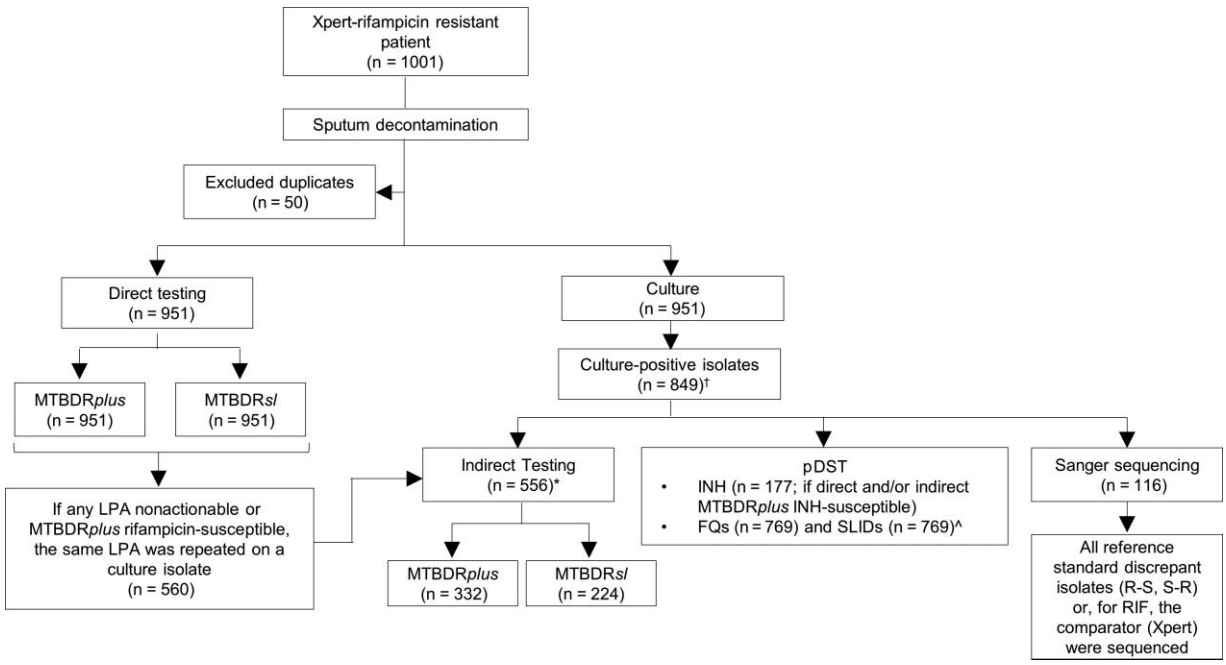

Figure 1. Testing flow diagram showing direct and indirect testing using MTBDR*plus* and MTBDR*sl* and the use of reference standard phenotypic testing for second-line drugs, irrespective of the LPA result. Prior to the study, the flow of tests were the same except MTBDR*sl* was not used and MTBDR*plus* was only done directly if the specimen was smear-positive. *4 direct nonactionables were culture-negative and unable to be tested indirectly. †102 Xpert-positives were not culture-positive and hence did not have an isolate available. ^80 isolates were contaminated upon regrowth for FQ and SLID pDST. Abbreviations: FQ, fluoroquinolones; INH, isoniazid; LPA, line probe assay; MDR, multidrug-resistant; pDST, phenotypic drug susceptibility testing; R, resistant; RIF, rifampicin; S, susceptible; SLID, second-line injectable drugs; Xpert, Xpert MTB/RIF.

and reflexed for MTBDR*sl* testing. All TUB-band negative, indeterminate for 1 or both drugs were reported as invalid (MTBDR*plus*/MTBDR*sl*); rifampicin-susceptible results were reported as discrepant and reflexed for indirect testing using a confirmed culture-positive isolate. All culture isolate results, except Sanger sequencing, formed part of indirect diagnostic workflows including Genotype MTBDR*plus,* MTBDR*sl*, and phenotypic drug susceptibility testing (pDST), and all valid results were reported immediately. Phenotypic DST was done on specimens with valid direct and indirect LPA results. All discrepant results for MTBDR*plus*/MTBDR*sl* with reference standard pDST were resolved with repeat testing on the cultured isolate. For discrepancies that remained even after repeat testing, sequencing was performed (Figure 1).

Sputum Collection and Preparation

In the Western Cape Province, 2 sputum samples were collected upfront for screening of presumptive TB per local guidelines [6]. Sputum processing and testing was done at the National Health Laboratory Service Green Point reference laboratory in Cape Town, South Africa. Pretreatment individuals who were first tested using Xpert MTB/RIF (version 4.3; Xpert) formed part of the then standard-of-care algorithm [18]. A paired sputum specimen from Xpert–RR individuals (n = 1001) was decontaminated using *n*-acetyl-L-cysteine-sodium hydroxide (final concentration, 1%) and the sediment resuspended in 2 mL phosphate buffer [19]. Auramine microscopy was performed. From decontaminated sputum, 0.5 mL was inoculated into a mycobacteria growth indicator tube (MGIT; Becton Dickinson) and incubated in a BACTEC MGIT960 instrument for ≤35 days (our programmatic standard of care due to space limitations).

DNA Extraction and Line Probe Assay Testing

DNA extracted per manufacturer's guidelines [20, 21] from resuspended sputum sediments was tested directly with MTBDR*plus* and MTBDR*sl* (version 2 of both) in parallel by a single operator irrespective of smear status. The GT blot (Hain Lifesciences) and Genoscan software (GS-001, Hain Lifesciences) were used to analyze results followed by operator visual confirmation. All invalid tests (direct testing) were repeated as recommended (the repeat result was reported in analyses). For specimens (direct testing) that were TB-negative per LPAs (ie, TUB-band negative), indeterminate for at least 1 locus, or with an LPA DST result discrepant with pDST, the corresponding isolate was tested using the same LPA (indirect testing). A total of 332 and 224 isolates were tested using MTBDR*plus* and MTBDR*sl*, respectively. The manufacturer-recommended 2.2°C/s ramp rate [17, 22] and ISO15189 standards were used. Results were interpreted per Supplementary Table 1.

TB and Phenotypic Drug Susceptibility Testing Reference Standards

MGIT960 culture positivity with MTBDR*plus* TUB-positivity was used for the detection of TB. Rifampicin pDST was not done. pDST was done programmatically for isoniazid, FQs, and second-line injectable drugs. Per the algorithm, only MTBDR*plus* RR, isoniazid-susceptible isolates received isoniazid pDST to ensure resistance was not excluded (we are hence unable to calculate MTBDR*plus*'s sensitivity, specificity, and positive predictive value (PPV) for isoniazid resistance). If direct MTBDR*plus* was nonactionable or isoniazid susceptible, indirect MTBDR*plus* testing was done and, only based on this result, was isoniazid pDST done (hence, only the negative predictive value (NPV) of indirect MTBDR*plus* for resistance was calculable). See the Supplementary Methods for more information.

Discrepant Analysis

Sanger sequencing was used as the composite reference standard to resolve discrepancies involving LPAs, pDST, and Xpert RR and MTBDR*plus* rifampicin-susceptible specimens (Supplementary Methods, Supplementary Table 6).

Implementation and Effect of Programmatic MTBDR*plus* and MTBDR*sl* Testing

We compared the diagnostic care cascade in the "before algorithm" (2 January 2012–30 December 2015) vs the "after algorithm" (1 June 2016- 30 September 2019) periods. In the before algorithm period, programmatic DST for isoniazid, FQs, and amikacin was done phenotypically. MTBDR*plus* (includes v1) was done routinely for both rifampicin and isoniazid directly in smear-positives or on culture isolates. In the after algorithm period, MTBDR*plus* and MTBDR*sl* (both version 2) were implemented programmatically and reported for potential patient management (see the Supplementary Methods for more detail on these periods).

Statistical Analyses

GraphPad Prism (version 6; GraphPad Software) and Stata (version 14.0; StataCorp; 2 sample proportion test and McNemar test) were used. *P* values ≤.05 were significant.

Ethics

This study was done in accordance with relevant guidelines and regulations approved by the Health Research Ethics Committee of Stellenbosch University and the Western Cape Province Department of Health. Permission was granted to access anonymized residual specimens collected as part of routine diagnostic practice, and informed consent waived.

RESULTS

Cohort Characteristics

Of 1001 Xpert RR sputa, 95% (951) were from unique patients, 89% (849) were confirmed culture-positive (93 were culture-negative and 10 culture-contaminated), and 81% (769) had a usable second-line pDST result (8%; 80 contaminated; Figure 1). Most individuals were male with smear-negative TB (Supplementary Table 2). In individuals with a known

human immunodeficiency virus (HIV) status, 50% (203 of 404) were living with HIV. Those living with HIV were more likely to be sputum smear-negative than those not living with HIV (59%, 120 of 203 vs 48%, 110 of 230; $P = .018$).

Smear Microscopy, Culture, and Phenotypic DST Results

Among the culture-positives, 44% (373 of 849) and 56% (476 of 849) were sputum smear-positive and smear-negative, respectively. Using MTBDR*plus*, 21% (177 of 849) and 60% (509 of 849) were classified as rifampicin-monoresistant and MDR (Figure 2). Using MTBDR*sl*, 5% (42 of 769), 1% (11 of 769), and 2% (19 of 769) were FQ-resistant, second- line injectable drug (SLID)-resistant, or both FQ- and SLID-resistant, respectively (Figure 3).

MTBDR*plus*

Nonactionables

Three percent (11 of 373) and 19% (92 of 476) of sputum smear-positives and smear-negatives had nonactionable results, respectively; of those, 70% (521 of 746) were phenotypically isoniazid resistant (Figure 2). Of the sputum smear-negative nonactionables, 18% (88 of 476) were due to a false-negative TB result and 1% (4 of 476) were due to an indeterminate call (Figure 2). Nonactionable results from indirect testing are provided in Supplementary Figure 1. No MTBDR*plus* invalid results occurred.

MTB

The sensitivity of MTBDR*plus* was 97% (363 of 373) and 82% (388 of 476; $P < .001$) for sputum smear-positive and smear-negative TB, respectively (Table 1).

Rifampicin

Ninety-one percent (686 of 746) of the Xpert RR patients whose direct MTBDR*plus* was actionable were MTBDR*plus* RR (24% [177 of 746] had MTBDR*plus*-defined rifampicin monoresistance). In a head-to-head comparison of direct MTBDR*plus* and Xpert actionable results, 8% (60 of 746) were Xpert-resistant MTBDR*plus*-susceptible, with most discrepants in smear-negative TB rather than in smear-positive TB (Figure 2). Overall, of the discrepants successfully sequenced (9 culture-contaminated, 3 nonamplifiable), 85% (22 of 26) resolved in favor of Xpert (Table 2). Indirect MTBDR*plus* results are provided in Supplementary Figure 1.

Isoniazid

Sixty-eight percent (509 of 746) of Xpert RR patients whose direct MTBDR*plus* was actionable had, per MTBDR*plus*, MDR and 2% (12 of 746) isoniazid monoresistance (the remainder were rifampicin-monoresistant). A total of 328 received indirect MTBDR*plus* testing, and 53% (177 of 328) were MTBDR*plus* RR, isoniazid-susceptible (Supplementary Figure 1). There were 17% (30 of 177) that were phenotypically resistant. We could only calculate MTBDR*plus*'s NPV for isoniazid resistance when done indirectly, which was 83% (147 of 177). When discrepant isoniazid results (indirect MTBDR*plus*-susceptible, pDST-resistant, n = 30) were analyzed, 80% (24 of 30) had usable sequences. Seventy-nine percent (19 of 24), all of which were sequencing wild-type, resolved in favor of MTBDR*plus* (Table 2), resulting in NPV increasing to 97% (166 of 171).

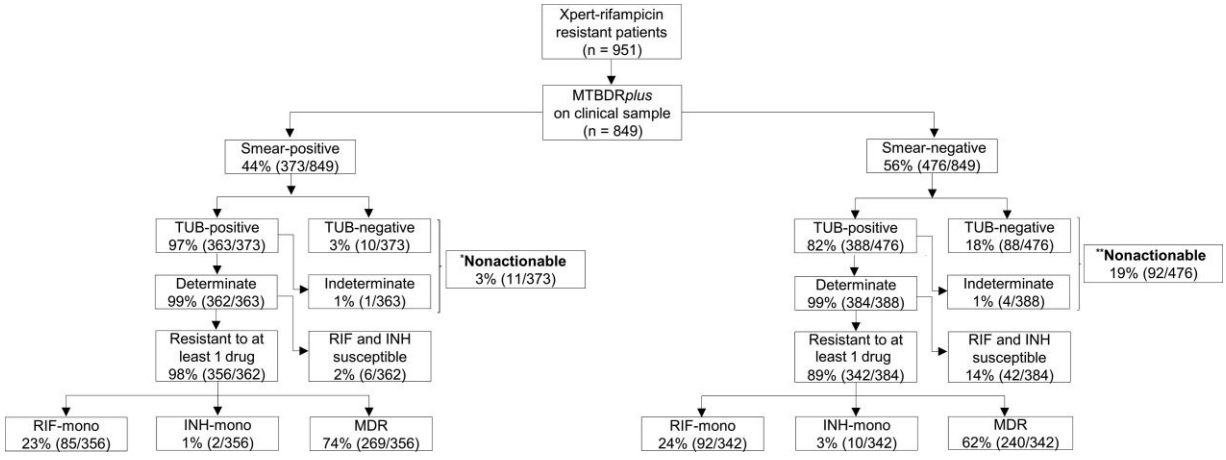

Figure 2. Direct MTBDR*plus* testing of sputum is successful in almost all smear-positives and most smear-negatives; however, it fails to generate a susceptibility result in a significant minority of smear-negatives (1 in 5), indicating that a failure to detect tuberculosis is the primary cause of drug resistance being missed (ie, nonactionable results). Furthermore, a significant minority of Xpert RIF-resistant patients do not have MDR per MTBDR*plus*, suggesting a continued role for isoniazid drug susceptibility testing. Importantly, in patients with actionable MTBDR*plus* results, sensitivity and specificity for resistance did not differ by smear status. Resistance classifications on the bottom 2 rows of boxes are per direct MTBDR*plus*. Of the 951 Xpert rifampicin-resistant patients, only 849 were confirmed culture-positive. *Indirect smear-positive MTBDR*plus* results: MDR (n = 7), RIF-mono (n = 0), INH-mono (n = 1), fully susceptible (n = 3), and nonactionable (n = 0). **Indirect smear-negative MTBDR*plus* results: MDR (n = 69), RIF-mono (n = 0), INH-mono (n = 3), fully susceptible (n = 20), and nonactionable (n = 0). Abbreviations: INH, isoniazid; mono, monoresistant; MDR, multidrug-resistant; RIF, rifampicin; TUB, TUB-band; Xpert, Xpert MTB/RIF.

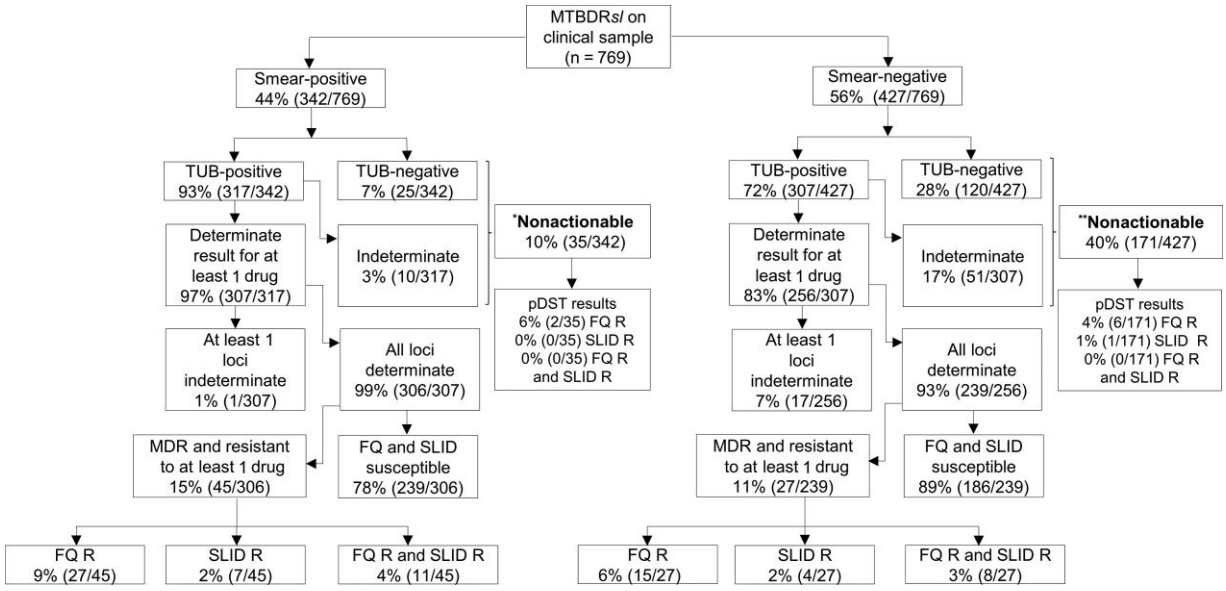

Figure 3. Although direct MTBDR*sl* testing of sputum is successful in most patients, it results in relatively high proportions of nonactionable results in smear-positives and especially in smear-negatives. MTBDR*sl* failed in 4 of 10 smear-negative patients with Xpert-diagnosed rifampicin resistance. As seen for MTBDR*plus*, a failure to generate an actionable result on smear-negatives was the primarily cause of missed resistance (as opposed to a false-negative susceptible result). Resistance classifications on the bottom 2 rows of boxes are per direct MTBDR*sl*. Of the 849 culture-positive patients, only 769 had usable pDST (80-contaminated). *Indirect smear-positive MTBDR*sl* results: FQ-R (n = 3), SLID-R (n = 0), FQ-R and SLID-R (n = 0), fully susceptible (n = 33), and nonactionable (n = 0). **Indirect smear-negative MTBDR*sl* results: FQ-R (n = 7), SLID-R (n = 4), FQ-R and SLID-R (n = 2), fully susceptible (n = 175), and nonactionable (n = 0). Abbreviations: FQ, fluoroquinolones; MDR, multidrug-resistant; pDST, phenotypic drug susceptibility testing; R, resistant; SLID, second-line injectable drug; TUB, TUB-band.

MTBDR*sl*

Nonactionable

When done directly, 10% (35 of 342) of sputum smear-positives and 40% (171 of 427) of smear-negatives were nonactionable (Figure 3). In addition, 4% (8 of 206), 0% (1 of 206), and 0% (0 of 206) of nonactionables were phenotypically resistant to FQs, SLIDs, or both FQs and SLIDs, respectively. Like MTBDR*plus* on sputum smear-negatives, most MTBDR*sl* smear-negative results were nonactionable due to a false-negative TB result (28%, 120 of 427) or an indeterminate result (17%, 51 of 427; Figure 3). A total of 28 MTBDR*sl* results were initially invalid prior to pDST (1%, 2 of 373 for sputum smear-positives vs 5%, 26 of 476 for sputum smear-negatives; $P < .001$; Supplementary Table 3), but all resolved upon retesting (and were hence ultimately not nonactionable). No indirect nonactionable results occurred (Supplementary Figure 2).

MTB

Sensitivity was 93% (347 of 373) and 73% (349 of 476; $P < .001$) for sputum smear-positive and smear-negative specimens, respectively (Table 1), and less than MTBDR*plus* in the same individuals (97%; 95% confidence interval [CI], 94%–98% vs 93%; 95% CI, 90%–95%; $P < .001$) for sputum smear-positives and (82%; 95% CI, 77%–84% vs 73%; 95% CI, 69%–77%; $P < .001$) for smear-negatives.

Fluoroquinolones

For direct sputum smear-positive and smear-negative testing, sensitivities were 89% (40 of 45) and 84% (31 of 37; $P = .105$) and specificities were 92% (180 of 195) and 93% (117 of 126; $P = .855$), respectively (Table 1, Figure 4). For indirect testing, sensitivity was 92% (12 of 13) and specificity was 100% (211 of 211; Supplementary Table 4). When discrepant FQ results from direct testing were analyzed (MTBDR*sl*-resistant pDST-susceptible, n = 24; MTBDR*sl*-susceptible pDST-resistant, n = 11), 83% (29 of 35) generated usable sequences. Sixty-nine percent (20 of 29) of discrepancies favored MTBDR*sl* and 31% (9 of 29) favored pDST (Table 3). MTBDR*sl* falsely reported 2 specimens with *gyrA* S95T natural polymorphisms [24] as resistant through the absence of a wild-type band (WT3, MUT3C). After following discrepant analysis reclassification, sensitivities and specificities increased (Figure 4, Supplementary Table 5).

Second-Line Injectable Drugs

For direct testing in sputum smear-positives and smear-negatives, sensitivities were 86% (19 of 22) and 81% (13 of 16; $P = .011$), respectively, and specificities were 97% (205 of 212) and 88% (112 of 127; $P = .002$), respectively (Table 1, Figure 3). For indirect testing, sensitivity was 100% (6 of 6) and specificity was 100% (218 of 218; Supplementary Table 4, Figure 4). When direct MTBDR*sl*-pDST discrepant results (MTBDR*sl*-resistant

Table 1. Accuracy of Direct MTBDR*plus* and MTBDR*sl* Testing for Tuberculosis and Phenotypic Second-line Drug Resistance in Sputum of Xpert-Positive Rifampicin-Resistant Patients

		Overall		Smear-Positive		Smear-Negative	
Assay		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
MTBDR*plus*	Tuberculosis	88 (751/849) 86–90	43 (40/93) 32–53	97 (363/373) 94–98	36 (4/11) 10–69	82 (388/476) 77–84 [a]***P* < .001**	44 (36/82) 32–54 [a]*P* = .635
MTBDR*sl*	Tuberculosis	82 (696/849) 79–84 [b]***P* < .001**	51 (47/93) 32–54 [b]*P* = .303	93 (347/373) 90–95 [b]*P* = .006	73 (8/11) 39–93 [b]*P* = .086	73 (349/476) 69–77 [a]***P* < .001** [b]*P* = .002	48 (39/82) 36–58 [a]*P* = .117 [b]***P* < .001**
	Fluoroquinolones	87 (71/82) 77–93	93 (297/321) 90–96	89 (40/45) 75–96	92 (180/195) 88–96	84 (31/37) 67–93 [a]*P* = .105	93 (117/126) 89–98 [a]*P* = .855
	Second-line injectable drugs	84 (32/38) 68–93 [c]*P* = .720	94 (317/339) 90–95 [c]*P* = .820	86 (19/22) 65–97 [c]*P* = .001	97 (205/212) 93–98 [c]*P* = .108	81 (13/16) 54–95 [a]*P* = .011 [c]*P* = .821	88 (112/127) 81–93 [a]*P* = .002 [c]*P* = .052
	Fluoroquinolone and second-line injectable drugs	70 (19/27) 69–98	97 (257/264) 94–98	85 (11/13) 54–98	97(165/169) 94–99	57 (8/14) 28–82 [a]*P* = .118	97 (92/95) 91–99 [a]*P* = .701

Data are % (n/N), 95% confidence interval. All *P* values which were statistically significant appeared in bold.

[a]Within-row comparisons between smear statuses.

[b]Within-column comparisons for MTBDR*sl* vs MTBDR*plus*.

[c]Within-column comparisons for second-line injectable drugs vs fluoroquinolones.

pDST-susceptible, n = 22 and MTBDR*sl*-susceptible pDST-resistant, n = 6) were analyzed, 43% (12 of 28) had sequenceable isolate DNA. In contrast to FQs, most discrepancies (67%, 8 of 12) resolved in favor of pDST (Table 3, Supplementary Table 7). Following reclassification, sensitivity and specificity increased (Figure 4, Supplementary Table 5).

Table 2. Sequencing of MTBDR*plus* Targets (*rpoB, katG, inhA* Promoter Region) Done to Resolve Discrepant Results Either Between MTBDR*plus* and Xpert (Rifampicin) or MTBDR*plus* and Phenotype (Isoniazid)

				Sequencing				
	Locus	MTBDR*plus*	Comparator Result	Mutation	No. of Isolates	No. With Heteroresistance	Susceptibility Result	Resolved in Favor of Line Probe Assay or Comparator
Rifampicin	*rpoB*[a] (n = 29)	S	R	S531L	8	1	R	Xpert
		…	…	H526Y	2	0	R	Xpert
		…	…	D516V	3	1	R	Xpert
		…	…	Q513P	1	0	R	Xpert
		…	…	L511P[b]	8 (1 Double mutant with D485N)	1	R	Xpert
		…	…	WT	4	0	S	MTBDR*plus*
	…	…	…	NR	3	…	…	…
	Discrepant resolution by sequencing			85% (22/26) resistant (resolved in favor of Xpert) 15% (4/26) susceptible (resolved in favor of MTBDR*plus*)				
Isoniazid	*katG*[c] (n = 24)	S	R	G312C	1	…	R	pDST
	…	…	…	S315T	3	…	R	pDST
	…	…	…	WT	19	…	S	MTBDR*plus*
		…				NR 1		
	inhA promoter[c] (n = 24)	S	R	–8 T/C WT	1 23	…	R, S	pDST MTBDR*plus*
…	Discrepant resolution by sequencing			21% (5/24) resistant (resolved in favor of pDST) 79% (19/24) susceptible (resolved in favor of MTBDR*plus*)				

Sequencing suggested Xpert is more sensitive for rifampicin resistance than MTBDR*plus*. MTBDR*plus* detected mutations known to cause isoniazid resistance better than pDST. See Supplementary Methods for how line probe assay results were categorized as discrepant.

Abbreviations: NR, not reportable (did not amplify for sequencing); pDST, phenotypic drug susceptibility testing; R, resistant; S, susceptible; WT, wild type; Xpert, Xpert MTB/RIF.

[a]Only Xpert rifampicin-resistant and MTBDR*plus* rifampicin-susceptible discrepant sputa were sequenced from the isolate.

[b]L511P is considered borderline by the World Health Organization, which recommends that people found with this mutation be classified as resistant [23].

[c]Discrepant isolates sequenced included only MTBDR*plus*-susceptible that were phenotypic-resistant (due to contemporaneous programmatic algorithm).

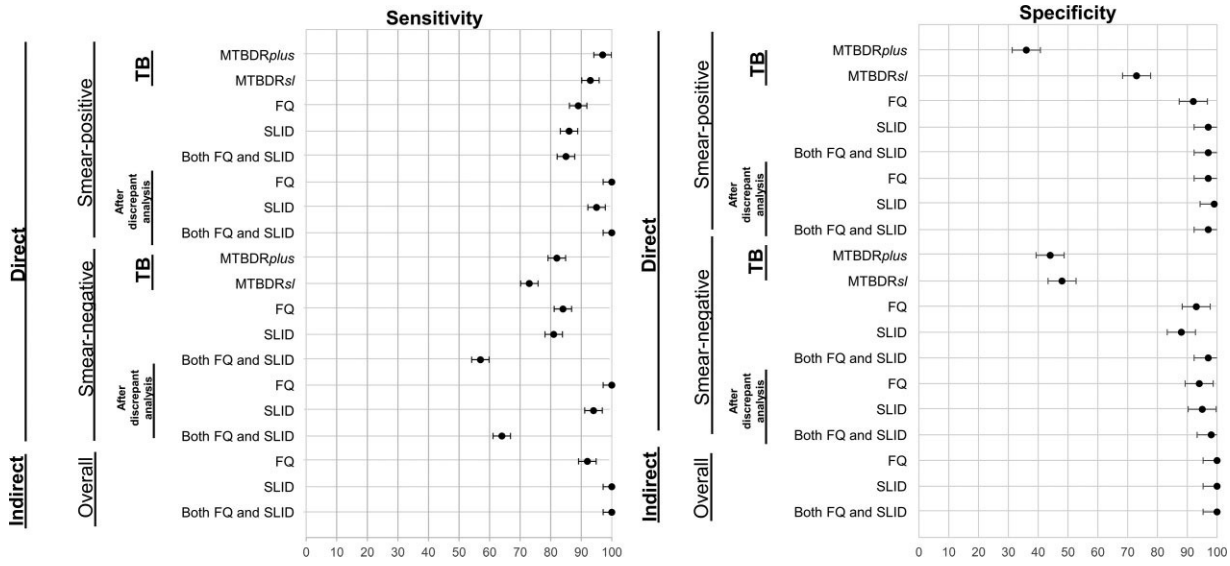

Figure 4. Selected summary forest plots showing sensitivity and specificity estimates (with 95% confidence intervals) for MTBDR*plus* and MTBDR*sl*. Importantly, only patients first detected as TB positive (top 2 rows) can generate an actionable line probe assay drug susceptibility testing result. Estimates for smear-negatives were lower than for smear-positives and, overall, estimates for SLIDs were lower than for FQs. All estimates improved in favor of LPAs after discrepant resolution. Abbreviations: FQ, fluoroquinolones; SLID, second-line injectable drugs; TB, tuberculosis.

Table 3. Sequencing of MTBDR*sl* Targets (*gyrA, rrs*) to Resolve Results Discrepant With Phenotypic Drug Susceptibility Testing

				Sequencing			
	Locus	MTBDR*sl*	pDST	Mutation	No. of Isolates	Susceptibility Result	Resolved in Favor of Line Probe Assay or pDST
Fluoroquinolones	***gyrA*** (n = 11)	S	R	G81C[a]	1	S	MTBDR*sl*
	…	…	…	A88T	1	R	pDST
	…	…	…	WT	9	S	MTBDR*sl*
	(n = 24)	R	S	A88T	1	R	MTBDR*sl*
		…	…	C86T	1	R	MTBDR*sl*
		…	…	D89N	1	R	MTBDR*sl*
		…	…	A90V	4	R	MTBDR*sl*
		…	…	S91P	1	R	MTBDR*sl*
		…	…	D94G	2	R	MTBDR*sl*
		…	…	S95T[b]	2	S	pDST
		…	…	WT	6	S	pDST
		…	…	NR	6	…	…
	Discrepant resolution by sequencing				69% (20/29) in favor of MTBDR*sl* 31% (9/29) in favor of pDST		
Second-line injectable drugs	*rrs* (n = 6)	S	R	WT	3	…	S MTBDR*sl*
	…	…	…	NR	3	…	… …
	(n = 22)	R	S	WT	8	…	S pDST
	…	…	…	A1401G	1	…	R MTBDR*sl*
	…	…	…	NR	13	…	… …
	…	…	…	…	…	…	… …
	Discrepant resolution by sequencing				33% (4/12) in favor of MTBDR*sl* 67% (8/12) in favor of pDST		

Most fluoroquinolone discrepants resolved in favor of MTBDR*sl*, whereas most second-line injectable drug discrepants resolved in favor of pDST.

Abbreviations: NR, number of specimens that did not amplify for sequencing; pDST, phenotypic drug susceptibility testing; R, resistant; S, susceptible; WT, wild-type.

[a]G81C, silent mutation.

[b]S95T, does not cause resistance [23, 24].

Table 4. Comparison of Key Care Cascade Gaps for the Diagnosis of Drug Resistance Before and After the Implementation of Improved Molecular Diagnostics for Resistance Beyond Rifampicin

…	Retrospective Period MTBDR*plus* Only on Smear-Positives Second-line DST by pDST Only			Prospective Period MTBDR*plus* and MTBDR*sl* Irrespective of Smear Status Second-line pDST Still Done		
	Overall (n = 2938)	Smear-Positive (n = 1674)	Smear-Negative (n = 1264)	Overall (n = 799)	Smear-positive (n = 416)	Smear-negative (n = 383)
On treatment without receiving any second-line DST	23 (668/2938)	21 (357/1674)	25 (311/1264) [a]*P* = .358	5 (40/799) [b]***P* < .001**	2 (7/416) [b]***P* < .001**	9 (33/383) [b]***P* < .001** [b]***P* < .001**
MTBDR*plus* direct testing	N/A	100 (1674/1674)	N/A	100 (799/799)	100 (416/416)	100 (383/383)
With an actionable result	N/A	79 (1317/1674)	N/A	99 (797/799)	100 (416/416)	99 (381/383) [a]*P* = .140
Without an actionable result	N/A	21 (357/1674)	N/A	0 (2/799)	0 (0/416)	1 (2/383)
MTBDR*sl* direct testing	N/A	N/A	N/A	100 (799/799)	100 (416/416)	100 (383/383)
With an actionable result	N/A	N/A	N/A	78 (622/799)	91 (380/416)	63 (242/383) [a]***P* < .001**
Without an actionable result	N/A	N/A	N/A	22 (177/799)	9 (36/416)	37 (141/383) [a]***P* < .001**
Days to result (actionable or nonactionable)	N/A	N/A	N/A	6 (5–7)	6 (5–7)	6 (5–7) [a]***P* < .001**
MTBDR*sl* indirect testing	N/A	N/A	N/A	22 (177/177)	9 (36/36)	37 (141/141)
With an actionable result	N/A	N/A	N/A	22 (177/177)	9 (36/36)	37 (141/141)
Without an actionable result	N/A	N/A	N/A	0	0	0
Days to result (actionable or nonactionable)	N/A	N/A	N/A	22 (16–26)	16 (13–22)	22 (18–27) [a]*P* = .081
pDST	77 (2270/2938)	79 (1317/1674)	75 (953/1264) [a]*P* = .358	94 (750/799) [b]***P* < .001**	96 (400/416) [b]***P* < .001**	91 (350/383) [a]*P* = .500 [b]***P* < .001**
Days to result (interquartile range)	37 (35–46)	33 (29–38)	42 (36–50) [a]***P* < .001**	30 (27–36) [b]***P* < .001**	28 (25–35) [b]***P* < .001**	34 (30–40) [a]***P* < .001** [b]***P* < .001**
Overall, second-line DST	…	…	…	…	…	…
Patients who required second-line DST on isolates (indirect MTBDR*sl* or pDST) when direct MTBDR*sl* was nonactionable	0	0	0	22 (177/799)	9 (36/416)	37 (141/383) [a]***P* < .001**
Days to first actionable second-line DST result (direct MTBDR*sl*, indirect MTBDR*sl*, or pDST)	37 (35–46)	33 (29–38)	42 (36–50) [a]***P* < .001**	6 (5–7)	6 (5–7)	6 (5–7) [a]***P* < .001**

Implementation of first-line MTBDR*plus* testing on Xpert rifampicin-resistant sputum to include smear-negatives and MTBDR*sl* testing on all sputum resulted in a greater proportion of patients receiving second-line DST, reduced reliance on culture, and reduced turnaround time. The Supplementary Methods section contains more information on these periods. Data are median (interquartile range) or % (n/N). All *P* values which were statistically significant appeared in bold.

Abbreviations: DST, drug susceptibility testing; N/A, nonapplicable; pDST, phenotypic drug susceptibility testing.

[a]Comparisons within rows and between columns by same smear status.

[b]Comparisons within rows in retrospective vs prospective periods.

Joint FQ and SLID Resistance

For sputum smear-positives and smear-negatives, direct sensitivities were 85% (11 of 13) and 57% (8 of 14; $P = .118$), respectively, and specificities were 97% (165 of 169) and 97% (92 of 95; $P = .701$), respectively (Table 1, Figure 3). Indirect testing sensitivity and specificity were very high (Supplementary Table 4, Figure 4). Like that observed for the individual drug classes, after discrepancy resolution, MTBDR*sl* sensitivity and specificity increased (Supplementary Table 5).

Diagnosis Care Cascade Gaps in Before and After Periods

We compared programmatic data from the period immediately preceding the study (before period when MTBDR*plus* was the only LPA done directly, only on sputum smear-positives, and the only second-line testing was pDST) to a similar period after the start of study testing (after period; both LPAs were done, at a minimum, directly and reported for routine patient management). With MTBDR*sl* implementation, the proportion of individuals on treatment without second-line DST results decreased from 23% (668 of 2938) to 5% (40 of 799; $P < .001$; Table 4), and second-line DST results were available more quickly (33 [29–38] to 16 [13–22] days for smear-positives and 42 [36–50] to 22 [18–27] days for smear-negatives), even after factoring in many smear-negatives with direct nonactionable results that required subculture for further testing compared with smear-positives (37%, 143 of 383 vs 9%, 36 of 416; $P < .001$; Table 4).

DISCUSSION

There are limited data on nonactionable results, accuracy, and effect of rapid molecular assays for the diagnosis of resistance

beyond rifampicin, especially on smear-negative sputum. To address this, we performed a large-scale evaluation of the newest-generation LPAs in a routine programmatic setting, did comprehensive reference standard testing, and compared care cascade data before and after. Definitive data on MTBDR*sl*'s performance on smear-negative specimens is essential as the need for FQ susceptibility testing increases and new tools such as Xpert MTB/XDR remain expensive (cost per cartridge $19.80, at least $3860 to upgrade existing modules [25]).

Our key findings include that 19% and 40% of smear-negative individuals tested by MTBDR*plus* and MTBDR*sl* were nonactionable, respectively, resulting in many individuals with resistance missed; about 25% of Xpert RR patients have MTBDR*plus*-defined isoniazid susceptibility; and deployment of direct LPA testing was associated with improvements in days to diagnosis, more individuals receiving DST, and reduced culture reliance.

MTBDR*sl* had almost double the nonactionable result rate of MTBDR*plus* in smear-negatives for TB detection, causing diagnostic and treatment delays. Our data highlight the suboptimal ability of reflex DSTs to detect TB even in individuals already identified as TB-positive by frontline tests. This information loss will persist as the limit of detection of new frontline tests outstrips that of reflex tests (Xpert MTB/RIF Ultra vs Xpert MTB/XDR). We recommend that all studies that evaluate reflex test report this key metric (nonactionable results).

In Xpert RR specimens that were MTBDR*plus* rifampicin-susceptible, Xpert was correct more frequently than MTBDR*plus* [26, 27]. Possible reasons include heteroresistance and variants not included in MTBDR*plus*. These findings question diagnostic algorithms that use MTBDR*plus* to confirm Xpert-detected rifampicin resistance [7, 27, 28].

Importantly, MTBDR*plus* has value for isoniazid-susceptibility detection. Our data suggest that isoniazid is likely effective in 25% of Xpert RR individuals. In agreement with that observed in the Democratic Republic of the Congo [29] and Iran [30], we recommend that RR TB not be automatically assumed to be MDR and all Xpert RR individuals receive isoniazid DST (which should always be done as isoniazid resistance prevalence is globally in excess of that of rifampicin [31].

Fluoroquinolones are key components of new regimens, and SLIDs such as amikacin remain important. Although important new tools such as Xpert MTB/XDR are emerging [32], MTBDR*sl* is already established in many laboratories worldwide. The sensitivity and specificity for FQ on smear-negatives were 84% and 93%, respectively. High MTBDR*sl* sensitivity (81%) was observed on smear-negatives for SLID; however, specificity was less (88%); both improved after discrepant analysis. Importantly, in contrast to FQs, most SLID MTBDR*sl*-pDST discrepant results resolved in favor of pDST-confirmed susceptibility.

In the after period, we found significant improvements in the proportion of people who had any second-line DST results (such individuals are thus more likely to start effective treatment) and time to result. Such real-world data regarding the programmatic impact of TB diagnostics are scarce but important. With the scale-up of second-line LPAs, individual with smear-negative TB still suffered from unacceptably long times to diagnosis. This subset of individuals should be targeted for interventions in order to accelerate treatment initiation, such as new expensive assays such as Xpert MTB/XDR or Deeplex Myc-TB (Genoscreen) [8, 33].

A strength and limitation of our study is the programmatic context of the study, permitting it to be large and the results reported for potential patient management within the South African care cascade. However, this meant that the study was constrained by contemporary diagnostic algorithms, which affected specimen and meta-data availability given the suboptimal quality of care common in high-volume resource-scarce settings.

Time to DST results associated with LPA scale-up may vary across other provinces within South Africa as, unlike in the Western Cape, only 1 specimen is collected initially for presumptive TB and a second sputum specimen is dependent on an individual returning to a clinic (this may affect generalizability). We were unable to do pDST for rifampicin and isoniazid; however, our primary objective was to evaluate LPA performance for second-line drugs. We also did targeted sequencing rather than whole-genome sequencing, and discrepant analyses may have missed rare noncanonical variants; however, WHO-recommended second-line pDST was done in all isolates [34].

LPA use in our programmatic laboratory was associated with improvements in the care cascade, and patient-important outcomes remained suboptimal. Until next-generation reflex DSTs are widely available, expanded LPA testing remains key to the successful scale-up of new regimens, despite important paucibacillary specimen performance caveats.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. S. P., M. dV., G. T., and R.W. conceptualized the experiments. T. D., S. P., B. D., and R.V. assisted with data curation. S.P. performed formal analysis and methodology and wrote the original draft. A. D. S., L. A. S., and E. S. assisted S.P. with the investigation. All authors reviewed and edited the manuscript.

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Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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Chapter 3

To test or not? Xpert MTB/RIF as an alternative to smear microscopy to guide line probe assay testing for drug-resistant tuberculosis

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Key findings:

Using Xpert generated routine data we identified a rule-in cut-off threshold for C_{Tmin}, semi-quantitation category and smear grade. We provided an ideal framework for preventing unnecessary downstream testing which would result in invalid LPA results. We have shown that LPAs can be broadened to encompass more patients who are smear-negative. Xpert semiquantitation category is preferable to informing reflex LPA testing rather than smear status.

Candidate's role:

Assisted in conception and design of study; clinical data collection, performing and running of all molecular and phenotypic tests for study; data interpretation, data analysis and preparation of manuscript.

To test or not? Xpert MTB/RIF as an alternative to smear microscopy to guide line probe assay testing for drug-resistant tuberculosis

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Abstract

Background: Xpert MTB/RIF (Xpert) revolutionised tuberculosis (TB) diagnosis, however, laboratory decision making on whether widely-used reflex drug susceptibility assays (MTBDR*plus*, MTBDR*sl*) are done on specimens is often based on smear microscopy status. Existing Xpert quantitative information may be useful.

Method: We performed receiver operator characteristic (ROC) curve analyses using sputum bacterial load measures [smear microscopy grade, Xpert semi-quantitation category and minimum cycle threshold (C_{Tmin}) values] for the classification of "likely non-actionable" (not resistant or susceptible) line probe assays results, which may reduce wasteful testing. We evaluated the ratio of actionable-to-non-actionable results and pay-offs with LPA-detectable missed isoniazid and fluoroquinolone resistance.

Findings: Smear-negatives were more likely than smear-positives to generate a non-actionable MTBDR*plus* [23% (133/559) vs. 4% (15/381)] or MTBDR*sl* [39% (220/559) vs. 12% (47/381)] result, however, excluding smear-negatives would result in missed rapid resistance diagnoses [e.g., only 51% (273/537) of LPA-diagnosable isoniazid resistance detected if smear-negatives omitted]. Within smear-negatives, testing ≥"medium" specimens had a high ratio of actionable-to-non-actionable results (12.8 or a 4-fold improvement vs. test all for MTBDR*plus,* 4.5 or 3-fold improvement for MTBDR*sl*), which would capture 64% (168/264) and 77% (34/44) of LPA-detectable resistance. If C_{Tmin} were used, greater resolution and higher ratios offset against resistance detection decrements are obtained.

Conclusion: Routinely-generated Xpert quantitative information is associated with the ratio of actionable-to-non-actionable LPA results and permits identification of smear-negatives specimens in whom this ratio may prove acceptably high to laboratories, enabling an expansion of direct DST. Surrogates better than smear should be used for guiding downstream reflex DST.

Words: 250/250

Introduction

Reflex drug susceptibility testing (DST) should be done immediately in all rifampicin-resistant tuberculosis (TB) cases to enable rapid effective treatment. Achieving this depends on testing specimens, including those that are smear-negative, directly. However, the widely-used World Health Organization (WHO)-endorsed line probe assays (LPAs) MTBDR*plus* and especially MTBDR*sl* (both Bruker, Germany) perform sub-optimally on paucibacillary specimens (1); often failing to generate an actionable (resistance or susceptible) result. Culture is hence often required to generate material for testing; however, culture is costly and slow.

Non-actionable results for the latest iterations of MTBDR*plus* and MTBDR*sl* occur in ~24 and ~40% of Xpert MTB/RIF (Xpert)-positive smear-negative patients, and can be a bigger cause of missed resistance than diminished sensitivity (2). Laboratories can avoid doing LPAs completely on smear-negative specimens, however, this reduces rapid diagnosis (associated downsides including delays, care cascade loss, and difficulty confirming eligibility for new regimens) or, if LPAs are done on smear-negatives, wasteful expenditure (consumables and labour to do MTBDR*sl* cost ~$50 per assay (3)), care cascade loss (a request for an additional specimen, which is often unsuccessfully fulfilled, is typically only triggered once the LPA is known to be non-actionable), and reduced user confidence can all result. Therefore, despite the WHO recommendation that MTBDR*sl* be done on smear-negative specimens, direct LPA testing is often, even in well-resourced settings, in reality limited to smear-positive specimens (4, 5). This undermines LPAs' potential impact, which remain the only widely-deployed molecular DSTs for first- and second-line resistance. LPAs may work well on some smear-negatives; however, as smear microscopy is a crude and insensitive categorical measure of bacterial load, laboratories are unable to identify this subset upfront prior to LPA testing (6).

We hypothesised that, in situations where Xpert is a frontline TB test, its quantitative information could be used to exclude *a priori* certain specimens from unnecessary LPA testing; thereby permitting LPAs to be applied more efficiently (i.e., on specimens with a reduced non-actionable result risk) and, if not already done in smear-negative specimens, LPAs could potentially expanded to include some.

In other words, pre-existing quantitative information routinely generated by Xpert could be used to improve LPA-based laboratory decision making and the drug-resistant TB care cascade. We also evaluate if smear grade would be more useful than smear status (positive or negative) as Xpert is not universally available.

Methods

Microbiology

We analysed Auramine smear microscopy, Xpert MTB/RIF (v4.3), MTBDR*plus* and MTBDR*sl* (both v2) results from 951 patients programmatically-diagnosed with Xpert rifampicin-resistant TB from 01/06/2016-30/09/2019 at a high-volume laboratory in a previously-described cohort (7). All patients had sputum tested directly with both LPAs irrespective of smear status.

Analyses

We did receiver operator characteristic (ROC) curve analyses (GraphPad v6, USA) using different sputum bacterial load measures to classify if MTBDR*plus* or MTBDR*sl* were non-actionable (not resistant or susceptible; defined as when bands corresponding to the amplification control or TB detection are absent or, if both present, ≥1 drug class locus control band was absent). Smear microscopy grade (defined per (8)), Xpert semi-quantitation category and minimum cycle threshold (C_{Tmin}) values (rounded to nearest integer) were analysed, and sensitivity and specificity (95% binomial confidence intervals) for the detection of non-actionable results evaluated. We identified thresholds corresponding to Youden's index (9), rule-out (≥95% sensitivity; almost all non-actionables correctly identified) and rule-in (≥95% specificity; almost all actionables correctly identified) scenarios; expecting rule-in to be most appropriate because it would not incorrectly exclude patients from the benefits of rapid LPA testing. We calculated, at each threshold, how many actionable results are generated before a non-actionable is encountered (ratio of actionable-to-non-actionable results) and how maximising this ratio was offset against missed LPA-based isoniazid and fluoroquinolone diagnoses. All analyses were done overall and, where possible, after smear status stratification.

Ethics

This study was approved by the Health Research Ethics Committee of Stellenbosch University (N16/04/045) and Western Province Department of Health (2016/RP18/637).

Results

Non-actionable LPAs and missed resistance diagnoses stratified by smear status and grade

Overall non-actionable result rates irrespective of smear status for MTBDR*plus* and MTBDR*sl* were 19% (148/792) and 40% (267/673) (ratios of actionable-to-non-actionable results of 5.4 and 2.5, respectively). Smear-negative specimens were, compared to smear-positives, more likely to generate a non-actionable MTBDR*plus* [23% (133/559) vs. 4% (15/381); p=0.001] or MTBDR*sl* [39% (220/559) vs. 12% (47/381); p<0.001] result (ratios of 3.2 and 24.4 for MTBDR*plus* and 1.5 and 7.1 for MTBDR*sl*, respectively). Non-actionable results, a receiver operating characteristic (ROC) curve of smear grade to detect non-actionable results, and the balance between the number of actionable results per non-actionable result and missed rapid drug resistance diagnoses are in **Figure 1** (positive and negative predictive values in **Supplementary Figure 1**).

MTBDRplus

Using smear-negativity as a threshold to identify non-actionables had a sensitivity and specificity of 90% (133/148) and 54% (426/792), respectively. Most non-actionable results occurred in smear-negatives (**Figure 1A**), but smear grade had suboptimal AUC for predicting non-actionable results (**Figure 1B**). The actionable-to-non-actionable ratio improves as increasing grades are used to exclude specimens (≤that grade) from testing, however, this is offset against missed resistance (**Figure 1C**). For example, to improve this ratio to 21.5 (threshold ≤scanty or, in other words, any smear-positive tested), only 51% (273/537) of LPA-diagnosable isoniazid resistance would be detected (**Supplementary Table 1**).

MTBDRsl

Smear negativity had a sensitivity and specificity of 83% (220/266) and 54% (339/674) for non-actionable results (p=0.049 and p=0.183 vs. MTBDR*plus*, respectively). The ratio of actionable-to-non-actionable MTBDR*sl* results was worse than MTBDR*plus*'s, driven by more frequent non-actionable results in smear-negatives [39% (220/559) vs. 23% (133/559) for MTBDR*plus*, p<0.001]. For example, MTBDR*sl*'s highest ratio was 16 (**Figure 1D**) whereas for MTBDR*plus* it was 109,

representing a ~7-fold improvement. If smear-negative specimens were excluded from MTBDR*sl*, only 58% (60/104) of LPA-diagnosable fluroquinolone resistance would be detected (**Supplementary Table 1**).

Xpert MTB/RIF semi-quantitation category

All patients

MTBDR*plus*: Like smear grade, non-actionable results were more frequent at lower semi-quantitation categories (**Figure 2A**), however, non-actionable results rates in the "very low" and "low" categories were higher than in smear-negative patients [49% (62/126) and 29% (62/210) in each category respectively vs. 23% (133/559; $p<0.001$ and $p=0.103$) in smear-negatives]. Semi-quantitation category had higher AUC than smear grade, yet no semi-quantitation category threshold approached the rule-in criterion (~95% specificity, **Figure 2B**). The largest improvement in the ratio of actionables-to-non-actionables occurred when specimens in the lowest two semi-quantitation categories were excluded (increasing from 5.4 when all tested to 24.2 if ≥medium tested) and this was accompanied by a relatively small reduction in detected resistance (~20%) (**Figure 2C**). In other words, if ≥medium was used, ~5-fold fewer non-actionables would occur and 78% (419/537) of potentially detectable resistance would still be detected.

MTBDR*sl*: Like MTBDR*plus,* MTBDR*sl* non-actionable rates in the "very low" and "low" categories were higher than in smear-negative patients [71% (90/126) and 49% (102/210) in each category respectively vs. 39% (220/559; $p<0.001$ and $p=0.021$)]. Like observed within smear grades, MTBDR*sl* never obtained similar actionable-to-non-actionable result ratios to MTBDR*plus* when specimens with the same semi-quantitation category were compared. Importantly, if ≥medium was used (specimens less than this excluded as "likely non-actionable), this ratio would improve from 2.5 to 7.2 (~3-fold improvement) with 88% (92/104) of potentially detectable resistance still detected (**Figure 2D**).

Smear-negatives

MTBDR*plus*: If laboratories that do not test smear-negative patients wish to partly expand testing, they may test smear-negatives who are ≥medium (ratio 12.8 vs. 3.2 for the test all strategy or 4-fold improvement), which would still capture 64% (168/264) of detectable resistance (**Figure 2E).** Within smear-negatives, 20% (114/559), 33% (182/559), 36% (200/559), and 11% (63/559) were "very low", "low", "medium", and "high", respectively; meaning that, in our setting, 47% (263/559) of smear-negatives would be ≥medium (this rule hence does not apply to a minority of individuals).

MTBDR*sl*: Similarly, if MTBDR*sl* was done on ≥medium smear-negatives, the ratio would improve from 1.5 for the test all strategy to 4.5 (3-fold improvement), with 77% (34/44) of detectable resistance still detected (**Figure 2F).**

By Xpert MTB/RIF C_{Tmin}

All patients

MTBDR*plus*: C_{Tmin} had, compared to semi-quantitation category and smear grade, higher AUC for detecting non-actionable results **(Figure 3A)** and was the only sputum bacillary load readout capable of meeting the rule-in criterion. 11% (86/792) of patients were C_{Tmin} ≥29 and this threshold had 95% (750/792) specificity, meaning 5% (42/792) of actionables would be misclassified as "likely non-actionable" and hence excluded from MTBDR*plus* **(Supplementary Table 1)**. Sensitivity was 30% (44/148), meaning 44 non-actionables would be correctly classified as "likely non-actionable" (non-actionables reduced by a third). NPV was 80% (750/854) meaning that, for every ten patients C_{Tmin} <29 (hence classified as "likely actionable"), eight would indeed be actionable and other two non-actionable (false-negative). PPV was 51% (44/86), meaning approximately half of patients C_{Tmin} ≥29 (hence classified as non-actionable), would indeed be non-actionable and the others actionable (false-positive) **(Supplementary Figure 1)**. Ratios of actionable-to-non-actionable results like those for semiquantiation category were obtained, peaking at approximately 77 (C_{Tmin} 12; estimates less than this C_{Tmin} are imprecise due to few specimens with very low C_{Tmin}s) **(Figure 3B)**. At this threshold where specimens C_{Tmin} ≥29 are excluded as "likely non-actionable", this ratio would be 6.4 (slightly

improved from the test-all ratio of 5.4) and this would come at the cost of missing 5% (25/537) of potentially detectable resistance.

MTBDR*sl:* 19% (129/674) of patients had C_{Tmin} ≥28, which had a rule-in specificity of 95% (638/674), meaning 5% (36/674) of actionables would be misclassified as "likely non-actionable" **(Figure 3A)**. Sensitivity was 34% (90/266); hence 90 non-actionables would be correctly classified as "likely non-actionable", permitting a one third reduction in non-actionables. NPV was like that for MTBDR*plus* **(Supplementary Figure 1)** but PPV higher [71% (90/126; p=0.003 vs. MTBDR*plus*), meaning approximately 7/10 people with C_{Tmin} ≥28 (hence classified as non-actionable), would indeed be non-actionable and the other 3/10 actionable (false-positive). Ratios of actionable-to-non-actionables results by C_{Tmin} peaked at approximately 38 (C_{Tmin} 16), less than half that of MTBDR*plus*. At the threshold where specimens with C_{Tmin} ≥28 are excluded as "likely non-actionable", this ratio would be 7.0 (compared to the test-all ratio of 5.0, 1.4-fold or 40% improvement) and would result in missing only 4% (4/104) of potentially detectable resistance.

Smear-negative patients

MTBDR*plus*: Compared to overall, C_{Tmin} had less AUC in smear-negatives but similar rule-in threshold **(Figure 3D)**. Even at the same C_{Tmin}s, lower actionable-to-non-actionable ratios occurred in smear-negatives **(Figure 3E**; for example, 13.8 vs. 24 overall at C_{Tmin} 20,). If the rule-in threshold of C_{Tmin} <29 was used, this ratio was 4.4 (compared to 3.2 for the test-all smear-negatives strategy, representing a 38% improvement) and resulted in 91% (241/264) of potentially detectable resistance captured. Furthermore, ratios ≥10 were possible, permitting MTBDR*plus* to be expanded to at least some smear-negatives (C_{Tmin} <23; 67% (177/264) of smear-negatives and 67% (177/264) of LPA-detectable resistance was C_{Tmin} <23.

MTBDR*sl*: If the rule-in threshold of C_{Tmin} <29 was used, this ratio was 1.7 (compared to 1.5 for the test-all smear-negatives strategy, a 13% improvement) and resulted in 93% (41/44) of potentially detectable resistance captured. MTBDR*sl* on specimens with C_{Tmin} <19 would have a ratio of 5.4 **(Figure 3F)**, which may be more acceptable in settings where smear-negative testing is not routinely

done. This ratio was more than the test-all strategy (3.6-fold improvement) and use of ≥medium semi-quantitation category (ratio of 4.5). 36% (119/332) of smear-negatives were C_{Tmin} <19, corresponding to 45% (24/44) of detectable resistance. Predictive values of this approach in smear-negatives, including for MTBDR*plus*, are in **Supplementary Figure 1.**

Discussion

LPAs are WHO-recommended first- and second-line rapid DSTs, however, they are not always done directly on specimens in which they may provide an actionable resistant or susceptible result, in part due to elevated non-actionable result risk in smear-negatives; depriving patients of early diagnoses. Although better DSTs, especially for second-line resistance, are doubtlessly required, the use of existing, widely-available test technologies should be optimised.

Our key findings are 1) testing C_{Tmin} <29 smear-negative specimens with MTBDR*plus* would reduce non-actionable rates by a third yet permit >90% of LPA-detectable isoniazid resistance to still be detected, 2) if C_{Tmin} <23 were used, more than ten MTBDR*plus* actionable results would occur before a non-actionable result occurs in smear-negatives (compared to three actionable per non-actionable ordinarily) and two-thirds of resistance still detected, 3) for MTBDR*sl* testing of smear-negatives, which usually results in 1.5 actionables per non-actionable result, more than five actionables per non-actionable are attainable, permitting 45% of detectable fluroquinolone resistance to be detected, and 4) in settings where C_{Tmin}s are unavailable, Xpert semiquantation category (≥medium, which would expand LPA testing to almost half of smear-negatives) has utility. These data provide a framework with example thresholds of how testing of smear-negatives using the LPAs can be made more efficient (more actionables per non-actionable), which may enable the expansion of LPA testing to such specimens where there previously was none. This has implications for minimising care cascade gaps.

Although our findings permit using Xpert to rationally expand the use of existing LPAs to certain paucibacillary specimens that may be ordinarily excluded, we affirm that where resources allow isoniazid and fluroquinolones DST should always be attempted directly on any TB-positive rifampicin-resistant specimen irrespective of smear microscopy status (10). Hence, our findings will primarily be interest to settings where direct MTBDR*plus* or MTBDR*sl* testing of smear-negatives is not always done, despite guidance to the contrary for MTBDR*sl* (1, 11). The precise threshold used will depend on locally acceptable ratios of actionable-to-non-actionable results versus the proportion of potentially detectable isoniazid or fluoroquinolone resistance laboratories are comfortable

243 excluding from the potential benefits of direct LPA testing (such individuals would still get DST done
244 on cultured bacilli using phenotypic or genotypic methods).

245 Our findings also demonstrate that, where WHO-recommended rapid molecular diagnostic tests are
246 available, smear microscopy, which comes at additional expense and is less accurate at informing
247 when "likely actionable" LPA testing should occur, is increasingly redundant for guiding downstream
248 laboratory decision making given the range of Xpert C_{Tmin}s (and to a lesser extent semiquantitation
249 categories) within smear-negatives. We therefore suggest PCR test quantitative readouts are used
250 where not all TB-positive specimens undergo automatically reflex DST (importantly, this includes
251 MTBDR*plus* for isoniazid resistance, given the prevalence of rifampicin mono-resistant TB (2, 12)).

252 This method serve as a framework for reflex DSTs other than the LPAs, such as Xpert MTB/XDR
253 (13) and FluoroType MTBDR (14) and others (15). Future work should include Ultra (as opposed to
254 Xpert). Furthermore, the principle of applying molecular (as opposed to visual) quantitative
255 information to determine downstream DST algorithms is agnostic to the frontline test. In other words,
256 our general approach could be applied to other frontline TB tests (16, 17) including the Truenat assays
257 (18). Importantly, such frontline tests are increasingly targeting multicopy genes that genotypic DSTs
258 do not include, resulting in large limit of detection differences. Thus, knowing which TB-positive
259 specimens may proceed onward to downstream DST with high actionable result likelihood is a need
260 that will persist.

261 A strength and limitation is that of our study is from a programmatic context, which permitted large
262 sample size, however, the exact thresholds used may require validation in other settings or
263 laboratories. Furthermore, balancing high non-actionable result rates and missed rapid resistance
264 diagnoses is hugely complex, affected by diverse laboratory, clinical, and health provider. Our study
265 was therefore intended to demonstrate proof-of-concept and illustrate what, purely from a laboratory
266 perspective, such payoffs may look like. It is important different settings choose thresholds that suit
267 them, and we have now provided information that may aid in such decision making.

268 In summary, we have demonstrated how LPAs may be expanded to include individuals who represent
269 a significant proportion of smear-negative patients. Xpert C_{Tmin}s or, failing that, Xpert

semiquantitation category is superior to informing reflex LPA testing than smear status, and the utility of molecular quantitative information generated already as part of the TB diagnostic process for informing other reflex tests requires consideration.

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Author Contributions

SP, MdV, GT, and RW conceived experiments. TD and SP provided specimens and data. SP conducted experiments and analysed data. All authors reviewed the manuscript and provided critical input.

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Figure legends

Figure 1. Smear grade's association with non-actionable LPA results, its ability to discriminate "likely non-actionable" from "likely actionable" results (if ≤ each grade) and pay-offs between the ratio of actionable-to-non-actionable results with the overall proportion of LPA-detected resistance. (A) Non-actionable results were more frequent at lower than higher grades and more so for MTBDR*sl* than MTBDR*plus*. In-column percentages reflect the proportion patients with a non-actionable result. **(B)** Smear grade had moderate AUCs for identifying "likely non-actionable" results (dashed lines 95% CIs) but no grade approached 95% specificity. **(C)** and **(D)** show the ratio of actionable-to-non-actionable results (solid lines, left y-axes) and how this improves as specimens with a certain smear grade (or greater) are tested by MTBDR*plus* or MTBDR*sl* respectively.
Abbreviations: AUCs-area under curve, CI-confidence intervals, FQ^R-fluroquinolone resistance, INH^R-isoniazid resistance, LPA-line probe assay, P-positive, SC-scanty, Xpert-Xpert MTB/RIF.

Figure 2. Xpert semi-quantitation category, non-actionable LPA results, and associated pay-offs with missed resistance as specimens ≤ specific semi-quantitation categories are excluded due to being flagged as "likely non-actionable". (A) Trends for semi-quantitation category mirrored those for smear grade. **(B)** This translated into excellent AUCs for discriminating "likely non-actionable results" (dashed lines 95% CIs) but no optimal rule-in threshold was identifiable. **(C)** and **(D)** shows the ratio of actionable-to-non-actionable results (solid lines, left y-axes) and how this improves as specimens with higher semi-quantitation categories are tested by MTBDR*plus* or MTBDR*sl*, respectively. **(E)** and **(F)** are limited to smear-negative specimens. Abbreviations: AUCs-area under curve, CI-confidence intervals, FQ^R-fluoroquinolone resistance, H-high, INH^R-isoniazid resistance, LPA-line probe assay, L-low, M-medium, NPV-negative predictive value, P-positive, PPV-positive predictive value, VL-very low, Xpert-Xpert MTB/RIF.

Figure 3. Xpert C_{Tmin}'s ability to discriminate "likely non-actionable" from "likely actionable" LPA results. (A) A ROC curve for all specimens showing AUCs (dashed lines 95% CIs, rule-in thresholds shown) and, in **(B)** and **(C)**, pay-offs between the ratios of actionable-to-non-actionable results and missed resistance for MTBDR*plus* and MTBDR*sl*. **(D-E)** are the same but restricted to smear-negative patients. Ratios were highest at low C_{Tmin} and slowly decreased as LPA testing was expanded to include samples with higher C_{Tmin}s, which had the upside of increasing detected resistance. AUCs and these ratios were less for smear-negative vs. all patients. Above C_{Tmin} x-axes are Xpert semiquantitation categories. Abbreviations: AUC-area under curve, CI-confidence intervals, C_{Tmin}-cycle threshold (minimum), FQs-fluoroquinolones, INH-isoniazid, LPA-line probe assay, NPV-negative predictive value, P-positive, PPV-positive predictive value, ROC-receiver operator characteristic, Xpert-Xpert MTB/RIF.

Figure 1.

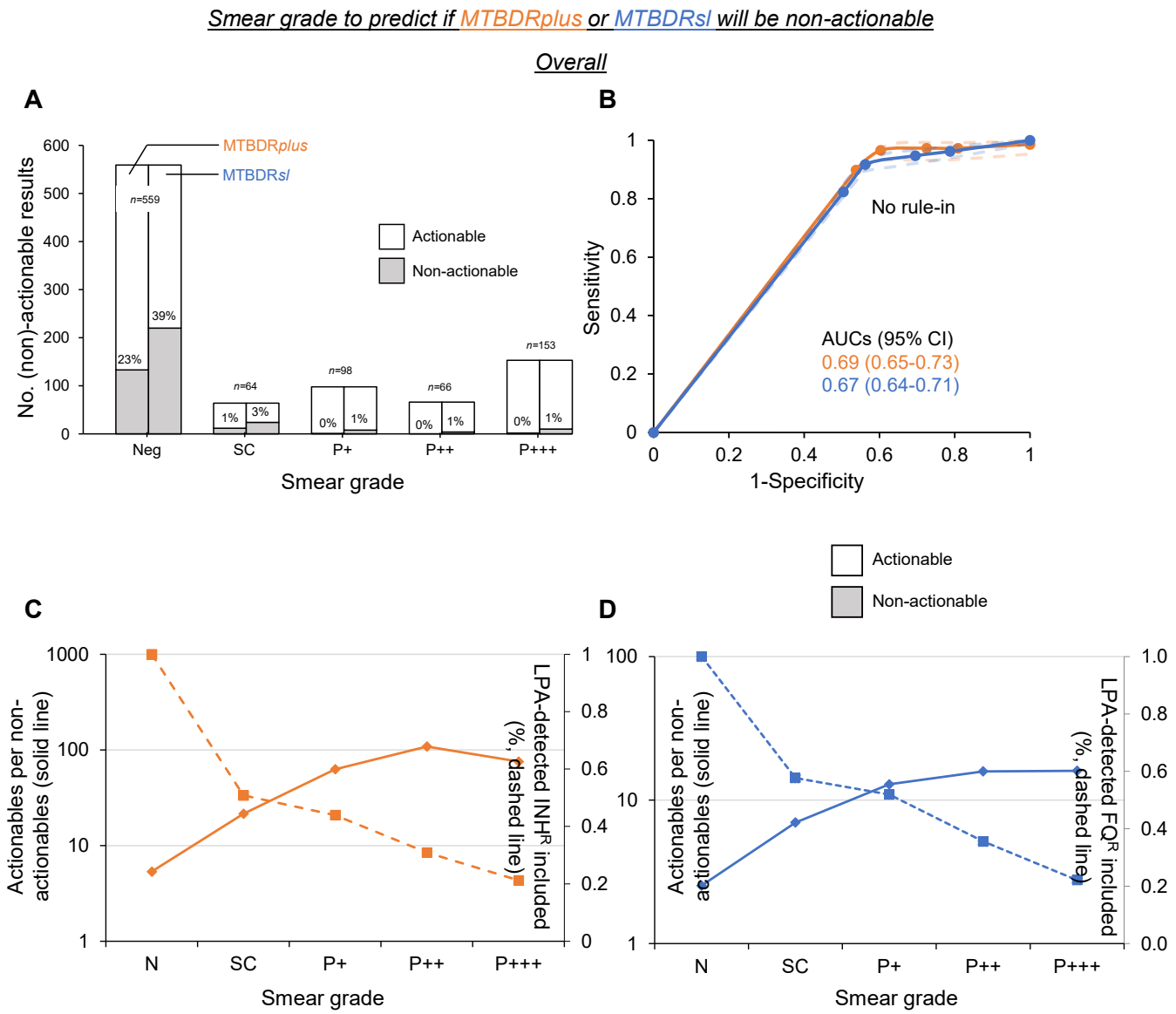


Figure 2.

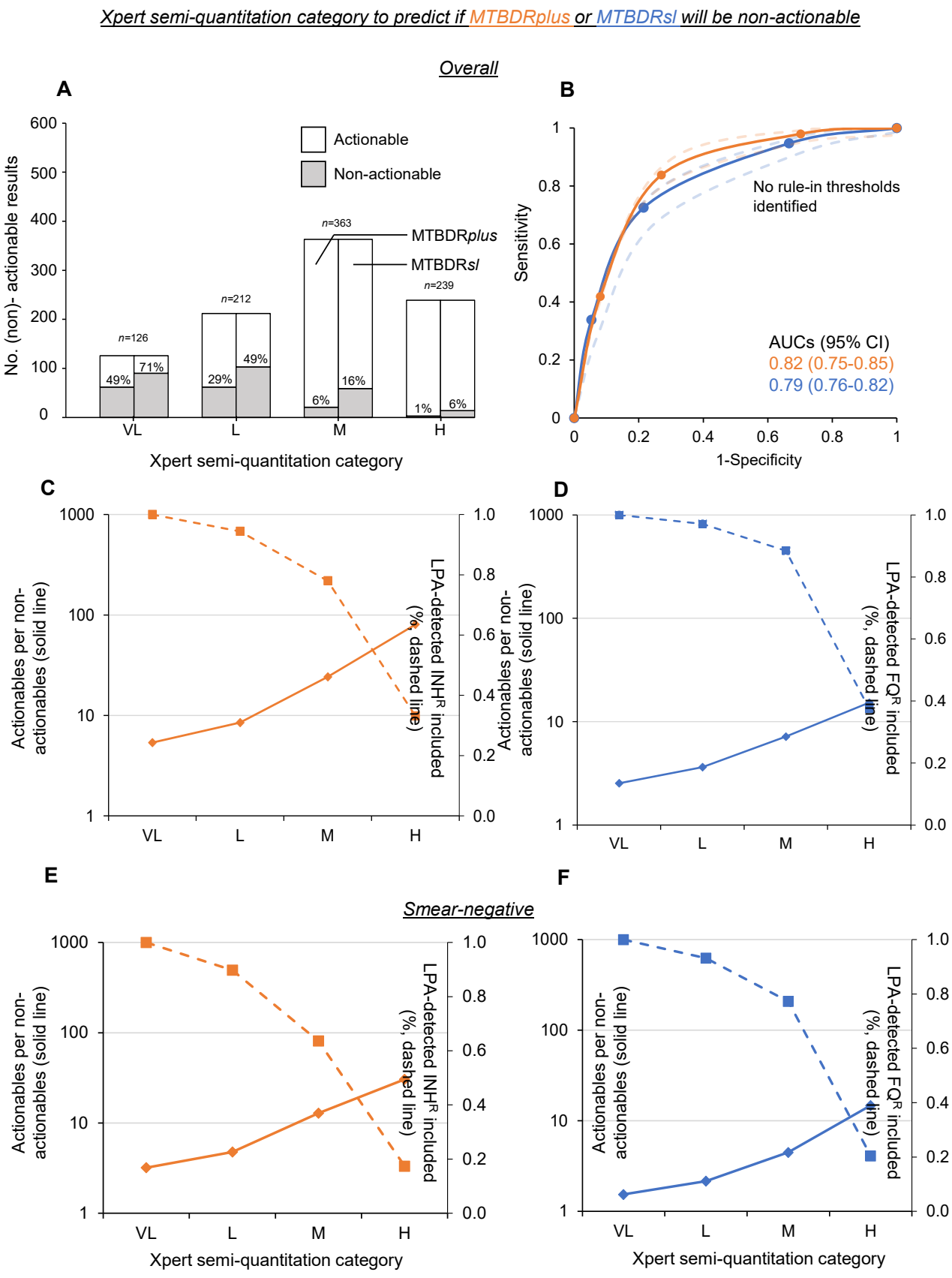
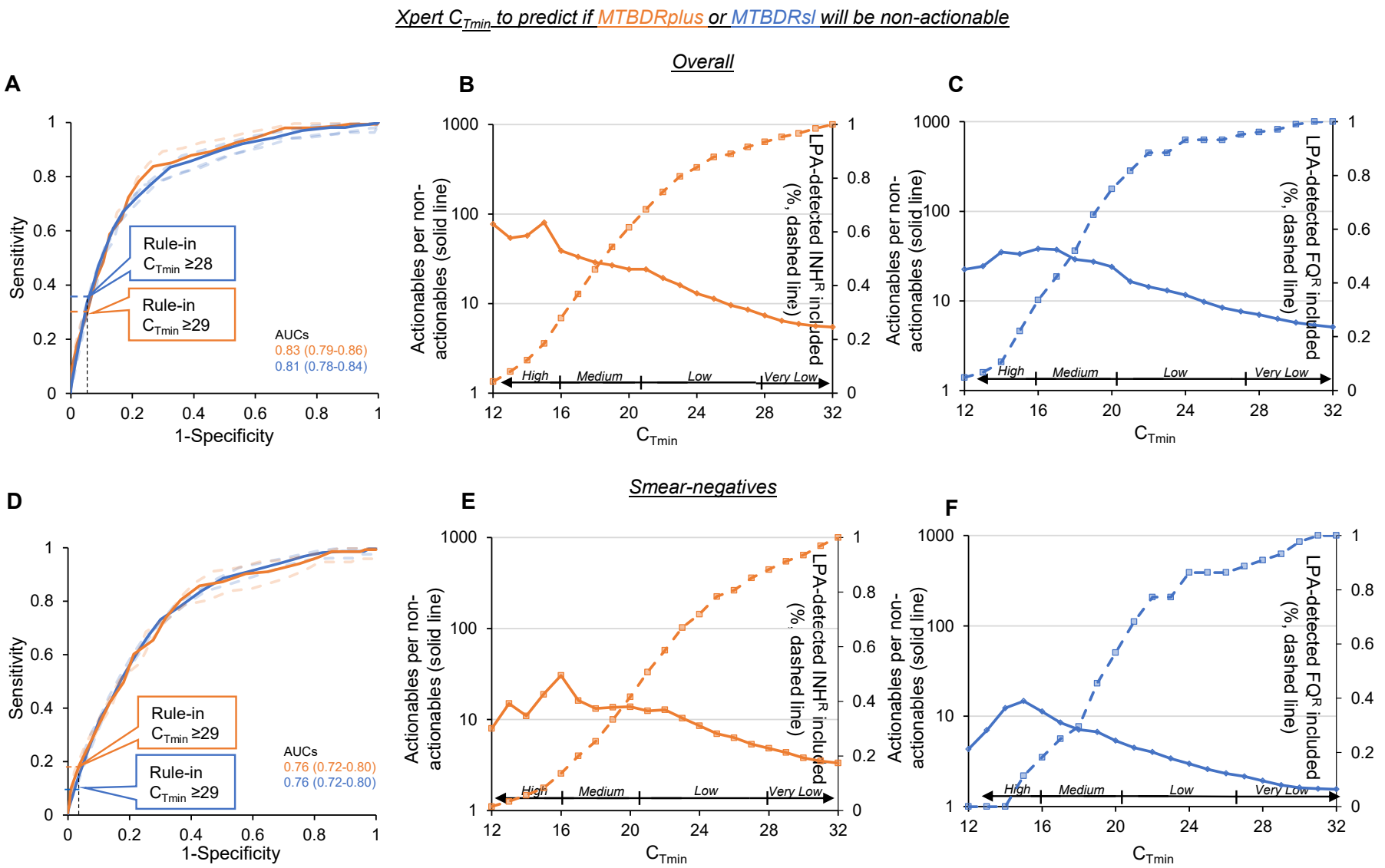


Figure 3



Chapter 4

Xpert MTB/XDR for detection of pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin

Pillay, S., Davies, G.R., Chaplin, M., De Vos, M., Warren, R., Steingart, K.R. and Theron, G., 2021. Web Annex 4.15. Low complexity automated NAATs: Diagnostic accuracy for detection of resistance to isoniazid and second-line anti-TB agents. A systematic review. *WHO consolidated guidelines on tuberculosis. Module 3: diagnosis–rapid diagnostics for tuberculosis detection*, p.432. Available at: https://www.ncbi.nlm.nih.gov/books/NBK572348/bin/webannex4-et10.pdf

Pillay S, Davies GR, Chaplin M, De Vos M, Schumacher SG, Warren R, Steingart KR, Theron G. Xpert MTB/XDR for detection of pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin. Cochrane Database of Systematic Reviews. 2021(6). https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD014841/full

Pillay, S., Steingart, K.R., Davies, G.R., Chaplin, M., De Vos, M., Schumacher, S.G., Warren, R. and Theron, G., 2022. Xpert MTB/XDR for detection of pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin. *Cochrane Database of Systematic Reviews*, (5). doi: 10.1002/14651858.CD014841

Publication status: Web annexure 4.15, protocol and systematic review published.

Key findings:

Findings in the review showed high accuracy estimates for the detection of FQ and INH resistance. Detection of resistance for ETH was suboptimal. The number of non-actionable results were low in the study.

Candidate's role:

Assisted in writing of protocol, screening of studies, capturing of data, selection of studies and data analysis, interpretation of results and preparation of manuscript.

Web Annex 4.15. Low complexity automated NAATs: Diagnostic accuracy for detection of resistance to isoniazid and second-line anti-TB agents. A systematic review

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EXECUTIVE SUMMARY

Xpert® MTB/XDR Assay (Xpert MTB/XDR, Cepheid, Sunnyvale, USA) detects MTBC (Mycobacterium tuberculosis complex) DNA and genomic mutations associated with resistance to isoniazid, fluoroquinolones, ethionamide, and second-line injectable drugs (amikacin, kanamycin, capreomycin) in a single cartridge. Xpert MTB/XDR is intended for use as a reflex test in clinical specimens (unprocessed sputum or concentrated sputum sediments) already determined to be MTBC-positive. The test is included in a class of diagnostic technologies that are cartridge-based and of low complexity.

The proposed role for the test is to be used as an initial test for resistance to isoniazid and second-line drugs (replacement for line probe assays and pDST as initial tests). Favorable characteristics of Xpert MTB/XDR include rapidity (less than 90 minutes for a result), ease-of-use (same familiar process as Xpert MTB/RIF and Xpert Ultra), and detection of resistance directly in clinical specimens.

This systematic review summarizes the current literature on the accuracy of Xpert MTB/XDR for detection of resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin as part of a World Health Organization process to develop guidelines for use of the test. This review does not include molecular drug susceptibility testing (DST) for kanamycin and capreomycin because, with the adoption of the new treatment regimens using all-oral medicines, the second-line injectable drugs are less relevant. We include molecular DST for amikacin because, of the second-line injectable drugs, amikacin is preferentially included on longer regimens when susceptibility has been demonstrated and adequate measures to monitor for adverse reactions can be ensured.

To identify studies, we searched multiple databases up to 6 September 2020 without language restriction. Two review authors independently assessed studies for eligibility. Two review authors independently extracted data from the included studies.

We stratified analyses by population, irrespective of rifampicin resistance and with detected rifampicin resistance, and target condition. We combined data using meta-analysis by fitting the bivariate random effects model. We performed all analyses stratified by type of reference standard, phenotypic DST (pDST), genotypic DST (gDST), and composite reference standard. For multicentre studies, we performed meta-analyses at the centre level (i.e. treating each centre as a separate study). We excluded MTBC-negative, MTBC-non-determinate, and inconclusive drug resistant results from analyses of diagnostic test accuracy. We performed sensitivity analyses by repeating the meta-analyses and excluding data from the manufacturer.

We identified three unpublished studies: Cepheid 2020, DIAMA 2020, and FIND 2020. All studies involved adults. One study evaluated archived frozen specimens and two studies evaluated sputum using a cross-sectional, prospective study design. The studies were in Benin, Cameroon, China, New Delhi, Moldova, Mumbai, and South Africa.

We did not identify any studies that assessed the accuracy of Xpert MTB/XDR for drug resistance in children.

As assessed by QUADAS-2, in the patient selection domain two studies were at low risk of bias and one study at unclear risk of bias because the manner of participant selection was not reported. In the reference standard domain, studies had low risk of bias for resistance to isoniazid, fluoroquinolones, and amikacin, and high risk of bias for resistance to ethionamide (for both pDST and gDST).

Xpert MTB/XDR for isoniazid resistance in people with microbiologically confirmed pulmonary tuberculosis irrespective of rifampicin resistance, pDST
Xpert MTB/XDR pooled sensitivity and specificity (95% confidence interval) were 94.2% (89.3 to 97.0) and 98.0% (95.2 to 99.2) (3 studies, 1605 participants, 61.9% with isoniazid resistance; high-certainty evidence for sensitivity and specificity).

Results of these studies indicate that in theory, of 1000 people where 50 have isoniazid resistance, 66 would be Xpert MTB/XDR-positive: of these, 19 (29%) would not have isoniazid resistance (false-positives) and 934 would be Xpert MTB/XDR-negative: of these, 3 (0%) would have isoniazid resistance (false-negatives).

Xpert MTB/XDR for fluoroquinolone resistance in people with microbiologically confirmed pulmonary tuberculosis irrespective of rifampicin resistance, pDST
Xpert MTB/XDR pooled sensitivity and specificity were 93.1% (88.0 to 96.1) and 98.3% (94.5 to 99.5.) (3 studies, 1337 participants, 28.7.% with fluoroquinolone resistance; high-certainty evidence for sensitivity and moderate-certainty evidence for specificity).

Results of these studies indicate that in theory, of 1000 people where 50 have fluoroquinolone resistance, 63 would be Xpert MTB/XDR-positive: of these, 16 (25%) would not have fluoroquinolone resistance (false-positives) and 937 would be Xpert MTB/XDR-negative: of these, 3 (0%) would have fluoroquinolone resistance (false-negatives).

Xpert MTB/XDR for ethionamide resistance in people with microbiologically confirmed pulmonary tuberculosis irrespective of rifampicin resistance, pDST
Xpert MTB/XDR pooled sensitivity and specificity were 56.6% (41.8 to 70.3) and 97.1% (91.9. to 99.0) (2 studies, 838 participants, 52.5% with ethionamide resistance; low-certainty evidence for sensitivity and moderate-certainty evidence for specificity).

Results of these studies indicate that in theory, of 1000 people where 50 have ethionamide resistance, 56 would be Xpert MTB/XDR-positive: of these, 28 (50%) would not have ethionamide resistance (false-positives) and 944 would be Xpert MTB/XDR-negative: of these, 22 (2%) would have ethionamide resistance (false-negatives).

Xpert MTB/XDR for ethionamide resistance in people with microbiologically confirmed pulmonary tuberculosis irrespective of rifampicin resistance, gDST
Xpert MTB/XDR pooled sensitivity and specificity were 96.4% (92.2 to 98.3) and 100.0% (82.5. to 100.0) (2 studies, 1001 participants, 28.0% with ethionamide resistance; moderate-certainty evidence for sensitivity and very low-certainty evidence for specificity).

Xpert MTB/XDR for amikacin resistance in people with microbiologically confirmed pulmonary tuberculosis irrespective of rifampicin resistance, pDST
Xpert MTB/XDR pooled sensitivity and specificity were 89.1% (80.9. to 94.1) and 99.5% (96.9 to 99.9) (2 studies, 1008 participants, 15.0% with amikacin resistance; high-certainty evidence for sensitivity and specificity).

Results of these studies indicate that in theory, of 1000 people where 50 have amikacin resistance, 50 would be Xpert MTB/XDR-positive: of these, 5 (10%) would not have amikacin resistance (false-positives) and 950 would be Xpert MTB/XDR-negative: of these, 5 (1%) would have amikacin resistance (false-negatives).

For each drug, Xpert MTB/XDR pooled sensitivity and specificity estimates were similar in people irrespective of rifampicin resistance and people with detected rifampicin resistance. However, owing to enrolment criteria in the studies, we note that most participants were rifampicin resistant in all analyses.

The sensitivity analyses made little difference to any of the findings.

Authors' conclusions

- For resistance to isoniazid, in people irrespective of rifampicin resistance, Xpert MTB/XDR sensitivity was 94.2% against a reference standard of pDST.
- For resistance to fluoroquinolones, in people irrespective of rifampicin resistance, Xpert MTB/XDR sensitivity was 93.1% against a reference standard of pDST.
- For resistance to ethionamide, in people irrespective of rifampicin resistance, Xpert MTB/XDR sensitivity was 56.6% against a reference standard of pDST.
- For resistance to amikacin, in people irrespective of rifampicin resistance, Xpert MTB/XDR sensitivity was 89.1% against a reference standard of pDST.
- MTB/XDR specificity was > 97.0% in nearly all analyses.

The impact of Xpert MTB/XDR is expected to be affected by several factors, including the health care infrastructure, access to other diagnostic tests, the ability of the index test to detect tuberculosis (which is required for DST), and the prevalence of resistance to a given drug. Given that the test targets a limited number of resistance variants in specific genes, the test may perform differently in different settings. These results should, therefore, be interpreted with caution.

Future studies should assess the accuracy of Xpert MTB/XDR in different population groups, including children and people living with HIV. In addition, studies should assess the accuracy of Xpert MTB/XDR in different geological settings, in smear-negative specimens, and with different types of clinical specimens.

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BACKGROUND

Early recognition and improved characterisation of tuberculosis drug resistance is a prerequisite for the rapid delivery of novel regimens to those who could benefit from them. For MDR/rifampicin-resistant-tuberculosis, the arrival of novel or repurposed drugs such as bedaquiline, clofazimine, and linezolid has revolutionized the efficacy of longer regimens, dispensing with the need for injectable drugs, and promising to deliver shorter all-oral regimens. Fluoroquinolones have an essential role and are also important for protecting second-line drugs like bedaquiline (WHO Consolidated Guidelines (Module 4) 2020).

While the availability of drug susceptibility testing using culture-based and molecular methods is increasing, coverage and availability of these technologies varies widely. For example, globally in 2019, only 59% of bacteriologically confirmed new tuberculosis cases were tested for rifampicin resistance. Among patients with rifampicin resistance, 71% were tested for resistance to fluoroquinolones, though coverage varied from around 35% in the Western Pacific to nearly 90% in Europe (WHO Global tuberculosis report 2020).

The development and scale-up of Xpert MTB/RIF was a major step toward improving tuberculosis and rifampicin resistance detection globally. The assay simultaneously tests for both conditions and offers a mostly automated hands-off solution deployable in many high tuberculosis burden settings. Xpert MTB/RIF has, however, been met with limitations. Of 48 high-burden countries,[5] only 18 countries (38%) reported that a WHO-recommended rapid diagnostic (which includes Xpert MTB/RIF) had been used as the initial test for more than half of their patients with tuberculosis (WHO Global tuberculosis report 2020).

The status quo for isoniazid susceptibility testing is worse. Although in high MDR-TB settings, the presence of rifampicin resistance alone has served as a proxy for MDR-TB and the basis for treatment decisions, emerging data suggest that, in some settings, rifampicin resistance testing has suboptimal specificity for MDR-TB (WHO Global tuberculosis report 2020). This means that testing for resistance to isoniazid (a critical first-line drug) is increasingly important. For instance, a study in the eastern Democratic Republic of the Congo found one in five rifampicin-resistant patients to be isoniazid susceptible when tested using the MTBDR*plus* line probe assay (Bismwa 2020), and the most recent South African National Survey of Drug Resistance found hotspots of rifampicin mono-resistance, where the prevalence ratio of such cases exceeded that of MDR-TB by as much as 30% (NICD 2016). Conversely, isoniazid resistance in the presence of rifampicin susceptibility (isoniazid mono-resistance) is also increasingly recognised as another emerging challenge in managing tuberculosis as it is an important enabler of MDR-TB (Sulis 2020).

Globally in 2019, 13% of new tuberculosis cases and 17% of previously treated tuberculosis cases had isoniazid resistance (WHO Global tuberculosis report 2020), yet isoniazid susceptibility testing is only generally done in patients who are rifampicin resistant. One reason for this is that genetic testing for isoniazid resistance is more complicated than testing for rifampicin resistance owing to a greater variety of resistance-associated variants (including large deletions) across several genes (including loci in *katG*, i*nhA*, and *ahpC*). Information on these mutations may not be routinely available in lower resource settings despite evidence showing that isoniazid resistance is associated with a three-fold increased risk of poor treatment outcomes (Espinal 2000) and hence should be treated with an intensified regimen including a fluoroquinolone (WHO Consolidated Guidelines (Module 4) 2020). Wider implementation of this modified regimen may reduce the risk of treatment failure and emergence of MDR-TB.

Though individualisation of MDR-TB treatment regimens according to susceptibility testing is promoted by guidance, gaps in infrastructure and personnel to support culture-based approaches may in part explain why, of an estimated 465,000 new cases of MDR/rifampicin-resistant-tuberculosis

[5] Forty-eight countries are in one or more of the three lists of high TB, TB/HIV and MDR-TB burden countries.

annually, only 44% were detected and notified (WHO Global tuberculosis report 2020). The WHO recommends that rapid techniques be used as the initial diagnostic tests to detect tuberculosis and rifampicin resistance in order to minimize delays in starting appropriate treatment (WHO Consolidated Guidelines (Module 3) 2020). The multiplexed nature of these new technologies theoretically permits susceptibility to be detected accurately and comprehensively for a single drug (where variants in multiple genes may cause resistance) and to several different drugs, each with their own sets of distinct resistance determinants. The flexibility of this technology offers the possibility of simultaneous detection of high confidence resistance causing mutations important for multiple drugs other than rifampicin.

This systematic review evaluated newly-developed rapid technologies that detect resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin.

A glossary of terms is provided in Appendix 1.

Index test(s)

The index tests are rapid, cartridge-based nucleic acid amplification tests, of low complexity, for detection of resistance to isoniazid and second-line anti-tuberculosis drugs.

We define a *cartridge-based test* as one that may use single or multiple specimens and most reagents are enclosed in a disposable sealed container to which a clinical specimen is added. Almost all processes (such as DNA extraction and/or polymerase chain reaction (PCR) procedures) are performed within the container linked to the diagnostic platform. Cartridge-based tests may require an initial manual specimen treatment step prior to transfer of the material requiring testing into the cartridge.

Low complexity refers to a situation where no special infrastructure is required and basic laboratory skills are suitable to run the test, however, equipment may still be required.

Xpert® MTB/XDR Assay (Xpert MTB/XDR, Cepheid, Sunnyvale, USA) is the main index test in this review. Evidence on MeltPro® XDR-TB (MeltPro, Xiamen Zeesan Biotech Co., Ltd., China) provided by the manufacturer is summarized separately in Supplement A. No independent evaluations of MeltPro were identified.

Xpert MTB/XDR detects MTBC (*Mycobacterium tuberculosis* complex) DNA and genomic mutations associated with resistance to isoniazid, fluoroquinolones, ethionamide, and second-line injectable drugs (amikacin, kanamycin, capreomycin) in a single cartridge. This review does not include molecular drug susceptibility testing (DST) for kanamycin and capreomycin because, with the adoption of the new treatment regimens using all-oral medicines, the second-line injectable drugs are less relevant. We include molecular DST for amikacin because, of the second-line injectable drugs, amikacin is preferentially included in longer regimens when susceptibility has been demonstrated and adequate measures to monitor for adverse reactions can be ensured (Bainomugisa 2020; WHO Consolidated Guidelines (Module 4) 2020).

Xpert MTB/XDR is intended for use as a reflex test in specimens (unprocessed sputum or concentrated sputum sediments) determined to be MTBC-positive (Cepheid package insert 2020). The test could also be done on cultured isolates; however, this is not stated by the manufacturer as an intended use case. Several advantages of the assay are proposed.

- Faster time to result for molecular DST.
- Results in < 90 minutes.
- Same easy-to-use process as Xpert MTB/RIF Ultra.
- Run on existing GeneXpert® platforms equipped with 10-colour modules.

The limit of detection for *Mycobacterium tuberculosis* by Xpert MTB/XDR (136 CFU/mL in unprocessed sputum) (Cepheid package insert 2020) is similar to that of Xpert MTB/RIF (112.6 CFU/mL), but higher than that of Xpert Ultra (15.6 CFU/mL) (Chakravorty 2017). The manufacturer states that "Specimens with "MTB Trace DETECTED" results when tested with the Xpert MTB/RIF Ultra Assay are expected to be below the limit of detection of the MTB/XDR Assay and are not recommended for testing with the Xpert MTB/XDR Assay," (Cepheid package insert 2020). As with Xpert MTB/RIF and Xpert Ultra, Xpert MTB/XDR detects both live and dead bacteria (Cepheid report 2020).

The following information is from the Cepheid package insert (Cepheid package insert 2020).
- Regarding isoniazid, Xpert MTB/XDR bases detection of resistance on mutations in defined regions of the *katG* and *fabG1* genes, *oxyR-ahpC* intergenic region and *inhA* promoter region of the MTB genome.

- Regarding fluoroquinolones, Xpert MTB/XDR bases detection of resistance on mutations in the *gyrA* and g*yrB* quinolone resistance determining regions of the MTB genome.

- Regarding ethionamide, Xpert MTB/XDR bases detection of resistance on mutations in the i*nhA* promoter region of the MTB genome. In addition, it is noted that "mutations conferring ethionamide resistance are reported to be present in genomic regions not targeted by the Xpert MTB/XDR assay". Of interest, Brossier and colleagues found that 22/47 (47%) of ethionamide-resistant clinical isolates had mutations in *ethA*. Hence, the absence of mutations in the *inhA* promoter region does not preclude ethionamide resistance (Brossier 2011). Cepheid acknowledges that reporting ethionamide resistance based only on the detection of the *inhA* promoter mutations is a known limitation that may limit sensitivity though specificity may be unaffected.

- Regarding amikacin, Xpert MTB/XDR bases detection of resistance on mutations in a defined region of *rrs* of the MTB genome.

Table 1. Drug related gene targets, codon regions, and nucleotide sequences that determine presence of variants associated with drug resistance in the Xpert MTB/XDR assay

Drug	Gene target	Codon regions	Nucleotide
Isoniazid	*inhA* promoter (also used for tuberculosis detection)	not applicable	-1 to -32 intergenic region
	katG	311-319	939-957
	fabG1	199-210	597-630
	oxyR-ahpC intergenic region	not applicable	-5 to -50 intergenic region (or -47 to -92) *
Ethionamide	*inhA* promoter	not applicable	-1 to -32 intergenic region
Fluoroquinolones	*gyrA*	87-95	261-285
	gyrB	531-544 (or 493-505) *	1596-1632
Amikacin, Kanamycin, Capreomycin	*rrs*		1396-1417
	eis promoter	not applicable	-6 to -42 intergenic region

*Codon numbering system according to Camus JC, Pryor MJ, Médigue C, Cole ST. Re-annotation of the genome sequence of *Mycobacterium tuberculosis* H37Rv. Microbiology (Reading). 2002;148(Pt 10):2967-2973, as reported in Cepheid, Clinical evaluation of the Xpert® MTBXDR assay, Report R244C2 Xpert MTB/XDR Rev 1.0.

Xpert MTB/XDR can report results as MTB NOT DETECTED or MTB DETECTED. If results are reported as MTB DETECTED, each drug is reported as resistance DETECTED or NOT DETECTED. If results are reported as MTB NOT DETECTED, INVALID, ERROR, or NO RESULT, then no DST results are reported, Appendix 2.

Clinical pathway

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A clinical pathway presents a framework for developing recommendations about the use of a test and may assist in assessing the effect of a new test on management decisions and patient-important outcomes (Gopalakrishna 2016). We considered several clinical scenarios in Appendix 3,

In this systematic review, the intended use of Xpert MTB/XDR is for diagnosis of drug resistance. The role of the test would be a replacement test for culture-based phenotypic DST in people diagnosed with tuberculosis irrespective of rifampicin resistance or with detected rifampicin resistance.
The downstream consequences of testing include the following:

True-positive: people would benefit from rapid diagnosis and early initiation of appropriate tuberculosis treatment.

True-negative: people would be spared unnecessary treatment and would benefit from reassurance and pursuit of an alternative diagnosis.

False-positive: people would likely experience anxiety, morbidity from additional testing, possible delays in further diagnostic evaluation, and prolonged and unnecessary treatment with drugs that may have lower bactericidal activity than second-line regimens and often have serious adverse effects.

False-negatives: are at an increased risk of patient morbidity and mortality, and continued risk of community transmission of drug-resistant tuberculosis.

Review objective

To estimate the diagnostic accuracy of Xpert MTB/XDR on sputum for the diagnosis of the following conditions in people with microbiologically confirmed pulmonary tuberculosis.[6]

- Isoniazid resistance.
- Fluoroquinolone resistance.
- Ethionamide resistance.
- Amikacin resistance.

[6] We initially included an objective "to estimate the diagnostic accuracy of cartridge-based assays to diagnose pulmonary tuberculosis in people with signs and symptoms of pulmonary tuberculosis". However, we did not identify any studies that directly addressed this question. Therefore, this objective and the corresponding PICO questions were removed (Guideline Development Group Meeting, 1 November 2020), see Supplement B. The studies included in this review were designed to evaluate the manufacturer's intended use of Xpert MTB/XDR as a reflex test for a specimen (unprocessed sputum or concentrated sputum sediments) that is determined to be MTB positive (Cepheid package insert 2020).

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METHODS

Types of studies

We included cross-sectional studies and cohort studies that assessed the diagnostic accuracy of the index test. We included diagnostic accuracy studies in which cases and controls were sampled from a single source population (referred to as a single gate design). We excluded case-control studies where cases and controls were sampled from different populations (referred to as a two-gate design). The latter type of study is prone to bias, particularly when a study enrols participants with severe disease and healthy participants without disease (Rutjes 2005). We included studies where the reference standard was performed after the index test and those where the reference standard was performed before the index test. We only included studies that reported data comparing the index test to an acceptable reference standard (defined below) from which we could extract true positive (TP), true negative (TN), false positive (FP), and false negative (FN) values.

Participants

We included people of any age, HIV positive or negative, with microbiologically confirmed pulmonary tuberculosis. Participants with tuberculosis were included irrespective of rifampicin resistance (with or without rifampicin resistance, or rifampicin resistance unknown) or with detected rifampicin resistance. We included studies that assessed the diagnostic accuracy of the index test using sputum, consistent with the intended use of the manufacturer, and studies from all types of health facilities and all laboratory levels (peripheral, intermediate, and central) from all countries.

Index test

Xpert MTB/XDR is the main index test in this review. Evidence on MeltPro® XDR-TB (MeltPro, Xiamen Zeesan Biotech Co., Ltd., China) provided by the manufacturer is summarized separately in Supplement A.

Target conditions

We included four target conditions:

1. Isoniazid resistance.
2. Fluoroquinolone resistance.
3. Ethionamide resistance.
4. Amikacin resistance.

Reference standards

We included a microbiological reference standard (MRS) and a composite reference standard (CRS).

The microbiological reference standards were phenotypic DST (pDST) alone and genotypic DST (gDST) alone.

The composite reference standard was pDST and gDST, where at least one component test is positive.

In the methodological assessment using QUADAS-2, we took into account the reliability of these different reference standards for individual drugs (Heyckendorf 2017; WHO Critical concentrations 2018).

Outcomes

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).
Cure - *we did not identify any studies that reported data for this outcome.*
Mortality - *we did not identify any studies that reported data for this outcome.*
Time to diagnosis - *we did not identify any studies that reported data for this outcome.*
Time to start treatment - *we did not identify any studies that reported data for this outcome.*

Search methods

We searched the following databases: Ovid MEDLINE (OVID, 1946-present) and Embase (OVID, 1947-present), for studies evaluating cartridge-based tests using tuberculosis, pulmonary AND Xpert, GeneXpert, Truenat, Cartridge, Point-of-Care Systems, Drug Susceptibility Test, isoniazid resistance, fluoroquinolone resistance, and second-line injectable drug resistance as search terms. We also searched Clinicaltrials.gov and the WHO ICTRP for trials in progress. Searches were run up to 6 September 2020 without language restriction, Appendix 4. On 4 November 2020, we ran an additional search using the search terms Zeesan and MeltPro.

We contacted researchers at FIND, the WHO Global Tuberculosis Programme, the manufacturer, and other experts in the field of tuberculosis diagnostics for information on ongoing and unpublished studies. We reviewed data submitted via the WHO public call.

Data collection and analysis

Selection of studies

We used Covidence to manage the selection of studies (Covidence 2017). Two review authors independently assessed studies for eligibility. We resolved disagreements by discussion with a third review author. We illustrated the study selection process in a PRISMA diagram (Moher 2009).

Data extraction

Two review authors independently extracted data from the reports, including: author, publication year, study design, country(ies)/sites where study was located, clinical setting, population characteristics, the number of TP, FP, FN, and TN values with respect to the reference standard, and inconclusive test results. We resolved disagreements by discussion with a third review author.

Assessment of methodological quality

Two review authors working independently assessed methodological quality using QUADAS-2 tailored to this review, Appendix 5. We resolved disagreements by discussion with a third review author.

Statistical analysis and data synthesis

We stratified analyses by population and target condition. Within each stratum, for example, detection of isoniazid resistance, we plotted estimates of the studies' observed sensitivities and specificities in forest plots with 95% confidence intervals (CIs) and in receiver operating characteristic (ROC) space using Review Manager (RevMan). Where adequate data were available, we combined data using meta-analysis by fitting the bivariate random effects model (Macaskill 2010; Reitsma 2005), using Stata with the metandi and xtmelogit commands (Stata 2019). When a bivariate random effects model could not be fit owing to few studies or sparse data, we instead specified two univariate random effects models (Takwoingi 2015). In situations where all studies in a meta-analysis reported a sensitivity of 100% or specificity of 100%, we used simple pooling by summing up the numbers of true positives and total resistant cases to calculate sensitivity or the numbers of true negatives and total susceptible cases to calculate specificity, as required. We performed all analyses stratified by type of reference standard.

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For multicentre studies, we anticipated that there would be variability in terms of how laboratory practices were carried out between different centres. For this reason, when data were available, we performed meta-analyses at the centre level (i.e. treating each centre as a separate study).

We excluded MTBC-negative and inconclusive test results from analyses of diagnostic test accuracy.

Inconclusive index test results

The manufacturer defines two types of inconclusive results, non-determinate and indeterminate.

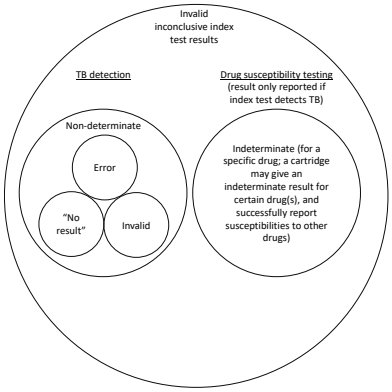

Figure. 1. Overview of different types of inconclusive results for Xpert MTB/XDR.

A non-determinate Xpert MTB/XDR test result is one that results in an INVALID, ERROR, or NO RESULT and can be due to an operator error, instrument, or cartridge issue (Cepheid package insert 2020). These three options are automatically generated, including the one called NO RESULT. The underlying reason for a non-determinate result is often not specified. The non-determinate Xpert MTB/XDR test results pertain only to the detection of MTBC, not to the detection of drug resistance.

A non-determinate result is distinct from MTB NOT DETECTED as shown below in Figure 2.

Drug Class	Result Call
N/A	INVALID/ERROR/NO RESULT
	MTB DETECTED
	MTB NOT DETECTED

Figure 2. Interpretation of non-determinate results and their relation to MTB DETECTED and MTB NOT Detected

An indeterminate Xpert MTB/XDR test result is one that indicates that resistance to a given drug could not definitively be detected based on the test's algorithm (Cepheid package insert 2020). This means that, based on quality control criteria, the test was not able to confidently report this particular result and the software suppressed the reporting of this. Indeterminate Xpert MTB/XDR test results pertain only to the detection of resistance to anti-tuberculosis drugs.

In addition, when data were available, we reported when the index test did not detect tuberculosis to begin with (missed cases).

We used the following approach to describe the different types of results.

Xpert MTB/XDR MTB NOT DETECTED
Among specimens with pDST results available, we determined the percentage that were Xpert MTB/XDR MTB NOT DETECTED. Among specimens with results reported as Xpert MTB/XDR

MTB NOT DETECTED, we further determined the percentage that were resistant or susceptible according to pDST.

Xpert MTB/XDR NON-DETERMINATE
Among the specimens initially tested, we determined the percentage of Xpert MTB/XDR NON-DETERMINATE results and, of these, the number of ERROR, INVALID, and NO RESULT results. We also determined the percentage of non-determinate results remaining following retesting.

Xpert MTB/XDR INDETERMINATE
Among specimens reporting Xpert MTB/XDR MTB DETECTED, we determined the percentage that were Xpert MTB/XDR INDETERMINATE (as drug resistance is only evaluated when MTB is detected). Among specimens with results reported as Xpert MTB/XDR INDETERMINATE, we further determined the percentage that were resistant or susceptible, according to pDST.

Investigations of heterogeneity

For each target condition, we investigated heterogeneity through visual examination of forest plots of sensitivity and specificity.

Sensitivity analyses

We performed sensitivity analyses by limiting inclusion in the meta-analysis to studies that were not designed or conducted by the manufacturer, therefore, we excluded Cepheid 2020.

Summary of findings and assessment of the certainty of the evidence (GRADE)

We assessed the certainty of evidence using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach for diagnostic studies (Schünemann 2008; Schünemann 2016). As recommended, we rated the certainty of evidence as either high (not downgraded), moderate (downgraded by one level), low (downgraded by two levels), or very low (downgraded by more than two levels) based on five domains: risk of bias, indirectness, inconsistency, imprecision, and publication bias. For each outcome, the certainty of evidence started as high for cross-sectional or cohort studies that enrolled participants with diagnostic uncertainty. When we found a reason for downgrading, we used our judgement to classify the reason as either serious (downgraded by one level) or very serious (downgraded by two levels). At least two review authors discussed judgments and applied GRADE in the following way (GRADEpro GDT 2015; Schünemann 2020a; Schünemann 2020b).

- Risk of bias: we used QUADAS-2 to assess risk of bias.
- Indirectness: we assessed indirectness in relation to the population (including disease spectrum), setting, interventions, and outcomes (accuracy measures). We used the prevalence of the condition as a guide to whether there was indirectness in the population.
- Inconsistency: GRADE recommends downgrading for unexplained inconsistency in sensitivity and specificity estimates.
- Imprecision: we considered a precise estimate to be one that would allow a clinically meaningful decision. We considered the width of the 95% CI and asked ourselves, ‘Would we make a different decision if the lower or upper boundary of the CI represented the truth?' In addition, we worked out projected ranges for TP, FN, TN, and FP for the prevalence of resistance to a given drug and made judgements on imprecision from these calculations.
- Publication bias: we considered the comprehensiveness of the literature search, outreach to researchers in tuberculosis, evidence identified from the WHO public call, and assistance from the WHO in identifying studies. Through these sources, we

identified several unpublished studies, but no publications. We graded publication bias as undetected.

RESULTS

Results of the search

We identified and screened a total of 1,649 records. Of these, we excluded 1620 for relevance to the topic. We retrieved 29 full text articles, including unpublished reports, and excluded 26 mainly because they were not rapid, low-complexity cartridge-based tests. We identified three unpublished studies for inclusion in the review, Cepheid 2020, DIAMA 2020, and FIND 2020. Appendix 6 shows the flow of studies in the review. A list of included and excluded studies is provided in Appendix 7.

Methodological quality of included studies

In the patient selection domain, we considered two studies (67%) to have low risk of bias and one study to have unclear risk of bias because we were unsure about the manner of participant selection (Cepheid 2020). Regarding applicability for patient selection, we considered all studies to have low concern.

In the index test domain, we considered all studies to have low risk of bias and low concern about applicability.

In the reference standard domain, we considered risk of bias separately for each drug and each reference standard. For resistance to isoniazid, fluoroquinolones, and amikacin, for pDST and gDST, we considered all studies have low risk of bias. For resistance to ethionamide, we considered all studies to have high risk of bias. For pDST, this was owing to considerable overlap in the minimum inhibitory concentration (MIC)s of *M tuberculosis* isolates with and without resistance-causing variants. For gDST, this was because no study included all loci required, e*thA*, *ethR*, and *inhA* promoter. Regarding applicability, for the reference standard domain, we considered all studies to have low concern.

In the flow and timing domain, we considered two studies to have low risk of bias and one study to have high risk of bias because not all participants were included in the analysis (DIAMA 2020). A summary table showing risk of bias and applicability concerns is included with each PICO question.

Findings

Study characteristics

The studies were in Benin, Cameroon, China, New Delhi, Moldova, Mumbai, Rwanda, and South Africa. We present key characteristics of the included studies in the Characteristics of included studies table, Appendix 8.

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PICO questions

Should MTB/XDR assay on sputum be used to diagnose isoniazid resistance in patients with microbiologically confirmed pulmonary TB?

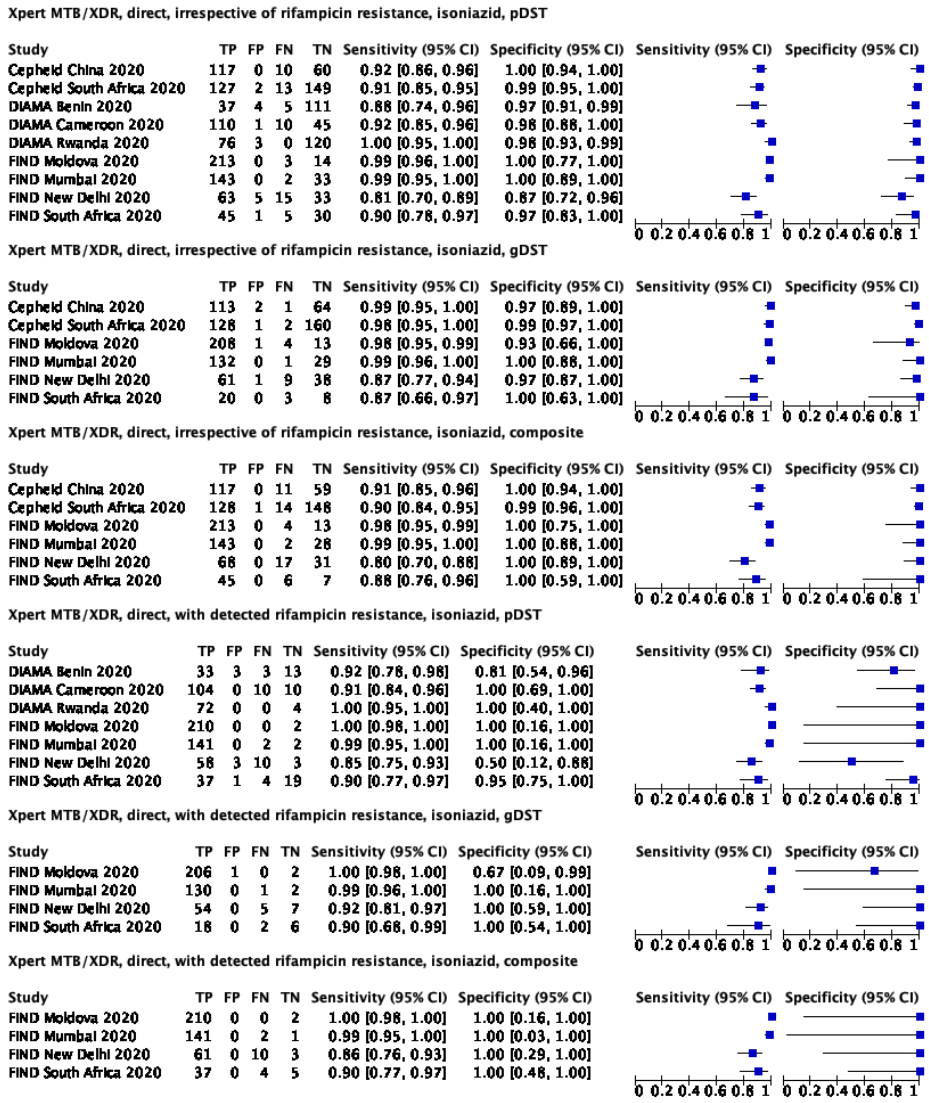

Figure 3. Forest plots of Xpert MTB/XDR sensitivity and specificity for detection of isoniazid resistance, by population and reference standard. Direct refers to testing directly on sputum.

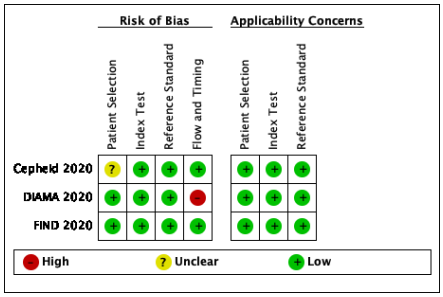

Figure 4. Xpert MTB/XDR, isoniazid resistance, risk of bias and applicability concerns.

Should MTB/XDR assay on sputum be used to diagnose fluoroquinolone resistance in patients with microbiologically confirmed pulmonary TB?

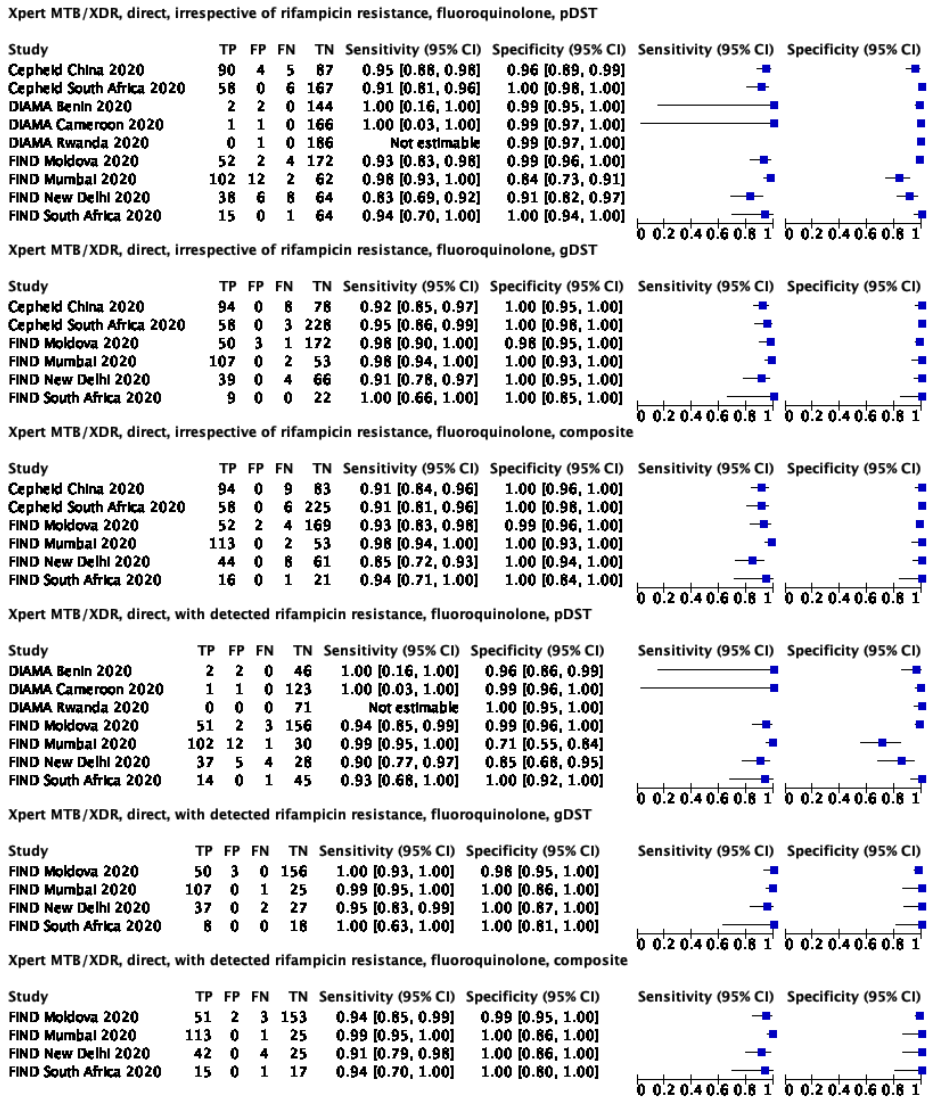

Figure 5. Forest plots of MTB/XDR sensitivity and specificity for detection of resistance to fluoroquinolones by population and reference standard. Direct refers to testing directly on sputum.

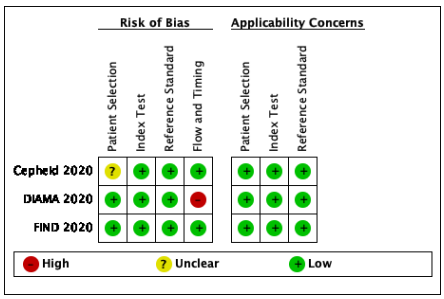

Figure 6. Xpert MTB/XDR, fluoroquinolone resistance, risk of bias and applicability concerns.

Should MTB/XDR assay on sputum be used to diagnose ethionamide resistance in patients with microbiologically confirmed pulmonary TB?

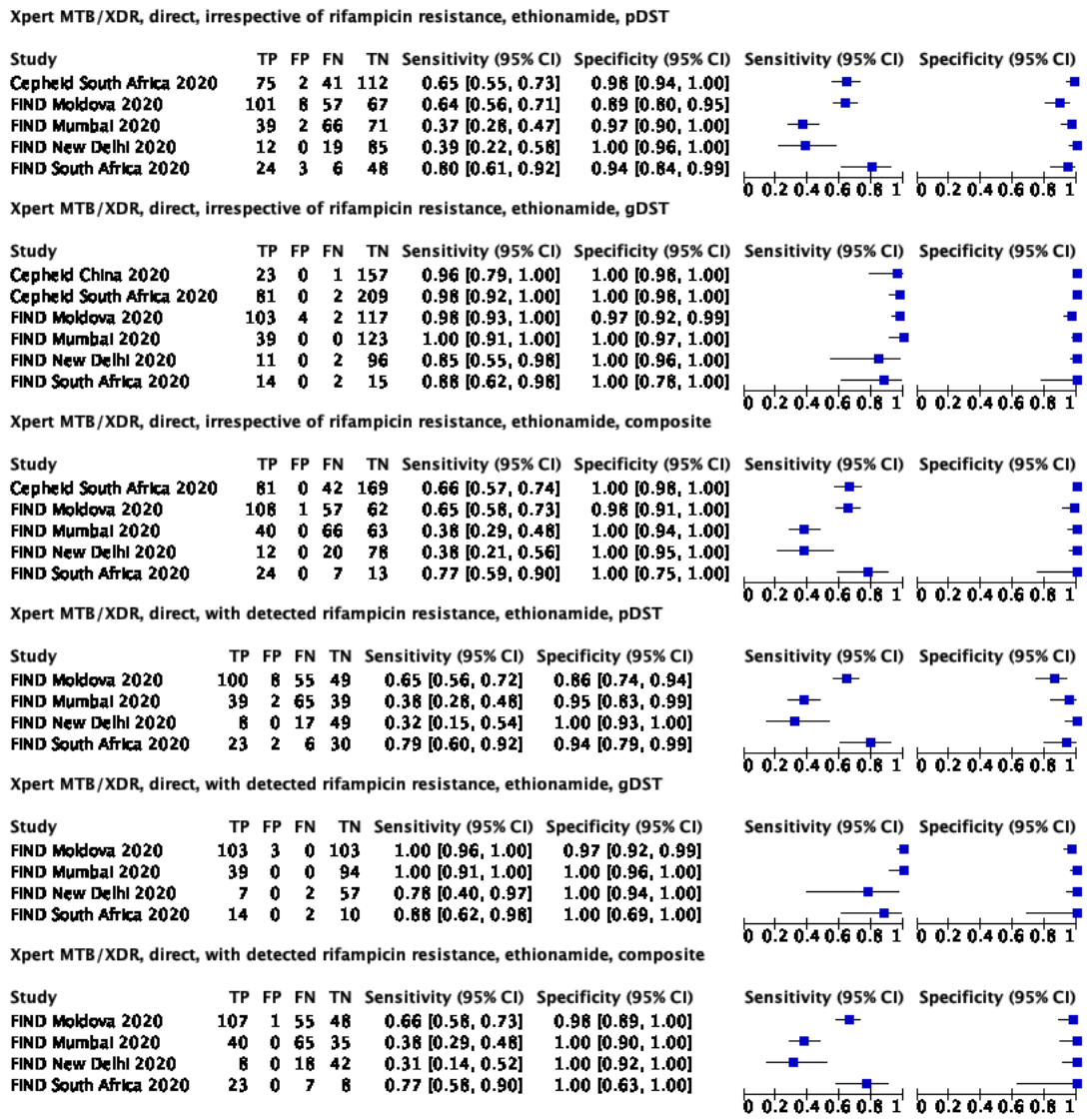

Figure 7. Forest plots of MTB/XDR sensitivity and specificity for detection of resistance to ethionamide by population and reference standard. Direct refers to testing directly on sputum.

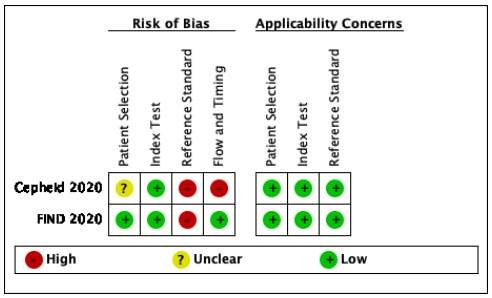

Figure 8. Xpert MTB/XDR, ethionamide resistance, risk of bias and applicability concerns.

Should MTB/XDR assay on sputum be used to diagnose amikacin resistance in patients with microbiologically confirmed pulmonary TB?

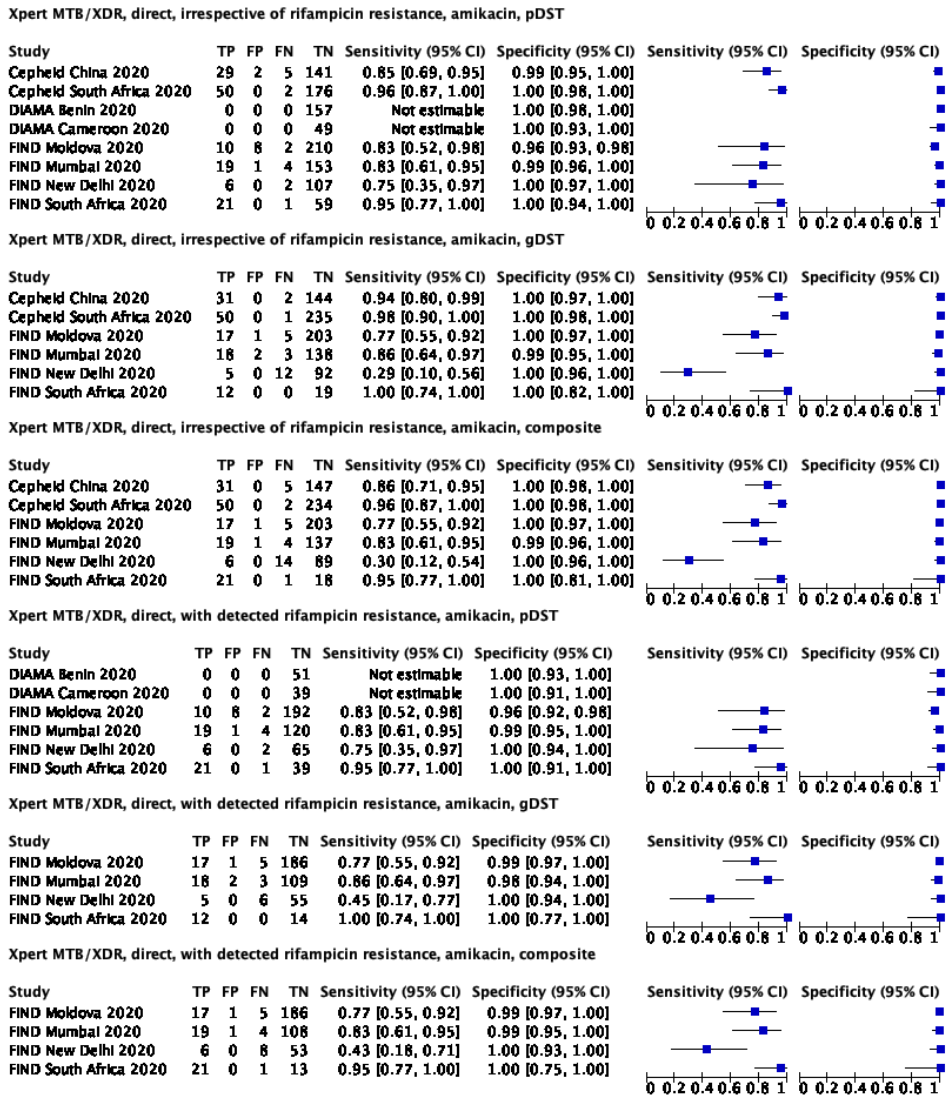

Figure 9. Forest plots of MTB/XDR sensitivity and specificity for detection of resistance to amikacin by population and reference standard. Direct refers to testing directly on sputum.

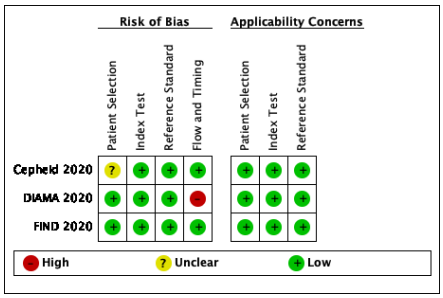

Figure 10. Xpert MTB/XDR, amikacin resistance, risk of bias and applicability concerns.

Table 2. Performance of Xpert MTB/XDR for resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin

Drug	Reference standard	No. studies (participants)	No. (%) with drug resistance	Pooled sensitivity % (95% CI)	Pooled specificity % (95% CI)	Positive predictive value % (95% CI) [1]	Negative predictive value % (95% CI) [1]

Irrespective of rifampicin resistance							
INH	pDST	3 (1605)	994 (61.9)	94.2 (89.3 to 97.0)	98.0 (95.2 to 99.2)	71.3 (50.1 to 86.0)	99.7 (99.4 to 99.8)
INH	gDST	2 (999)	682 (68.3)	97.3 (92.8 to 99.0)	98.4 (95.9 to 99.3)	75.6 (55.4 to 88.6)	99.9 (99.6 to 100.0)
INH	Composite	2 (1055)	768 (72.8)	93.6 (86.5 to 97.1)	99.7 (96.6 to 100.0)	94.2 (58.6 to 99.5)	99.7 (99.3 to 99.8)
With detected rifampicin resistance							
INH	pDST	2 (744)	684 (91.9)	97.2 (89.7 to 99.3)	91.5 (68.5 to 98.1)	83.0 (51.2 to 95.8)	99.1 (96.6 to 99.8)
INH	gDST	1 (434)	416 (95.9)	98.4 (88.9 to 99.8)	97.5 (27.1 to 100.0)	94.5 (15.4 to 99.9)	99.5 (96.6 to 99.9)
INH	Composite	1 (476)	465 (97.7)	97.6 (84.7 to 99.7)	100.0 (74.1 to 100.0)	100.0 (58.0 to 100.0)	99.3 (95.2 to 99.9)
Irrespective of rifampicin resistance							
FQ	pDST	3 (1337)	384 (28.7)	93.1 (88.0 to 96.1)	98.3 (94.5 to 99.5)	74.6 (46.8 to 90.7)	99.7 (99.4 to 99.8)
FQ	gDST	2 (997)	375 (37.6)	95.7 (91.8 to 97.7)	99.9 (92.0 to 100.0)	97.5 (36.9 to 100.0)	99.8 (99.6 to 99.9)
FQ	Composite	2 (1021)	407 (39.9)	92.8 (88.1 to 95.8)	99.8 (96.0 to 100.0)	95.5 (54.4 to 99.7)	99.6 (99.4 to 99.8)
With detected rifampicin resistance							
FQ	pDST	2 (666)	216 (32.4)	95.2 (89.1 to 98.0)	96.6 (87.2 to 99.2)	92.4 (75.4 to 97.9)	98.5 (96.7 to 99.4)
FQ	gDST	1 (434)	205 (47.2)	98.6 (94.3 to 99.7)	98.8 (94.7 to 99.7)	97.2 (88.6 to 99.4)	99.6 (98.2 to 99.9)
FQ	Composite	1 (452)	230 (50.9)	96.0 (90.6 to 98.4)	99.1 (96.2 to 99.8)	97.9 (91.3 to 99.5)	98.8 (97.2 to 99.5)
Irrespective of rifampicin resistance							
ETO	pDST	2 (838)	440 (52.5)	56.6 (41.8 to 70.3)	97.1 (91.9 to 99.0)	50.9 (28.6 to 72.8)	97.8 (97.0 to 98.4)
ETO	gDST	2 (1001)	280 (28.0)	96.4 (92.2 to 98.3)	100.0 (82.5 to 100.0)	99.6 (19.5 to 100)	96.5 (92.7 to 98.4)
ETO	Composite	2 (843)	457 (54.2)	57.1 (42.8 to 70.2)	99.8 (95.3 to 100.0)	94.7 (39.9 to 99.8)	97.9 (97.1 to 98.5)
With detected rifampicin resistance							
ETO	pDST	1 (492)	313 (63.6)	51.7 (33.1 to 69.8)	94.8 (84.8 to 98.3)	81.0 (62.2 to 91.7)	86.7 (81.9 to 90.4)
ETO	gDST	1 (434)	167 (38.5)	98.0 (74.2 to 99.9)	99.7 (83.5 to 100.0)	99.3 (68.6 to 100.0)	99.4 (91.2 to 100.0)
ETO	Composite	1 (457)	323 (70.7)	53.1 (34.7 to 70.7)	99.5 (87.0 to 100.0)	98.0 (63.9 to 99.9)	87.6 (82.6 to 91.3)
Irrespective of rifampicin resistance							
AMK	pDST	2 (1008)	151 (15.0)	89.1 (80.9 to 94.1)	99.5 (96.9 to 99.9)	90.1 (59.0 to 98.3)	99.5 (99 to 99.7)
AMK	gDST	2 (990)	156 (15.8)	89.5 (64.5 to 97.6)	99.7 (98.4 to 99.9)	93.3 (73.9 to 98.6)	99.5 (97.9 to 99.9)
AMK	Composite	2 (1005)	175 (17.4)	84.1 (63.0 to 94.3)	99.8 (99.0 to 99.9)	94.9 (81.1 to 98.8)	99.2 (98 to 99.7)
With detected rifampicin resistance							
AMK	pDST	1 (490)	65 (13.3)	86.1 (75.0 to 92.7)	98.9 (93.0 to 99.8)	97.2 (83.4 to 99.6)	95.9 (92.7 to 97.8)
AMK	gDST	1 (433)	66 (15.2)	81.1 (56.0 to 93.6)	99.2 (96.9 to 99.8)	97.8 (92.4 to 99.4)	94.6 (86.8 to 97.9)
AMK	Composite	1 (443)	81 (18.3)	79.0 (55.4 to 91.9)	99.5 (97.6 to 99.9)	98.4 (93.7 to 99.6)	94.0 (86.8 to 97.4)

Abbreviations: AMK: amikacin; CI: Confidence interval; standard; DST: drug susceptibility testing; ETO: ethionamide; FQ: fluoroquinolone; INH: isoniazid; pDST phenotypic DST; gDST: genotypic DST.

Notes: Within each multicentre study, when data were available, we performed meta-analyses at the centre level (i.e. treating each centre separately).
1. Prevalence for calculating predictive values: 5% in people irrespective of rifampicin resistance and 30% in people with detected rifampicin resistance.

As seen in Table 2, for each drug, Xpert MTB/XDR pooled sensitivity and specificity estimates were similar in people irrespective of rifampicin resistance and people with detected rifampicin resistance. However, owing to enrolment criteria in the studies, we note that most participants were rifampicin resistant in all analyses.

PICO questions

1. Should MTB/XDR assay on sputum be used to diagnose INH resistance in patients with microbiologically confirmed pulmonary TB, irrespective of resistance to RIF, MRS?
2. Should MTB/XDR assay on sputum be used to diagnose INH resistance in patients with microbiologically confirmed pulmonary TB, irrespective of resistance to RIF, CRS?
3. Should MTB/XDR assay on sputum be used to diagnose INH resistance in patients with microbiologically confirmed pulmonary TB, with detected resistance to RIF, MRS?
4. Should MTB/XDR assay on sputum be used to diagnose INH resistance in patients with microbiologically confirmed pulmonary TB, with detected resistance to RIF, CRS?
5. Should MTB/XDR assay on sputum be used to diagnose FQ resistance in patients with microbiologically confirmed pulmonary TB, irrespective of resistance to RIF, MRS?
6. Should MTB/XDR assay on sputum be used to diagnose FQ resistance in patients with microbiologically confirmed pulmonary TB, irrespective of resistance to RIF, CRS?
7. Should MTB/XDR assay on sputum be used to diagnose FQ resistance in patients with microbiologically confirmed pulmonary TB, with detected resistance to RIF, MRS?
8. Should MTB/XDR assay on sputum be used to diagnose FQ resistance in patients with microbiologically confirmed pulmonary TB, with detected resistance to RIF, CRS?
9. Should MTB/XDR assay on sputum be used to diagnose ETO resistance in patients with microbiologically confirmed pulmonary TB, irrespective of resistance to RIF, pDST?
10. Should MTB/XDR assay on sputum be used to diagnose ETO resistance in patients with microbiologically confirmed pulmonary TB, irrespective of resistance to RIF, gDST?
11. Should MTB/XDR assay on sputum be used to diagnose ETO resistance in patients with microbiologically confirmed pulmonary TB, irrespective of resistance to RIF, CRS?
12. Should MTB/XDR assay on sputum be used to diagnose ETO resistance in patients with microbiologically confirmed pulmonary TB, with detected resistance to RIF, pDST?
13. Should MTB/XDR assay on sputum be used to diagnose ETO resistance in patients with microbiologically confirmed pulmonary TB, with detected resistance to RIF, gDST?
14. Should MTB/XDR assay on sputum be used to diagnose ETO resistance in patients with microbiologically confirmed pulmonary TB, with detected resistance to RIF, CRS?
15. Should MTB/XDR assay on sputum be used to diagnose AMK resistance in patients with microbiologically confirmed pulmonary TB, irrespective of resistance to RIF, MRS?
16. Should MTB/XDR assay on sputum be used to diagnose AMK resistance in patients with microbiologically confirmed pulmonary TB, irrespective of resistance to RIF, CRS?
17. Should MTB/XDR assay on sputum be used to diagnose AMK resistance in patients with microbiologically confirmed pulmonary TB, with detected resistance to RIF, MRS?
18. Should MTB/XDR assay on sputum be used to diagnose AMK resistance in patients with microbiologically confirmed pulmonary TB, with detected resistance to RIF, CRS?

Table 3. GRADE Certainty of Evidence
See Supplement C. GRADE evidence profiles.

PICO	Drug	Population	Reference standard	No. studies (participants)	Pooled sensitivity % (95% CI)	Pooled specificity % (95% CI)	Certainty Evidence Sens	Certainty Evidence Spec	Explanations
1	INH	Irrespective rifampicin resistance	pDST	3 (1605)	94.2 (89.3, 97.0)	98.0 (95.2, 99.2)	Moderate	Moderate	Downgraded one level for indirectness for sensitivity and specificity
2	INH	Irrespective rifampicin resistance	CRS	2 (1055)	93.6 (86.5, 97.1)	99.7 (96.6, 100.0)	Moderate	Moderate	Downgraded one level for indirectness for sensitivity and specificity
3	INH	With detected rifampicin resistance	pDST	2 (744)	97.2 (89.7, 99.3)	91.5 (68.5, 98.1)	High	Low	Downgraded one level for inconsistency, and one level imprecision (specificity)
4	INH	With detected rifampicin resistance	CRS	1 (476)	97.6 (84.7, 99.7)	100.0 (74.1 100.0)	High	Low	Downgraded two levels for imprecision (specificity)
5	FQ	Irrespective rifampicin resistance	pDST	3 (1337)	93.1 (88.0, 96.1)	98.3 (94.5, 99.5)	High	Moderate	Downgraded one level for inconsistency (specificity)
6	FQ	Irrespective rifampicin resistance	CRS	2 (1021)	96.0 (90.6, 98.4)	99.1 (96.2, 99.8)	High	High	
7	FQ	With detected rifampicin resistance	pDST	2 (666)	95.2 (89.1, 98.0)	96.6 (87.2, 99.2)	High	Moderate	Downgraded one level for inconsistency (specificity)
8	FQ	With detected rifampicin resistance	CRS	1 (452)	96.0 (90.6 to 98.4)	99.1 (96.2 to 99.8)	High	High	
9	ETO	Irrespective rifampicin resistance	pDST	2 (838)	56.6 (41.8, 70.3)	97.1 (91.9, 99.0)	Low	Moderate	Downgraded one level for risk of bias, one level for inconsistency (sensitivity); downgraded one level for risk of bias (specificity)
10	ETO	Irrespective rifampicin resistance	gDST	2 (1001)	96.4 (92.2, 98.3)	100.0 (82.5, 100.0)	Low	Very Low	Downgraded two levels for risk of bias (sensitivity); downgraded two levels for risk of bias, one level for imprecision (specificity)
11	ETO	Irrespective rifampicin resistance	CRS	2 (843)	57.1 (42.8, 70.2)	99.8 (95.3, 100.0)	Low	Moderate	Downgraded one level for risk of bias one level for inconsistency (sensitivity); downgraded one

									level for risk of bias (specificity)
12	ETO	With detected rifampicin resistance	pDST	1 (492)	51.7 (33.1, 69.8)	94.8 (84.8, 98.3)	Very Low	Moderate	Downgraded one level for risk of bias, one level for inconsistency, one level for imprecision (sensitivity); downgraded one level for risk of bias (specificity)
13	ETO	With detected rifampicin resistance	gDST	1 (434)	98.0 (74.2, 99.9)	99.7 (83.5, 100.0)	Very Low	Very Low	Downgraded two levels for risk of bias, one level for imprecision (sensitivity and (specificity)
14	ETO	With detected rifampicin resistance	CRS	1 (457)	53.1 (34.7, 70.7)	99.5 (87.0, 100.0)	Very Low	Moderate	Downgraded one level for risk of bias, one level for inconsistency, one level for imprecision (sensitivity); downgraded one level for risk of bias (specificity)
15	AMK	Irrespective rifampicin resistance	pDST	2 (1008)	89.1 (80.9, 94.1)	99.5 (96.9, 99.9)	Moderate	High	Downgraded one level for risk of bias (sensitivity)
16	AMK	Irrespective rifampicin resistance	CRS	2 (1005)	84.1 (63.0, 94.3)	99.8 (99.0, t99.9)	Low	High	Downgraded one level for risk of bias, one level for inconsistency (sensitivity)
17	AMK	With detected rifampicin resistance	pDST	1 (490)	86.1 (75.0, 92.7)	98.9 (93.0, 99.8)	Low	High	Downgraded two levels for imprecision (sensitivity)
18	AMK	With detected rifampicin resistance	CRS	1 (443)	79.0 (55.4, 91.9)	99.5 (97.6, 99.9)	Low	High	Downgraded two levels for imprecision (sensitivity)

Abbreviations: AMK: amikacin; CI: Confidence interval; standard; DST: drug susceptibility testing; ETO: ethionamide; FQ: fluoroquinolone; INH: isoniazid; pDST phenotypic DST; gDST: genotypic DST

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Xpert MTB/XDR MTB NOT DETECTED and inconclusive test results

Xpert MTB/XDR MTB NOT DETECTED
Here we summarize results for Xpert MTB/XDR MTB NOT DETECTED and resistant cases therefore missed. Cepheid 2020 was the only study that reported this information.

Isoniazid

Of 530 specimens tested, 512 had pDST results available. Of these 512 specimens with pDST results available, 32 (6.3%) were Xpert MTB/XDR MTB NOT DETECTED.
By the pDST reference standard, of these 32 specimens, two (6.3%) were resistant and 30 (93.8%) were susceptible.

Fluoroquinolones

Of 530 specimens tested, 453 had pDST results available. Of these 453 specimens with pDST results available, 32 (7.1%), were Xpert MTB/XDR MTB NOT DETECTED.
By the pDST reference standard, of these 32 specimens, one (3.1%) was resistant and 31 (96.9%) were susceptible.

Ethionamide

Of 530 specimens tested, 260 had pDST results available. Of these 260 specimens with pDST results available, 30 (11.5%) were Xpert MTB/XDR MTB NOT DETECTED.
By the pDST reference standard, of these 30 specimens, two (6.7%) were resistant and 28 (93.3%) were susceptible.

Amikacin

Of 530 specimens tested, 445 had pDST results available. Of these 445 specimens, 32 (7.2%) were Xpert MTB/XDR MTB NOT DETECTED.
By the pDST reference standard, of these 32 specimens, 32 (100.0%) were susceptible.

Non-determinate test results
Here we provide a summary of non-determinate results and their pDST status.

Cepheid 2020
- Initial testing

Of 531 specimens tested, 15 resulted in non-determinate results after their Xpert testing. There were 10 “Error” results, one "Invalid” result, and four “No Result” results. *Therefore, the non-determinate rate upon initial testing was 2.8%.*
- Retesting

These 15 specimens were retested and 14 of the 15 gave valid results upon retest. One of the 15 retested specimens resulted in an “Error” following its repeat test. *Therefore, the non-determinate rate following retesting was 0.2% (1/531).*

FIND 2020
- Initial testing

Of 709 specimens tested, 21 resulted in non-determinate results after their initial Xpert tests. *Therefore, the non-determinate rate upon initial testing rate was 3.0% (21/709).*
- Retesting

Of these 21 specimens, 19 gave valid results upon retesting. *Therefore, the non-determinate rate following retesting was 0.3% (2/709).*

The phenotypic status of non-determinate results was not discernable for either study.

Indeterminate test results
Here we provide a summary of indeterminate results and their pDST status.

Isoniazid

Cepheid 2020
Of 530 specimens tested, 498 specimens had an Xpert MTB/XDR MTB DETECTED result. Of these 498 specimens, two (0.4%) had indeterminate results for detection of resistance.
By the pDST reference standard, of these two specimens, two (100%) were resistant and zero (0%) were susceptible.

FIND 2020
Of 709 specimens tested, 657 had an Xpert MTB/XDR MTB DETECTED result. Of these 657 specimens, two (0.3%) had indeterminate results for detection of resistance. None were indeterminate upon retesting.

Fluoroquinolones

Cepheid 2020
Of 530 specimens tested, 498 specimens had an Xpert MTB/XDR MTB DETECTED result. Of these 498 specimens, four (0.8%) had indeterminate results for detection of resistance.
By the pDST reference standard, of these four specimens, zero (0%) were resistant and four (100%) were susceptible.

FIND 2020
Of 709 specimens tested, 657 had an Xpert MTB/XDR MTB DETECTED result. Of these 657 specimens, nine (1.4%) had indeterminate results for detection of resistance. None were indeterminate upon retesting.

Ethionamide

Cepheid 2020
Of 530 specimens tested, 498 specimens had an Xpert MTB/XDR MTB DETECTED result. Of these 498 specimens, none (0%) had an indeterminate result for detection of resistance.

FIND 2020
Of 709 specimens tested, 657 had an Xpert MTB/XDR MTB Detected result. Of these 657 specimens, one (0.2%) had an indeterminate result for detection of resistance. This specimen was no longer indeterminate upon retesting.

Amikacin

Cepheid 2020
Of 530 specimens tested, 498 specimens had an Xpert MTB/XDR MTB DETECTED result. Of these 498 specimens, eight (1.6%) had indeterminate results for detection of resistance. By the pDST reference standard, of these eight specimens, zero (0%) were resistant and eight (100%) were susceptible.

FIND 2020
Of 709 specimens tested, 657 had an Xpert MTB/XDR MTB DETECTED result. Of these 657 specimens, 23 (3.5%) had indeterminate results for detection of resistance. One was indeterminate upon retesting.

pDST results could not be discerned for FIND 2020 indeterminates.

Sensitivity analyses

Table 4 presents the findings from sensitivity analyses that excluded data from the manufacturer. There are two rows of results presented for each drug. The first row presents the results of the meta-analysis including Cepheid 2020, and the subsequent row, the results of the sensitivity analysis excluding Cepheid 2020 (in bold).

These sensitivity analyses made little difference to any of the findings.

Table 4. Xpert MTB/XDR accuracy for drug resistance in people irrespective of rifampicin resistance, sensitivity analyses

Drug	Reference standard	No. studies (participants)	No. (%) with resistance to drug	Pooled sensitivity % (95% CI)	Pooled specificity % (95% CI)
Isoniazid	pDST	3 (1605)	994 (61.9)	94.2 (89.3 to 97.0)	98.0 (95.2 to 99.2)
Isoniazid, without Cepheid	**pDST**	**2 (1005)**	**685 (68.2)**	**96.0 (89.4 to 98.6)**	**97.1 (91.9 to 99.0)**
Fluoroquinolones	pDST	3 (1337)	384 (28.7)	93.1 (88.0 to 96.1)	98.3 (94.5 to 99.5)
Fluoroquinolones, without Cepheid	**pDST**	**2 (1112)**	**225 (20.1)**	**93.5 (83.4 to 97.6)**	**98.4 (94.3 to 99.5)**
Ethionamide	pDST	2 (838)	440 (52.5)	56.6 (41.8 to 70.3)	97.1 (91.9 to 99.0)
Ethionamide, without Cepheid	**pDST**	**1 (756)**	**324 (42.9)**	**53.1 (35.7 to 69.7)**	**96.5 (89.1 to 98.9)**
Amikacin	pDST	2 (1008)	151 (15.0)	89.1 (80.9 to 94.1)	99.5 (96.9 to 99.9)
Amikacin, without Cepheid	**pDST**	**1 (612)**	**65 (10.6)**	**86.1 (74.9 to 92.8)**	**99.3 (94.4 to 99.9)**

DISCUSSION

Summary of main results

This systematic review summarizes the current literature and included three unpublished studies on the accuracy of Xpert MTB/XDR for detection of resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin.

Xpert MTB/XDR for isoniazid resistance in people with microbiologically confirmed pulmonary tuberculosis irrespective of rifampicin resistance, pDST
Xpert MTB/XDR pooled sensitivity and specificity (95% confidence interval) were 94.2% (89.3 to 97.0) and 98.0% (95.2 to 99.2) (3 studies, 1605 participants, 61.9% with isoniazid resistance; high-certainty evidence for sensitivity and specificity).

Results of these studies indicate that in theory, of 1000 people where 50 have isoniazid resistance, 66 would be Xpert MTB/XDR-positive: of these, 19 (29%) would not have isoniazid resistance (false-positives) and 934 would be Xpert MTB/XDR-negative: of these, 3 (0%) would have isoniazid resistance (false-negatives).

Xpert MTB/XDR for fluoroquinolone resistance in people with microbiologically confirmed pulmonary tuberculosis irrespective of rifampicin resistance, pDST
Xpert MTB/XDR pooled sensitivity and specificity were 93.1% (88.0 to 96.1) and 98.3% (94.5 to 99.5.) (3 studies, 1337 participants, 28.7.% with fluoroquinolone resistance; high-certainty evidence for sensitivity and moderate-certainty evidence for specificity).

Results of these studies indicate that in theory, of 1000 people where 50 have fluoroquinolone resistance, 63 would be Xpert MTB/XDR-positive: of these, 16 (25%) would not have fluoroquinolone resistance (false-positives) and 937 would be Xpert MTB/XDR-negative: of these, 3 (0%) would have fluoroquinolone resistance (false-negatives).

Xpert MTB/XDR for ethionamide resistance in people with microbiologically confirmed pulmonary tuberculosis irrespective of rifampicin resistance, pDST
Xpert MTB/XDR pooled sensitivity and specificity were 56.6% (41.8 to 70.3) and 97.1% (91.9. to 99.0) (2 studies, 838 participants, 52.5% with ethionamide resistance; low-certainty evidence for sensitivity and moderate-certainty evidence for specificity).

Results of these studies indicate that in theory, of 1000 people where 50 have ethionamide resistance, 56 would be Xpert MTB/XDR-positive: of these, 28 (50%) would not have ethionamide resistance (false-positives) and 944 would be Xpert MTB/XDR-negative: of these, 22 (2%) would have ethionamide resistance (false-negatives).

Xpert MTB/XDR for ethionamide resistance in people with microbiologically confirmed pulmonary tuberculosis irrespective of rifampicin resistance, gDST
Xpert MTB/XDR pooled sensitivity and specificity were 96.4% (92.2 to 98.3) and 100.0% (82.5. to 100.0) (2 studies, 1001 participants, 28.0% with ethionamide resistance; moderate-certainty evidence for sensitivity and very low-certainty evidence for specificity).

Results of these studies indicate that in theory, of 1000 people where 50 have ethionamide resistance, 48 would be Xpert MTB/XDR-positive: of these, 0 (0%) would not have ethionamide resistance (false-positives) and 952 would be Xpert MTB/XDR-negative: of these, 2 (0%) would have ethionamide resistance (false-negatives).

Xpert MTB/XDR for amikacin resistance in people with microbiologically confirmed pulmonary tuberculosis irrespective of rifampicin resistance, pDST

Xpert MTB/XDR pooled sensitivity and specificity were 89.1% (80.9. to 94.1) and 99.5% (96.9 to 99.9) (2 studies, 1008 participants, 15.0% with amikacin resistance; high-certainty evidence for sensitivity and specificity).

Results of these studies indicate that in theory, of 1000 people where 50 have amikacin resistance, 50 would be Xpert MTB/XDR-positive: of these, 5 (10%) would not have amikacin resistance (false-positives) and 950 would be Xpert MTB/XDR-negative: of these, 5 (1%) would have amikacin resistance (false-negatives).

AUTHORS' CONCLUSIONS

- For resistance to isoniazid, in people irrespective of rifampicin resistance, Xpert MTB/XDR sensitivity was 94.2% against a reference standard of pDST.
- For resistance to fluoroquinolones, in people irrespective of rifampicin resistance, Xpert MTB/XDR sensitivity was 93.1% against a reference standard of pDST.
- For resistance to ethionamide, in people irrespective of rifampicin resistance, Xpert MTB/XDR sensitivity was 56.6% against a reference standard of pDST and 96.4% against a reference standard of gDST. However, the gDST reference standard only included the *inhA* promoter.
- For resistance to amikacin, in people irrespective of rifampicin resistance, Xpert MTB/XDR sensitivity was 89.1% against a reference standard of pDST.
- Xpert MTB/XDR specificity was > 97.0% in nearly all analyses.
- Overall, for resistance to isoniazid and fluoroquinoles, Xpert MTB/XDR sensitivity estimates for individual studies were consistent against the different reference standards.
- Overall, for resistance to a given drug, indeterminate results were infrequent and mostly resolved with retesting.
- We were not always able to link the analyses to a specific clinical pathway scenario, especially for Scenario A (patients evaluated for tuberculosis) and Scenario D (patients on treatment).

The impact of Xpert MTB/XDR is expected to be affected by several factors, including the health care infrastructure, access to other diagnostic tests, the ability of the index test to detect tuberculosis (which is required for DST), and the prevalence of resistance to a given drug. Given that the test targets a limited number of resistance variants in specific genes, the test may perform differently in different settings. These results should, therefore, be interpreted with caution.

The 2020 World Health Organization consolidated guidelines on drug resistant tuberculosis treatment recognize the importance of later generation fluoroquinolones in all-oral regimens of shorter duration (WHO Consolidated Guidelines (Module 4) 2020**)**. The review findings suggest that Xpert MTB/XDR provides accurate results for detection of fluoroquinolone resistance and can assist with rapid initiation of an optimized treatment regimen.

Future studies should assess the accuracy of Xpert MTB/XDR for drug resistance in different population groups, including children and people living with HIV. In addition, studies should assess the accuracy of Xpert MTB/XDR in different geological settings, in smear-negative specimens, and with different types of clinical specimens. Guidance is needed for specimens that test “MTB Trace DETECTED” with the Xpert MTB/RIF Ultra Assay.

Studies should utilize a comprehensive composite reference standard for gDST using all known resistance-associated loci, not just those analyzed by the index test. Studies should include patients from different points on the clinical pathway. In addition, we suggest quantifying the impact of non-actionable results, especially in smear-negative specimens. Future studies should also assess the

diagnostic accuracy of Xpert MTB/XDR for pulmonary tuberculosis in adults, children, and people living with HIV and in people who are smear negative.

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Declarations of Interest

SP received funding from the World Health Organization Global Tuberculosis Programme, Geneva.
GRD has no conflicts to declare.
MDV is employed by the Foundation for Innovative New Diagnostics (FIND). FIND has conducted studies and published on Xpert MTB/RIF as part of a collaborative project between FIND, a Swiss non-profit, Cepheid, a US company, and academic partners. The product developed through this partnership was developed under a contract that obligated FIND to pay for development costs and trial costs and Cepheid to make the test available at specified preferential pricing to the public sector in low- and middle-income countries. In addition, FIND conducted studies for the Xpert MTB/RIF Ultra assay, which have also been published.
MC received funding from READ-It. READ-It aims to improve the evidence base and ensure its dissemination and helps to ensure healthcare problems relevant to low- and middle-income countries are addressed, and that people living in these countries are part of the process. READ-It (project number 300342-104) is funded by the Foreign, Commonwealth and Development Office (FCDO), UK.
RW has no conflicts to declare.
KRS received funding from the World Health Organization Global Tuberculosis Programme, Geneva. In addition, she has received financial support from Cochrane Infectious Diseases, Liverpool; McGill University, Montreal; Baylor College of Medicine, Houston; Stellenbosch University, Cape Town; Foundation for Innovative New Diagnostics (FIND), Geneva; and the World Health Organization Global Tuberculosis Programme, Geneva for preparation of related systematic reviews.
GT received funding from the World Health Organization Global Tuberculosis Programme, Geneva. In addition, he has received in-kind research consumable and equipment donations provided to employer by Cepheid to work on Xpert MTB/RIF and Xpert MTB/RIF Ultra (not Xpert MTB/XDR) for diagnostic accuracy evaluations for tuberculosis detection. These studies are on different products to those potentially considered for inclusion in this review.

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APPENDICES

Appendix 1. Glossary of terms

Amplification
Amplification is replication of a DNA fragment to generate copies. Both the original and the newly synthesized copies can be described as the amplicons.

Codon
A codon is a sequence of three DNA or RNA bases that corresponds to a specific amino acid or a signal to start or stop transcription or translation. The DNA in coding regions of the genome is read in groups of three bases (A, G, C, T).

Critical concentration
The critical concentration of an anti-tuberculous agent has been adopted and modified from international convention. The critical concentration is defined as the lowest concentration of an anti-tuberculosis agent *in vitro* that will inhibit the growth of 99% of phenotypically wild type strains of *M tuberculosis* complex.

Culture isolate
Culture isolate refers to *M tuberculosis* cells from a clinical specimen that have been grown. For tuberculosis diagnosis, a volume of the clinical specimen is processed and incubated under conditions that promote *M tuberculosis* growth. The cells that are grown are referred to a culture isolate.

DNA sequencing
DNA sequencing is a process to determine the nucleotide (A, G, C, T) sequence of fragments of DNA. By comparison of DNA sequences from distinct tuberculosis isolates, variations known as mutations can be identified. Some mutations in *M tuberculosis* are known to be associated with drug resistance.

Drug susceptibility testing
Drug susceptibility tests determine whether *M tuberculosis* cells are sensitive or resistant to antibiotics. Testing may be undertaken using phenotypic or genotypic analyses.

eis promoter
Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to second line injectable drugs, amikacin and kanamycin.

fabG1
Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to isoniazid.

Genotypic drug susceptibility testing (gDST)
Genotypic testing involves detecting predetermined mutations in DNA that are known to make the organism resistant to a drug. When mutations causing drug resistance are not known, genotypic DST is not useful.

gyrA
Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to fluoroquinolones.

gyrB
Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to fluoroquinolones.

Heteroresistance

Heteroresistance is defined as resistance to certain antibiotics in a subset of a larger microbial population that is generally considered to be susceptible to these antibiotics according to traditional phenotypic drug susceptibility testing.

Indeterminate test result

An indeterminate Xpert MTB/XDR test result is one that indicates that resistance to a given drug could not definitively be detected based on the test's algorithm.

***inhA* promoter**

Gene target included in the Xpert MTB/XDR test to detect MTB and resistance to isoniazid and ethionamide. Mutations in the *inhA* promoter region of TB are known to confer low level resistance to isoniazid and high-level cross resistance to ethionamide.

Intergenic region

Is a region of DNA sequence located between genes and a subset of noncoding DNA. Some intergenic regions act to control coding regions (genes) nearby.

katG

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to isoniazid.

Locus

A locus is the position of a genetic feature in the DNA sequence, like a genetic street address. Loci are standardized between genomes by reference to a common reference genome, such as H37Rv for *M tuberculosis*.

Microbiologically confirmed

Refers to a biological specimen that is positive by culture or a WHO-recommended rapid molecular test, such as Xpert MTB/RIF, Xpert Ultra, or Truenat MTB.

Mutation

A mutation is a change in a DNA sequence. Mutations can result from DNA copying mistakes made during cell division, exposure to ionizing radiation, exposure to chemicals called mutagens, or infection by viruses.

Non-determinate test result

A non-determinate Xpert MTB/XDR test result is one that results in an Error, Invalid, or No Result and can be due to an operator error, instrument, or cartridge issue.

***oxyR-ahpC* intergenic region**

Gene targets included in the Xpert MTB/XDR test to detect mutations that confer resistance to isoniazid.

Phenotypic drug susceptibility testing (pDST)

Phenotypic testing requires growth of *M tuberculosis* in the presence of antibiotics at a specific concentration that will inhibit the growth of a sensitive organism or have no impact on growth of a resistant organism.

Presumptive tuberculosis

Refers to a patient who presents with symptoms or signs suggestive of or compatible with tuberculosis.

Promoter region

A promoter region is a sequence of DNA where the transcriptional machinery binds before transcribing the DNA into RNA that may then be translated into an amino acid sequence.

Resistance-determining region

A region of the *M tuberculosis* genome where mutations commonly cause resistance to a specific drug.

rrs

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to second line injectable drugs, amikacin, kanamycin, and capreomycin.

Sanger sequencing

Technique for DNA sequencing based upon the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during *in vitro* DNA replication, also known as 'the chain termination method'.

Targeted gene sequencing

The process for detecting predetermined mutations in DNA or genomic regions.

Whole genome sequencing (WGS)

The process of determining the complete genome sequence for a given organism at one time through next generation sequencing methods. This method can determine the order of all nucleotides in a given genome and detect any variations relative to a reference genome using bioinformatics analyses.

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Appendix 2. Possible test results for each target in the Xpert MTB/XDR assay

Drug Class	Result Call
N/A	INVALID/ERROR/NO RESULT
	MTB DETECTED
	MTB NOT DETECTED
Isoniazid	Low INH Resistance DETECTED
	INH Resistance DETECTED
	INH Resistance NOT DETECTED
	INH Resistance INDETERMINATE
Fluoroquinolone	Low FLQ Resistance DETECTED
	FLQ Resistance DETECTED
	FLQ Resistance NOT DETECTED
	FLQ Resistance INDETERMINATE
Amikacin	AMK Resistance DETECTED
	AMK Resistance NOT DETECTED
	AMK Resistance INDETERMINATE
Kanamycin	KAN Resistance DETECTED
	KAN Resistance NOT DETECTED
	KAN Resistance INDETERMINATE
Capreomycin	CAP Resistance DETECTED
	CAP Resistance NOT DETECTED
	CAP Resistance INDETERMINATE
Ethionamide[a]	ETH Resistance DETECTED
	ETH Resistance NOT DETECTED

Positive results for the Xpert MTB/XDR assay can be MTB DETECTED and all resistance targets are NOT DETECTED, or MTB DETECTED and one or more of the resistance targets is DETECTED, or MTB DETECTED and/or one or more of the following resistance targets is INDETERMINATE.

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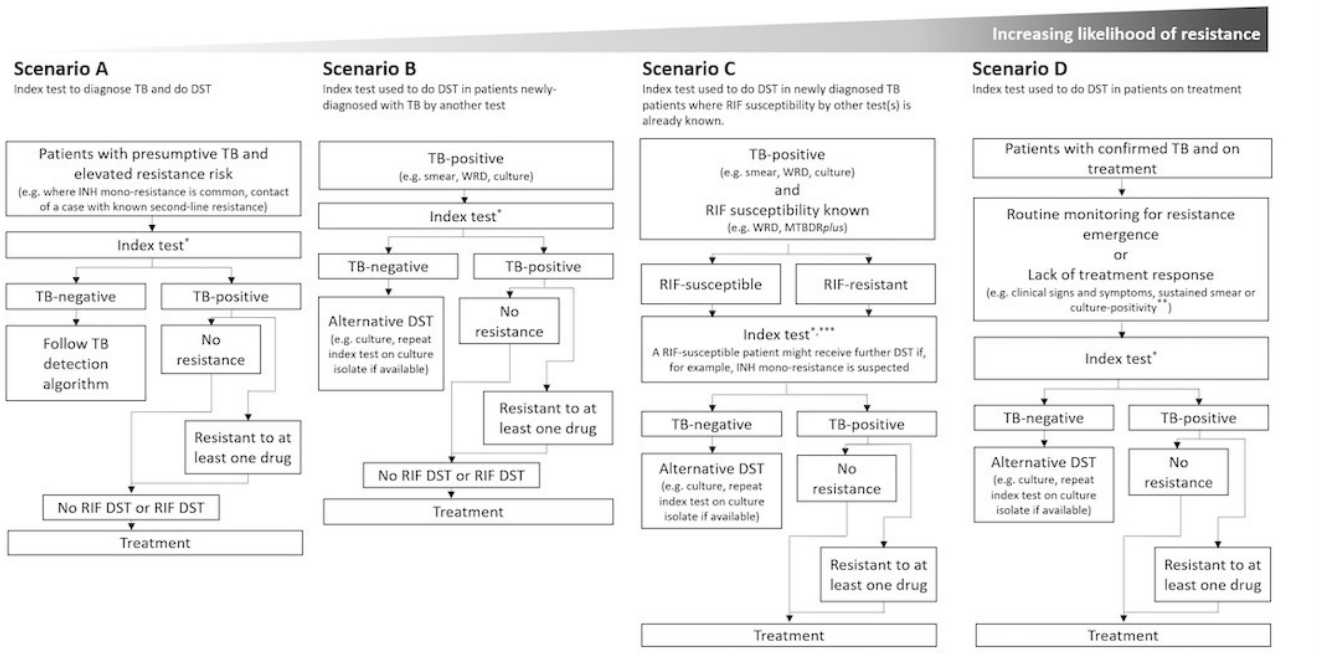
Appendix 3. Figure. Clinical pathway



The index test may be used in the following scenarios.
A. Index test used to diagnose tuberculosis and detect drug resistance.
B. Index test used to detect drug resistance in patients newly diagnosed with tuberculosis by another test where rifampicin susceptibility is unknown. Proposed role of Xpert MTB/XDR would be an initial test for resistance to isoniazid and second-line drugs (replacement for LPAs and culture-based DST as initial tests).
C. Index test used to detect drug resistance in patients newly diagnosed with tuberculosis and rifampicin resistance by other tests (although less likely, it is possible that the index test may still be done when documented rifampin susceptibility by other tests exists). Proposed role of Xpert MTB/XDR would be an initial test for resistance to isoniazid and second-line drugs (replacement for LPAs and culture-based DST as initial tests).
D. Index test used to detect drug resistance in patients on treatment. Proposed role of Xpert MTB/XDR would be a test used in combination with other tests for treatment monitoring (parallel testing).

Abbreviations: DST: drug susceptibility testing; RIF: rifampicin; TB: tuberculosis; WRD: WHO-recommended rapid diagnostic.

*Although direct testing is preferred for rapidity (which can be done on a raw specimen or a specimen remnant after some form of processing such as N-acetyl-L-cysteine (NALC)-NaOH decontamination), indirect testing using a cultured isolate could also be done (if, for example, a MTBC-positive reflex result is unavailable or culture has already been done due to diagnose tuberculosis).
**Xpert MTB/XDR may be considered in patients who were Xpert MTB/Ultra rifampicin susceptible prior to treatment and transitioned to Xpert MTB/Ultra rifampicin resistant while on treatment.
***Although index test use may be prioritised when risks of isoniazid- and/or second-line-resistance are elevated (in Scenario C if rifampicin resistance is first detected), it may also be applied irrespective of what the rifampicin susceptibility is, although we expect this to be less frequent.

Notes: 1) for all regimens, final composition will depend on other factors, including rifampicin susceptibility determined by an alternative test; 2) the timing of rifampicin DST can be before, in parallel, or after the index test is applied; and 3) for ease of presentation, tuberculosis and MTBC are treated equivalently.

Appendix 4. Search strategy

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Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to present>

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1 Extensively Drug-Resistant Tuberculosis/ or Tuberculosis, Multidrug-Resistant/ or Tuberculosis, Pulmonary/

2 (tuberculosis adj3 (lung or pulmonary)).mp.

3 (tuberculosis adj3 respiratory).mp.

4 (isoniazid resistance or isoniazid resistant).mp.

5 ((Fluoroquinolone adj3 resistance) or (Fluoroquinolone adj3 resistant)).mp.

6 (Second-line injectable drug adj3 resistance).mp.

7 (Second-line injectable drug adj 3 resistant).mp.

8 ((SLID adj3 resistance) or (SLID adj3 resistant)).mp.

9 (MDR-TB or XDR-TB).mp.

10 ((isoniazid or fluoroquinolone or "second-line injectable drug" or SLID) adj3 monoresist*).mp.

11 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10

12 (cartridge adj3 test*).mp.

13 cartridge*.ab. or cartridge*.ti.

14 (Molbio or Truenat or Cepheid or Xpert* or Bioneer or Hain).mp.

15 Genexpert*.mp.

16 exp Point-of-Care Systems/

17 drug susceptibility test*.mp.

18 12 or 13 or 14 or 15 or 16 or 17

19 11 and 18

20 limit 19 to yr="2015 -Current"

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Appendix 5. QUADAS-2

Domain 1: Patient selection
Detection of tuberculosis
Risk of bias: Could the selection of patients have introduced bias?

Signalling question 1: Was a consecutive or random sample of patients enrolled?
We answered yes if the study enrolled a consecutive or random sample of eligible patients; no if the study selected patients by convenience; and unclear if the study did not report the manner of patient selection or was not clearly reported.
Signalling question 2: Was a case-control design avoided?
We answered yes if the study enrolled patients with presumptive tuberculosis; no if the study enrolled cases with confirmed tuberculosis and controls from a healthy population; and unclear if we cannot tell. We consider that accuracy studies may have a cross-sectional design even when the reference standard is performed before the index test if both cases and controls are sampled from a single source population.
Signalling question 3: Did the study avoid inappropriate exclusions?
We answered yes if the study included both smear-positive and smear-negative individuals; no if the study included primarily or exclusively smear-positive or smear-negative patients; and unclear if we cannot tell. If at the time of specimen collection, the patient was on any form of tuberculosis treatment and if culture reference standard was used, we answered no because the bactericidal action of antibiotics can cause negative culture and positive PCR results.

Applicability: Are there concerns that the included patients and setting do not match the review question?
We answered low concern if patients were evaluated as outpatients (with either expectorated or induced sputum) in local hospitals or primary care centres. We answered high concern if patients were evaluated exclusively as inpatients in tertiary care centres. We answered unclear concern if the clinical setting was not reported or there was insufficient information to make a decision. We also answered unclear concern if testing was done at a central-level laboratory and the clinical setting was not reported if, for example, it was difficult to tell whether the laboratory provided services mainly to very sick patients or patients with a broader clinical spectrum of illness.

Detection of drug resistance
Risk of bias: could the selection of patients have introduced bias?

Signalling question 1: Was a consecutive or random sample of patients enrolled?
We answered the same as for detection of tuberculosis.
Signalling question 2: Was a case-control design avoided?
We answered yes if the study enrolled tuberculosis patients with suspected or sufficiently high pre-test probability (per WHO guidelines) for resistance to isoniazid, second-line drugs, or both isoniazid and second-line drugs; no if the study enrolled tuberculosis patients with confirmed pre-known resistance to the drug in question; and unclear for all other scenarios or if it was not clearly reported. We consider that accuracy studies may have a cross-sectional design even when the reference standard is performed before the index test if both cases and controls are sampled from a single source population.
Signalling question 3: did the study avoid inappropriate exclusions?
We answered yes for people who were previously treated for tuberculosis. we answered no if people who were previously treated were excluded. Patients previously tested for tuberculosis have a higher risk of having drug resistance and are likely to be the target population for initial use of the index tests. In people with samples known to be heteroresistant (a mix of susceptible and resistant tuberculosis strains in the specimen) were excluded, which is particularly relevant for fluoroquinolones, we answered no. We answered unclear if we cannot tell.

Applicability: Are there concerns that the included patients and setting do not match the review question?
We judged low concern if the selected clinical specimens or isolates match the review question, which reflects the way the test will be used in practice.
We judged high concern if the selected specimens or isolates did not represent those for whom the test will be used in practice, such as in individuals who do not require investigation for resistance to the drugs in question.
We will judge unclear concern if we cannot not tell.

Domain 2: Index test
Detection of tuberculosis

Risk of bias: could the conduct or interpretation of the index test have introduced bias?

Signalling question 1: were the index test results interpreted without knowledge of the results of the reference standard?
We answered this question yes for all studies where results are automatically generated and the user is provided with printable test results. Thus, there is no room for subjective interpretation of test results. For those assays, which require user interpretation, we answered yes if the reader of the assay was blinded to results of reference tests. We answered no if the reader of the assay was not blinded to the results of reference tests. If the specimens were from a biobank (repository that stores biological specimens) comprised of specimens with known second-line drug resistance and the identity of these specimens was known to the assay reader, we will also answer no unless the assay automatically generates results. We answered unclear if it was not stated in the paper or if the study authors failed to answer this question.
Signalling question 2: if a threshold was used, was it prespecified?
We answered yes for all studies.

Applicability: are there concerns that the index test, its conduct, or its interpretation differ from the review question?
Variations in test technology, execution, or interpretation may affect estimates of the diagnostic accuracy of a test. We will judge the study to be of low concern for applicability if the test was performed as recommended by the manufacturer. We judged the study to be of high concern if the test was applied differently than recommended by the manufacturer, for example if the test was applied to pooled sputa. We judged the study to be of unclear concern if we cannot tell.

Detection of drug resistance
Risk of bias: could the conduct or interpretation of the index test have introduced bias?

Signalling question 1. were the index test results interpreted without knowledge of the results of the reference standard?
We answered this question yes for all studies where results are automatically generated and the user is provided with printable test results, such as drug susceptibility testing run by MGIT 960 SIRE. For those assays which require user interpretation, such as Löwenstein–Jensen (LJ) drug susceptibility testing, we answered yes if the reader of the assay was blinded to results of reference tests. We answered no if the reader of the assay was not blinded to the results of reference tests. We answered unclear if it was not stated in the paper or if the study authors failed to answer this question.
Signalling question 2: if a threshold was used, was it prespecified?
We answered yes for all studies.

Applicability: are there concerns that the index test, its conduct, or its interpretation differ from the review question?
Same judgements as for detection of tuberculosis.

Domain 3: Reference standard
Detection of tuberculosis
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?

Signalling question 1: Is the reference standard likely to correctly classify the target condition?
We answered yes for all studies, since a microbiological reference standard for *M tuberculosis* identification was a criterion for inclusion in the review.
Signalling question 2: Were the reference standard results interpreted without knowledge of the results of the index test?
We answered yes if the reference test provided an automated result (for example, MGIT 960), blinding was explicitly stated, or it was clear that the reference standard was performed at a separate laboratory and/or performed by different people. We answered no if the study stated that the reference standard result was interpreted with knowledge of the index test result. We answered unclear if we could not tell.

Applicability: Are there concerns that the target condition as defined by the reference standard does not match the question? We answered high concern if a type of culture was not done as part of the reference standard, because studies that include only DNA-based tests do not directly measure live M tuberculosis. We answered low concern if culture was performed. We answered unclear concern if we cannot tell.

Detection of drug resistance
Risk of bias: could the reference standard, its conduct or its interpretation have introduced bias?

Signalling question 1: is the reference standard likely to correctly classify the target condition?
We answered these questions for each target condition separately by reference standard as follows.

Drug	pDST	gDST, targeted sequencing	Composite (pDST and gDST, targeted sequencing)	gDST, whole genome sequencing)	Composite (pDST and gDST, whole genome sequencing)
Isoniazid	Yes	Unclear if few loci are investigated, and yes, if all relevant loci are analysed. Loci required for yes*: katG*, *inhA* promoter, *oxyR-ahpC i*ntergenic region, and *fabG1*	Yes	Unclear if few loci are investigated, and yes, if all relevant loci are analysed. Loci required for yes*: katG*, *inhA* promoter, *oxyR-ahpC i*ntergenic region, and *fabG1*	Yes
Fluoroquinolone	Yes, will depend on critical concentration used for moxifloxacin*	Yes Loci required for yes: *gyrA* and *gyrB*	Yes	Yes Loci required for yes: *gyrA* and *gyrB*	Yes
Ethionamide	No, there is considerable overlap in the MICs of *M tuberculosis* isolates with and without resistance-causing variants.	Unclear if few loci are investigated, and yes, if all relevant loci are analysed Loci required for yes: *ethA*, *ethR*, and i*nhA* promoter No if only the *inhA* promoter was analysed	Unclear	Unclear if few loci are investigated, and yes, if all relevant loci are analysed. Loci required for yes: e*thA*, *ethR*, and i*nhA* promoter No if only the *inhA* promoter was analysed	Unclear
Amikacin	Yes	Yes, if all relevant loci are analysed Loci required for yes: *rrs* and *eis* promoter	Yes	Yes, if all relevant loci are analysed Loci required for yes: *rrs* and *eis* promoter	Yes

Abbreviations: gDST: genotypic drug susceptibility testing; pDST: phenotypic drug susceptibility testing.

*We used the currently-recommended WHO critical concentrations as a benchmark for judging risk of bias. For *M tuberculosis*, the antimicrobial susceptibility testing critical concentration is defined as the lowest concentration of an anti-tuberculosis agent in vitro that will inhibit the growth of 99% of phenotypically wild type strains of *M tuberculosis* complex", (WHO Critical concentrations 2018).

Signalling question 2: were the reference standard results interpreted without knowledge of the results of index test.
For pDST, we answered yes if the reference test provided an automated result (for example, if liquid culture is used as in MGIT 960 DST), blinding was explicitly stated, or it was clear that the reference test was performed at a separate laboratory, or performed by different people, or both. Of note, pDST on solid media is not automated. We answered no if the study stated that the reference standard result was interpreted with knowledge of the index test result. We answered unclear if we cannot tell. For gDST, we answered yes for all studies since the results for the reference standard are automated.

We added the following signalling question.
Signalling question 3: Were the index test and reference standard both done on material of the same type (clinical specimen or sediment, or isolate)?
Phenotypic DST (pDST) and genotypic DST (gDST) for reference standard testing can be done on an isolate that has undergone (potentially multiple rounds) of culture in drug-free media. This may lead to the depletion of

resistant strains present in the original specimen (which would have been used for the index test if direct testing was done) and cause discrepant results. We think this is an important question as it addresses heteroresistance, which often explains discordance between genotypic and phenotypic results.
For direct testing of a clinical specimen by the index test: we answered yes if the reference test was done directly on the same clinical specimen; no if the reference standard was done on a culture isolate; and unclear if we could not tell. For indirect testing of a culture isolate by the index test: we answered yes if the reference test was done on the same culture isolate (e.g. indirect sequencing); no if the reference standard was done on a different culture isolate, or specimen; and unclear if we could not tell.

Applicability: are there concerns that the target condition as defined by the reference standard does not match the question?
We judged applicability to be of low concern for all studies because specimens to be subsequently tested for drug resistance will have already been identified as *M tuberculosis* complex positive.

Domain 4: Flow and timing
Detection of tuberculosis
Risk of bias: could the patient flow have introduced bias?

Signalling question 1: was there an appropriate interval between the index test and reference standard?
We expect the reference standard test to be undertaken at the same time as the index test (i.e. each performed on a paired sample for most studies). However, we expected some studies to include specimens from patients who had received a reference test on an earlier sample. The sample applies to some culture isolates, whose drug susceptibility profile might have been confirmed prior to the index test being available. We answered yes if the tests were paired or were separated by a few days. We answered no if reference and index tests were not done on paired samples and were separated by several months. As patients suspected of second-line drug resistance are often on some form of anti-tuberculosis therapy, it is possible that variation in the microbial population of specimens collected at different time points may occur. We answered unclear if it was not stated in the paper or if the authors failed to answer this question.
Signalling question 2: did all patients receive the same reference standard?
We answered yes if the reference standard was applied to all patients or a random sample of patients, no if the reference standard was only applied to a selective group of patients, and unclear if it was not stated in the paper or if the authors failed to answer this question.
Signalling question 3: were all patients included in the analysis?
We determined the answer to this question by comparing the number of participants enrolled with the number of patients included in the 2 x 2 tables. We will note if the study authors reported the number of indeterminate assay results. We answered yes if the number of participants enrolled was clearly stated and corresponded to the number presented in the analysis or if exclusions were adequately described. We answered no if there were participants missing or excluded from the analysis and there was no explanation given. We answered unclear if not enough information was given to assess whether participants were excluded from the analysis.

Detection of drug resistance
We answered the same as for detection of tuberculosis.

Judgements for risk of bias assessments for a given domain
If we answered all signalling questions for a domain yes, then we judged risk of bias as low.
If we answered all or most signalling questions for a domain no, then we judged risk of bias as high.
If we answered only one signalling question for a domain no, we discussed further the risk of bias judgement.
If we answered all or most signalling questions for a domain unclear, then we judged risk of bias as unclear.
If we answered only one signalling question for a domain unclear, we discussed further the risk of bias judgement for the domain.
For reference standard domain, if either any of the reference standard had signalling no, we judged risk of bias as high.

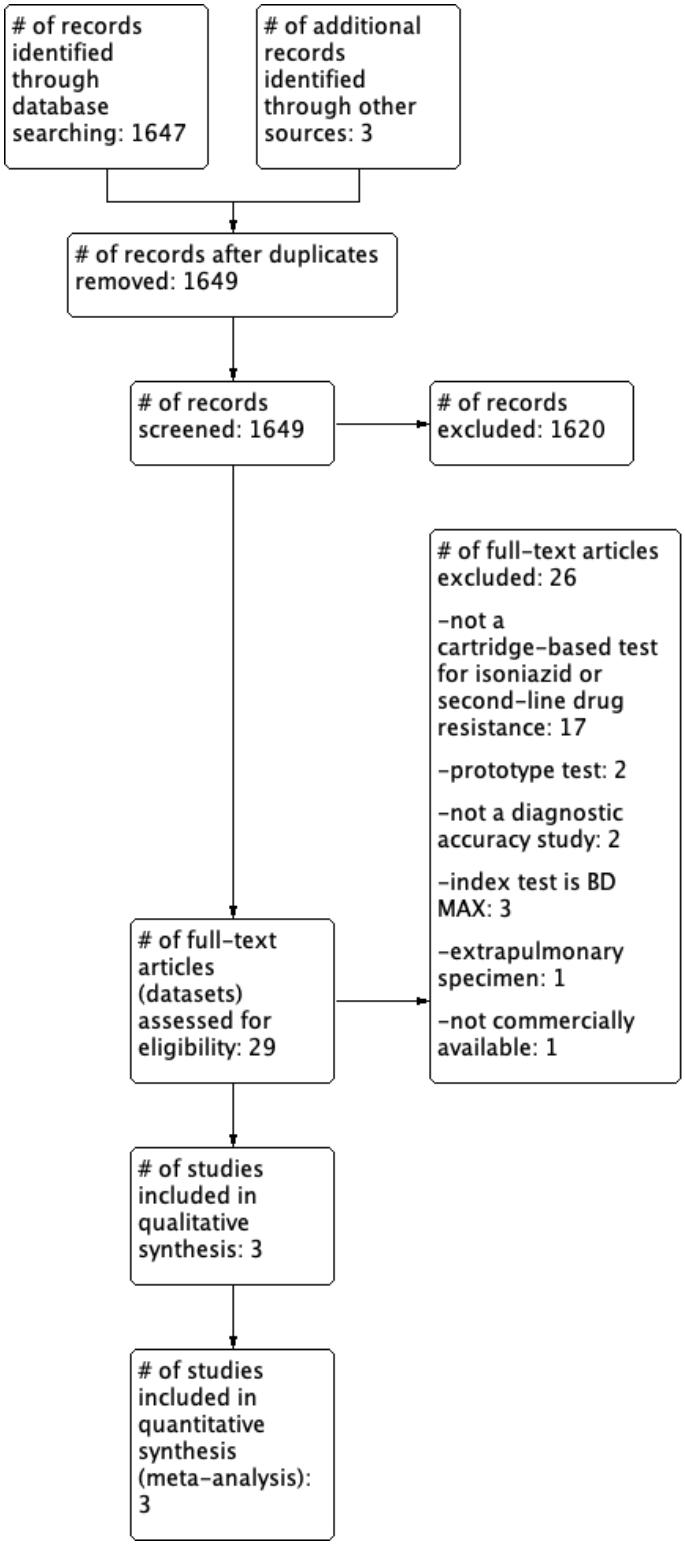
Critical concentrations and clinical breakpoints for medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant tuberculosis

Drug groups	Drug	LJ	7H10	7H11	MGIT
First-line	Isoniazid	0.2	0.2	0.2	0.1
Fluroquinolones	Levofloxacin	2.0	1.0	-	1.0
	Moxifloxacin (CC)	1.0	0.5	0.5	0.25
	Moxifloxacin (CB)	–	2.0	-	1.0

	Gatifloxacin	0.5	-	-	0.25
Second-line	Amikacin	30.0	2.0	-	1.0
Other	Ethionamide	40.0	5.0	10.0	5.0

Abbreviations: CB: critical breakpoint; CC: critical concentration

Appendix 6. Flow of studies in the review



Appendix 7. List of studies

Included studies

Cepheid 2020
Clinical evaluation of the Xpert® MTBXDR assay
Report R244C2 Xpert MTB/XDR Rev 1.0
Sponsor: Cepheid, Sunnyvale, USA

DIAMA 2020
DIAgnostics for Multidrug Resistant Tuberculosis in Africa
ClinicalTrials.gov Identifier: NCT03303963
Sponsor: Dissou Affolabi, Kigali, Rwanda

FIND 2020
Analytical Performance and Clinical Diagnostic Accuracy of the Xpert MTB/XDR Assay for TB and Expanded Resistance Detection, September 2020
ClinicalTrials.gov Identifier: NCT03728725
Sponsor: Foundation for Innovative New Diagnostics, Geneva, Switzerland

Excluded studies

1. Andreevskaya SN, Smirnova TG, Larionova EE, Andrievskaya IY, Chernousova LN, Ergeshov A. Isoniazid-resistant Mycobacterium tuberculosis: prevalence, resistance spectrum and genetic determinants of resistance. Bulletin of Russian State Medical University. 2020 (1):21-6. (Not a cartridge-based test for isoniazid or second-line drug resistance)
2. Beutler M, Plesnik S, Mihalic M, Olbrich L, Heinrich N, Schumacher S, et al. A pre-clinical validation plan to evaluate analytical sensitivities of molecular diagnostics such as BD MAX MDR-TB, Xpert MTB/Rif Ultra and FluoroType MTB. PLOS One. 2020;15(1):e0227215. (Not a diagnostic accuracy study)
3. Bisognin F, Lombardi G, Finelli C, Re MC, Dal Monte P. Simultaneous detection of Mycobacterium tuberculosis complex and resistance to rifampicin and isoniazid by MDR/MTB ELITe MGB R kit for the diagnosis of tuberculosis. PLOS One. 2020;15(5):e0232632. (Not a cartridge-based test for isoniazid or second-line drug resistance)
4. Broda A, Nikolayevskyy V, Casali N, Khan H, Bowker R, Blackwell G, et al. Experimental platform utilising melting curve technology for detection of mutations in Mycobacterium tuberculosis isolates. European Journal of Clinical Microbiology & Infectious Diseases. 2018;37(7):1273-9. (Not a cartridge-based test for isoniazid or second-line drug resistance)
5. Chakravorty S, Roh SS, Glass J, Smith LE, Simmons AM, Lund K, et al. Detection of isoniazid-, fluoroquinolone-, amikacin-, and kanamycin-resistant tuberculosis in an automated, multiplexed 10-Color assay suitable for point-of-care use. Journal of Clinical Microbiology. 2017;55(1):183-98. (Prototype test)
6. Chang Y, Kim S, Kim Y, Ei PW, Hwang D, Lee J, et al. Evaluation of the QuantaMatrix multiplexed assay platform for molecular diagnosis of multidrug- and extensively drug-resistant tuberculosis using clinical strains isolated in Myanmar. Annals of Laboratory Medicine. 2020;40(2):142-7. (Not a cartridge-based test for isoniazid or second-line drug resistance)
7. Chumpa N, Kawkitinarong K, Rotcheewaphan S, Sawatpanich A, Petsong S, Tumwasorn S, et al. Evaluation of Anyplex TM II MTB/MDR kit's performance to rapidly detect isoniazid and rifampicin resistant Mycobacterium tuberculosis from various clinical specimens. Molecular Biology Reports. 2020;47(4):2501-8. (Not a cartridge-based test for isoniazid or second-line drug resistance)
8. Ciesielczuk H, Kouvas N, North N, Buchanan R, Tiberi S. Evaluation of the BD MAX TM MDR-TB assay in a real-world setting for the diagnosis of pulmonary and extra-pulmonary TB. European Journal of Clinical Microbiology & Infectious Diseases.2020;39(7):1321-7. (Index test is BD MAX)
9. Foongladda S, Banu S, Pholwat S, Gratz J, O-Thong S, Nakkerd N, et al. Comparison of TaqMan(R) Array Card and MYCOTB(TM) with conventional phenotypic susceptibility testing in MDR-TB. International Journal of Tuberculosis and Lung Disease. 2016;20(8):1105-12. (Not a cartridge-based test for isoniazid or second-line drug resistance)
10. Galarza M, Fasabi M, Levano KS, Castillo E, Barreda N, Rodriguez M, et al. High-resolution melting analysis for molecular detection of multidrug resistance tuberculosis in Peruvian isolates. BMC Infectious Diseases. 2016;16:260. (Not a cartridge-based test for isoniazid or second-line drug resistance)
11. Han Y, Xiao N, Huang S, Qin M, Che N, Liu Z. The Application of Xpert Mycobacterium tuberculosis/rifampicin, quantitative polymerase chain reaction and high resolution melting curve in the diagnosis of superficial lymph node TB. Current Pharmaceutical Biotechnology. 2019;20(12):1044-54. (Extrapulmonary specimens)
12. Havlicek J, Dachsel B, Slickers P, Andres S, Beckert P, Feuerriegel S, et al. Rapid microarray-based detection of rifampin, isoniazid, and fluoroquinolone resistance in Mycobacterium tuberculosis by use of a single cartridge. Journal of Clinical Microbiology. 2018;56(2). (Not a cartridge-based test for isoniazid or second-line drug resistance)
13. Huang F, Dang L, Sun H, Yang H, Wu X. [A study of the value of three molecular diagnostic techniques in the diagnosis of tuberculosis]. Zhonghua jie he he hu xi za zhi = Zhonghua jiehe he huxi zazhi = Chinese Journal of

Tuberculosis and Respiratory Diseases. 2015;38(9):680-5. (Not a cartridge-based test for isoniazid or second-line drug resistance)
14. Kim S, Kim Y, Chang Y, Hirgo WK, Chang CL, Shim T-S, et al. Comparison of Quantamatrix multiplexed assay platform and GenoType MTBDR assay using smear-positive sputum specimens from patients with multidrug-resistant/extensively drug-resistant tuberculosis in South Korea. Frontiers in Microbiology. 2019;10:1075. (Not a cartridge-based test for ISONIAZID or second-line drug resistance)
15. Klotoe BJ, Molina-Moya B, Gomes HM, Gomgnimbou MK, Oliveira Suzarte L, Feres Saad MH, et al. TB-EFI, a novel 18-Plex microbead-based method for prediction of second-line drugs and ethambutol resistance in Mycobacterium tuberculosis complex. Journal of Microbiological Methods. 2018;152:10-7. (Not a cartridge-based test for isoniazid or second-line drug resistance)
16. Law ILG, Loo JFC, Kwok HC, Yeung HY, Leung CCH, Hui M, et al. Automated real-time detection of drug-resistant Mycobacterium tuberculosis on a lab-on-a-disc by recombinase polymerase amplification. Analytical Biochemistry. 2018;544:98-107. (Not a cartridge-based test for isoniazid or second-line drug resistance)
17. Lee YS, Kang MR, Jung H, Choi SB, Jo K-W, Shim TS. Performance of REBA MTB-XDR to detect extensively drug-resistant tuberculosis in an intermediate-burden country. Journal of Infection and Chemotherapy. 2015;21(5):346-51. (Not a cartridge-based test for isoniazid or second-line drug resistance)
18. Li Q, Ou XC, Pang Y, Xia H, Huang HR, Zhao B, et al. A novel automatic molecular test for detection of multidrug resistance tuberculosis in sputum specimen: A case control study. Tuberculosis (Edinburgh, Scotland). 2017;105:9-12. (Not commercially available)
19. Mokaddas EM, Ahmad S, Eldeen HS. GeneXpert MTB/RIF is superior to BBD Max MDR-TB for diagnosis of tuberculosis (TB) in a country with low incidence of multidrug-resistant TB (MDR-TB). Journal of Clinical Microbiology. 2019;57(6). (Index test is BD MAX)
20. Murray P, Cooper C, Maus C. Comparative Performance of BD MAX MDR-TB and Cepheid Xpert MTB/RIF Assays. Journal of Clinical Microbiology. 2019;57(9). (Not a diagnostic accuracy study)
21. Pang Y, Dong H, Tan Y, Deng Y, Cai X, Jing H, et al. Rapid diagnosis of MDR and XDR tuberculosis with the MeltPro TB assay in China. Scientific Reports. 2016;6:25330. (Not a cartridge-based test for isoniazid or second-line drug resistance)
22. Santos PFGD, Costa ERD, Ramalho DM, Rossetti ML, Barcellos RB, Nunes LdS, et al. Detection of tuberculosis drug resistance: a comparison by Mycobacterium tuberculosis MLPA assay versus Genotype RMTBDRplus. Memorias do Instituto Oswaldo Cruz. 2017;112(6):396-403. (Not a cartridge-based test for isoniazid or second-line drug resistance)
23. Shah M, Paradis S, Betz J, Beylis N, Bharadwaj R, Caceres T, et al. Multicenter study of the accuracy of the BD MAX TM MDR-TB assay for detection of Mycobacterium tuberculosis complex and mutations associated with resistance to rifampin and isoniazid. Clinical Infectious Diseases. 2019. (Index test is BD MAX)
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Appendix 8. Table. Characteristics of included studies

Study year	Countries/centres (High MDR Burden)	Study design	Number of patients (% detected RR)	Age of enrolment	PLHIV	Reference standard for drug resistance	Loci included in gDST reference standard
Cepheid 2020	China (yes) South Africa (yes)	cross-sectional, retrospective[1]	530 (47.9%)	≥ 15 years[2]	NR	pDST gDST composite	*katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, gyrA, gyrB, rrs*, *eis* promoter
DIAMA 2020	Benin (no) Cameroon (no) Rwanda (no)	cross-sectional, prospective	621 (48.3%)	≥ 15 years	13.3% Benin; 37.2% Rwanda	pDST	NA
FIND 2020	New Delhi (yes) Moldova (yes) Mumbai (yes) South Africa (yes)	cross-sectional, prospective	611 (80.9%)	≥ 18 years	17.5% overall, 87.1% South Africa	pDST gDST composite	*katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, gyrA, gyrB, rrs, eis* promoter

Abbreviations: NA: not applicable; NR: not reported; RR: rifampicin resistance

Footnotes

[1]. In some cases, the reference standard was performed before and in other cases after the index test.

[2]. One participant was 13 years old.

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SUPPLEMENTARY INFORMATION

Supplement A. MeltPro® XDR-TB

Background
On 30 October 2020, we were notified by the WHO Global Tuberculosis Programme about a clinical study conducted in China evaluating MeltPro® XDR-TB (Xiamen Zeesan Biotech Co., Ltd., China), a low complexity test for resistance to anti-tuberculosis drugs. The WHO provided us with a report summarizing the clinical study. We corresponded with study authors for additional information and clarifications.

MeltPro XDR-TB is a commercially available, low complexity test for detection of mutations associated with resistance to rifampicin, isoniazid, fluoroquinolones, and injectable second-line drugs. MeltPro XDR-TB is designed to detect drug resistance on specimens determined to be TB positive. MeltPro XDR-TB testing is performed using an all-in-one machine, Sanity 2.0, Figure 1. Manual pipetting is required in the preliminary sample preparation stage, and subsequent processes - nucleic acid extraction, sample loading, detection (i.e. real-time PCR), and interpretation of results - are all fully automatic. The detection of drug resistance is based on multi-color melting curve analysis.

Figure S1. Sanity 2.0

Regarding rifampicin, MeltPro XDR-TB bases detection of resistance on mutations in the *rpoB* gene.
Regarding isoniazid, MeltPro XDR-TB bases detection of resistance on mutations in the *ahpC* promoter region, *inhA* promoter region, and *katG gene.*
Regarding fluoroquinolones, MeltPro XDR-TB bases detection of resistance on mutations in the *gyrA* quinolone resistance determining region.
Regarding second-line injectable drugs, MeltPro XDR-TB bases detection of resistance on mutations in *rrs* gene and *eis* promoter region.

Search methods for identification of studies
On 4 November 2020, we ran an additional electronic search using the terms Zeesan and MeltPro. We did not identify any publications. In correspondence, the authors wrote, “We have not published relevant research reports yet. We expect to entrust the hospital with further clinical verification in the near future, and then publish relevant articles, “(personal communication, Lili Zheng, Xiamen Zeesan Biotech Co., Ltd, llzheng@zsandx.com, 10 November 2020).

Summary of the clinical study
This was a cross-sectional, prospective study in which participants were selected by convenience. The study aim was to evaluate and validate the performance of the MeltPro® XDR-TB test kit. The study authors did not collect information on participant characteristics, such as age, HIV status, and tuberculosis treatment history. Participants came from both outpatient and inpatient settings. The study was conducted in China, a high TB burden, high TB/HIV burden country, and high MDR-TB burden country.

Participants

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Sputum samples were selected from patients who had submitted specimens for culture and subsequent drug-susceptibility testing during the routine work of the clinical laboratory of the hospital. All selected samples were first tested by the MeltPro®MTB Test Kit (Xiamen Zeesan Biotech Co., Ltd.) and if found to be TB positive, the sample was eligible for inclusion. The authors stated, "In other words, samples selected for enrolment were by convenience." Samples were included if they were tuberculosis positive, had DST results, and at least 2 mL were available after the laboratory had completed other tests.

Sample size = 715
- patients with presumptive tuberculosis, n = 592, outpatient setting
- patients suspected of having XDR-TB or pre-XDR-TB, n = 123, drug-resistant ward, inpatient setting

Index test was MeltPro XDR-TB

Reference standard was MGIT DST

Outcomes were sensitivity and specificity

Indeterminate results were not included in the determination of sensitivity and specificity.

Sequencing was performed to resolve discordant index test and culture-based DST results.

Results

Methodological quality assessment
In the patient selection domain, we judged this study to be of high risk of bias because participants were selected by convenience. Regarding applicability, we rated this as unclear because demographic information was not reported. For the other QUADAS-2 domains, index test, reference standard, and flow and timing, we judged low risk of bias. Regarding applicability, we considered the index test and reference standard domains to be of low concern.

Findings

See Figure S2.

- MeltPro sensitivity was 85% for resistance to isoniazid in people with rifampicin susceptibility and 88% in people with rifampicin resistance.
- MeltPro sensitivity was 90% for resistance to fluoroquinolones in people with rifampicin susceptibility and 91% in people with rifampicin resistance.
- MeltPro sensitivity was 88% for resistance to fluoroquinolones in people with rifampicin susceptibility and 79% in people with rifampicin resistance.
- MeltPro specificity was ≥ 97% for all drugs in people with rifampicin susceptibility, but lower (86% to 90%) in people with rifampicin resistance.
- Inconclusive results: there were 27/715 (3.8%), 27/715 (3.8%) and 19/715 (3.4%) clinical sputum specimens without valid signals for isoniazid, fluoroquinolones, and amikacin, respectively, which the authors thought could be caused by low concentrations of tuberculosis bacteria.

Sequencing to resolve discordant results

Isoniazid: There were 18 samples whose DST was isoniazid sensitive but detected as isoniazid resistant by MeltPro XDR-TB. Sequencing results showed that all of them had mutations in the detection region of probes. There were 22 samples whose DST was isoniazid resistant but detected as

isoniazid sensitive by MeltPro XDR-TB. Sequencing results showed that none of them showed any mutation in the coverage area of the probes.
Fluoroquinolones: There were 20 samples whose DST was fluoroquinolone sensitive but detected as fluoroquinolone resistant by MeltPro XDR-TB. Sequencing results showed that all of them had mutations in *gyrA*. There were 10 samples whose DST was fluoroquinolone resistant but detected as fluoroquinolone sensitive by MeltPro XDR-TB. Sequencing results showed that two samples had D94G heterogenic mutation, while the remaining eight samples showed no mutation in the coverage area of the probe.
Amikacin: There were 20 samples whose DST was amikacin sensitive but detected as amikacin resistant by MeltPro XDR-TB. Sequencing results showed that all of them had mutations in *rrs* gene. There were 10 samples whose DST was amikacin resistant but detected as amikacin sensitive by MeltPro XDR-TB. Sequencing results showed that none of them showed any mutation in the coverage area of the probes.

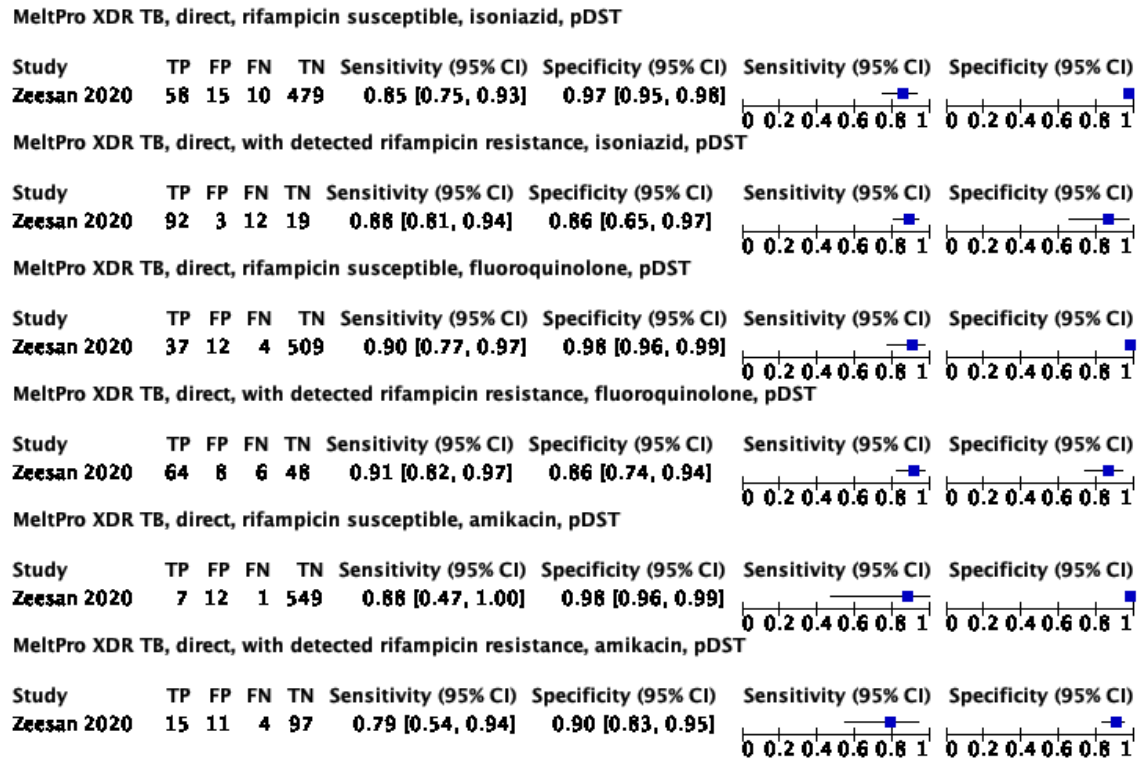

Figure S2. Forest plots of MeltPro XDR-TB sensitivity and specificity for detection of resistance to isoniazid, fluoroquinolones (levofloxacin), and amikacin by rifampicin resistance status.

Supplement B. Should Xpert MTB/XDR assay on sputum be used to diagnose PTB in people with signs and symptoms of TB?

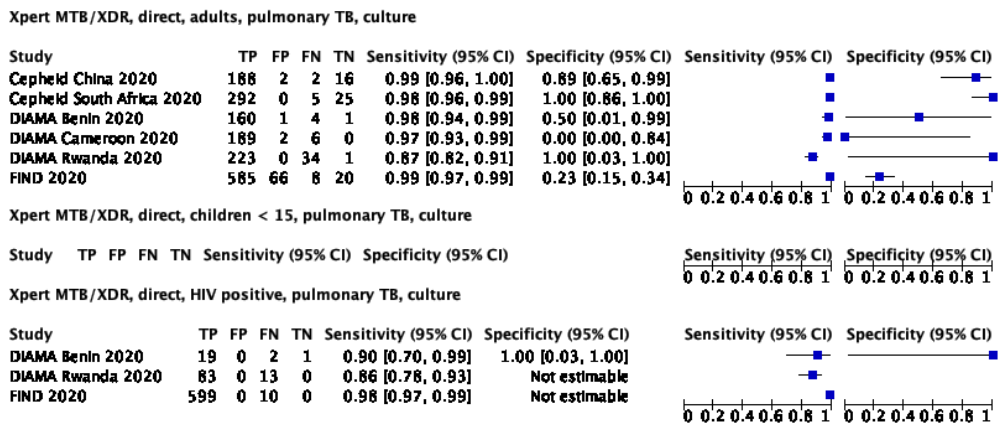

Figure. Forest plots of Xpert MTB/XDR sensitivity and specificity for the diagnosis of pulmonary tuberculosis by population, culture reference standard. Direct refers to testing directly on sputum

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Supplement C. GRADE evidence profiles

1. Question: Should MTB/XDR assay on sputum be used to diagnose INH resistance in patients with microbiologically confirmed pulmonary TB, irrespective of resistance to RIF, pDST?

Sensitivity	0.94 (95% CI: 0.89 to 0.97)
Specificity	0.98 (95% CI: 0.95 to 0.99)

Prevalences	2%	10%	15%

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested			Test accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 2%	pre-test probability of 10%	pre-test probability of 15%	
True positives (patients with INH resistance)	3 studies 994 patients	cross-sectional (cohort type accuracy study)	not serious	serious [a]	not serious [b]	not serious	none	19 (18 to 19)	94 (89 to 97)	141 (134 to 146)	⊕⊕⊕◯ MODERATE
False negatives (patients incorrectly classified as not having INH resistance)								1 (1 to 2)	6 (3 to 11)	9 (4 to 16)	
True negatives (patients without INH resistance)	3 studies 611 patients	cross-sectional (cohort type accuracy study)	not serious	serious [a]	not serious	not serious	none	960 (933 to 972)	882 (857 to 893)	833 (809 to 843)	⊕⊕⊕◯ MODERATE
False positives (patients incorrectly classified as having INH resistance)								20 (8 to 47)	18 (7 to 43)	17 (7 to 41)	

Explanations

a. We had several concerns about whether there is indirectness in the populations studied. First, the median prevalence of isoniazid resistance in the included studies was 67.2% (range, 26.8% (DIAMA, Benin) to 93.9% (FIND, Moldova), higher than the three prevalences in the GRADE table. Applicability to settings with a lower prevalence of isoniazid resistance comes with some uncertainty. Second, there are potential differences in the mutations present in isoniazid mono-resistant strains and MDR strains. That is, there are studies that suggest that a more diverse set of mutations can be found in mono-resistant strains that MDR strains. Third, although the population for this PICO question is 'irrespective of rifampicin resistance,' owing to enrollment criteria in the studies, we note that most participants were rifampicin resistant. We downgraded one level for indirectness.

b. Sensitivity estimates ranged from 81% (FIND, New Delhi) to 100% (DIAMA, Rwanda). Regarding the low sensitivity estimate in New Delhi, the study authors reported that sequencing did not show the presence of variants typically associated with resistance in many phenotypically isoniazid-resistant samples suggesting that variants not analyzed by Xpert MTB/XDR might play a role. We did not downgrade for inconsistency. This was a judgement.

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[Diagnostic Test Accuracy Protocol]

Xpert MTB/XDR for detection of pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin

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ABSTRACT

Objectives

This is a protocol for a Cochrane Review (diagnostic). The objectives are as follows:

To estimate the diagnostic accuracy of Xpert MTB/XDR for the detection of pulmonary tuberculosis in people with signs and symptoms of pulmonary tuberculosis.

To estimate the diagnostic accuracy of Xpert MTB/XDR for resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin in people irrespective of rifampicin resistance and people with detected rifampicin resistance. In these populations, pulmonary tuberculosis will have been detected by Xpert MTB/XDR (as it is a reflex test). Such populations typically will have received prior testing verifying tuberculosis with another WHO-approved test.

Secondary objectives

To compare the diagnostic accuracy of Xpert MTB/XDR by direct testing versus indirect testing (whereby Xpert MTB/XDR is performed on a *Mycobacterium tuberculosis* isolate grown from culture).

To investigate the effects of potential sources of heterogeneity on test accuracy.

For pulmonary tuberculosis, potential sources include HIV status, smear status, history of tuberculosis, treatment status (no treatment or currently on treatment), and treatment response status (culture conversion, yes or no).

For drug resistance, potential sources include the type of reference standard and history of tuberculosis treatment. In addition, for fluoroquinolone resistance, a potential source of heterogeneity is the specific drug (e.g. ofloxacin or moxifloxacin) used in the phenotypic culture-based DST (pDST) reference standard. We will also consider whether the WHO-recommended critical drug concentration was used for the pDST reference standard (WHO Critical Concentrations 2018; WHO Critical Concentrations 2021).

Regarding previously treated people, these investigations are important questions for clinical practice. For tuberculosis detection, studies have highlighted the challenges in interpreting Xpert MTB/RIF-positive and Xpert Ultra-positive results in previously treated people (Mishra

2020; Theron 2016a). As mentioned, for detection of drug resistance, previous treatment may increase the likelihood of having drug resistance.

BACKGROUND

A glossary of terms related to this Cochrane Protocol is provided in Appendix 1.

Tuberculosis is one of the top 10 causes of death worldwide; and people with tuberculosis are often poor and disadvantaged, have more limited access to health care, and often face stigma and discrimination (WHO Global Tuberculosis Report 2020). In 2019, 10 million people developed active tuberculosis disease (WHO Global Tuberculosis Report 2020). When tuberculosis is detected early and effectively treated, the disease is largely curable. However, the World Health Organization (WHO) estimates that nearly one-third of individuals with active tuberculosis go undiagnosed and unreported and do not receive the care they need (WHO Global Tuberculosis Report 2020). The gap is even wider for drug-resistant tuberculosis (Naidoo 2017; Subbaraman 2016; WHO Global Tuberculosis Report 2020). Globally, in 2019, there were 465,000 (estimated) new cases of rifampicin-resistant tuberculosis with three countries accounting for around one half of the cases: India (27%), China (14%), and the Russian Federation (8%) (WHO Global Tuberculosis Report 2020). However, only 38% of the number of people estimated to have drug-resistant tuberculosis were ultimately enrolled in multidrug-resistant tuberculosis (MDR-TB) treatment programmes and of these, only 57% were successfully treated (WHO Global Tuberculosis Report 2020).

Tuberculosis drug resistance is a critical public health problem presenting a major challenge for patients, healthcare workers, and health services. Importantly, drug-resistant tuberculosis threatens to impede progress towards the targets set by the End TB Strategy of the WHO (WHO End TB 2015), and the health-related targets described in United Nations Sustainable Development Goal 3 (United Nations Sustainable Development Goals 2030). MDR-TB (defined below) and extensively drug-resistant tuberculosis (XDR-TB, defined below) are responsible for almost a third of deaths owing to antimicrobial resistance globally (O'Neill 2016).

The following classification system is used for tuberculosis drug resistance (WHO Consolidated Guidelines (Module 4) 2020; WHO Extensively Drug-Resistant Tuberculosis 2021). Of note, in 2021, the WHO updated the definitions for XDR-TB and pre-XDR-TB to draw attention to the seriousness of these conditions and take into consideration new and repurposed drugs.

- Rifampicin-resistant tuberculosis is caused by *Mycobacterium tuberculosis (M tuberculosis*) strains resistant to rifampicin (resistance caused by mutations in a small region of the *rpoB* gene). These strains may be susceptible or resistant to isoniazid (i.e. MDR-TB), or resistant to other first-line or second-line tuberculosis medicines.
- Rifampicin-susceptible, isoniazid-resistant tuberculosis is tuberculosis caused by *M tuberculosis* strains resistant to isoniazid and susceptible to rifampicin.
- MDR-TB is tuberculosis caused by *M tuberculosis* strains that are resistant to at least rifampicin and isoniazid, two of the core tuberculosis medicines. A subset of people with rifampicin-resistant tuberculosis will have MDR-TB.
- Pre-XDR-TB is caused by *M tuberculosis* strains that fulfil the definition of MDR-TB or rifampicin-resistant tuberculosis, and which are also resistant to any fluoroquinolone. The fluoroquinolones include levofloxacin and moxifloxacin, because these are the fluoroquinolones currently recommended by WHO for inclusion in shorter and longer regimens.
- XDR-TB is caused by *M tuberculosis* strains that fulfil the definition of MDR-TB or rifampicin-resistant tuberculosis and which are also resistant to any fluoroquinolone and at least one additional Group A drug. The Group A drugs are currently levofloxacin or moxifloxacin, bedaquiline, and linezolid. Owing to the recent change in the definition, the present version of Xpert MTB/XDR is not capable of detecting WHO-defined XDR-TB.

MDR-TB/rifampicin-resistant tuberculosis

Globally, in 2019, 3% of new tuberculosis cases and 18% of previously treated tuberculosis cases had MDR-TB/rifampicin-resistant tuberculosis; the percentage of these cases that were MDR-TB was 78% (WHO Global Tuberculosis Report 2020). While the availability of drug susceptibility testing (DST) using culture-based and molecular methods is increasing, coverage and availability of these technologies varies widely. For example, globally in 2019, only 59% of bacteriologically confirmed new tuberculosis cases were tested for rifampicin resistance. Among people with rifampicin resistance, 71% were tested for resistance to fluoroquinolones, though coverage varied from around 35% in the Western Pacific to nearly 90% in Europe (WHO Global Tuberculosis Report 2020).

The development and scale-up of the Xpert MTB/RIF assay was a major step towards improving detection of tuberculosis and rifampicin resistance globally. The assay simultaneously tests for both conditions and consists of a mostly automated hands-off method making it feasible to position and scale in many high tuberculosis burden settings. Xpert MTB/RIF has, however, been met with limitations. In 2019, of 48 high tuberculosis burden countries, only 18 (38%) reported that a WHO-recommended rapid diagnostic (which includes Xpert MTB/RIF) had been used as the initial test for more than 50% of the tuberculosis cases who were notified (WHO Global Tuberculosis Report 2020). These 48 countries are in one or more of the three lists of high tuberculosis burden, high TB/HIV burden, and high MDR-TB burden countries.

Isoniazid mono-resistant tuberculosis

Globally in 2019, 13% of new tuberculosis cases and 17% of previously treated tuberculosis cases had isoniazid resistance (WHO Global Tuberculosis Report 2020), yet susceptibility testing for isoniazid (a critical first-line drug) is often only performed in people who are rifampicin resistant. Although in high MDR-TB settings the presence of rifampicin resistance alone has served as a proxy for MDR-TB and the basis for treatment decisions (Liu 2019; Nasiri 2018), emerging data suggest that in some settings, DST for rifampicin resistance has suboptimal specificity for MDR-TB. This means that testing for resistance to isoniazid is increasingly important. For example, one study in the eastern Democratic Republic of the Congo found one in five people with rifampicin resistance to be isoniazid susceptible when tested using the GenoType MTBDR*plus*, a line probe assay (Bisimwa 2020), and the most recent South African National Survey of Drug Resistance found hotspots of rifampicin mono-resistance, where the prevalence ratio of such cases exceeded that of MDR-TB by up to 30% (NICD 2016). Conversely, isoniazid resistance in the presence of rifampicin susceptibility (isoniazid mono-resistance)

is also increasingly recognized as another emerging challenge in managing tuberculosis as it is an important enabler of MDR-TB.

Susceptibility testing for isoniazid is more complicated than for rifampicin owing to a greater variety of resistance-associated variants (including large deletions) across several genes (e.g. loci in *katG*, *inhA*, and *ahpC*). Information on these mutations may not be routinely available in lower resource settings despite evidence showing that isoniazid resistance is associated with a three-fold increased risk of poor treatment outcomes (Espinal 2000), and should be treated with an intensified regimen including a fluoroquinolone (WHO Consolidated Guidelines (Module 4) 2020).

Treatment of tuberculosis

Tuberculosis treatment regimens must contain multiple drugs to which the organisms are susceptible to cure tuberculosis and avoid selection for drug resistance. Compared to treatment for drug-susceptible tuberculosis (tuberculosis caused by *M tuberculosis strains* not suspected or confirmed to be drug resistant), treatment for MDR-TB is longer and more complex, toxic, and expensive with a median cost per person of USD 5659 as against USD 860 for drug-susceptible tuberculosis (WHO Global Tuberculosis Report 2020). MDR-TB regimens may be standardized (all patients are treated with the same regimen) or individualized (patients receive only drugs to which laboratory testing confirms susceptibility). Individualized regimens have higher rates of treatment success (Orenstein 2009); however, until 2018, all MDR-TB regimens employed at least five second-line drugs for a duration of up to 24 months. This arduous regimen resulted in significant drug toxicity, suboptimal adherence, and substantial loss to follow-up (Walker 2019). Fluoroquinolones and aminoglycosides formed the backbone of such regimens and treatment outcomes are significantly worse in people infected with tuberculosis strains that exhibit resistance to one or both of these drug classes (Falzon 2013). However, the introduction of novel or repurposed drugs, such as bedaquiline, clofazimine, and linezolid, has revolutionized the efficacy of longer regimens, dispensing with the need for injectable drugs and promising to deliver shorter all-oral regimens (WHO Consolidated Guidelines (Module 4) 2020). In treating MDR-TB/rifampicin-resistant tuberculosis, fluoroquinolones have an essential role and are also important for protecting second-line drugs such as bedaquiline.

In a recent landmark clinical trial, a seven-drug shorter standardized regimen of nine to 12 months showed non-inferiority to longer regimens (Nunn 2019). Although, the seven-drug shorter standardized regimen saves patients from a year or more of daily tuberculosis drugs, it still requires four months of an injectable drug, associated with pain at the injection site and a potential for serious adverse events (e.g. hearing loss and kidney damage) (Churchyard 2019). Uptake of this regimen was initially limited because the regimen's efficacy may be impacted by undetected resistance to individual component drugs if DST is unavailable and, as mentioned, it still contains an injectable drug for the initial four months (WHO Global Tuberculosis Report 2020). Based on additional observational data, the WHO subsequently recommended that the injectable agent may be replaced by bedaquiline (WHO Rapid Communication 2019). Recently, a six-month three-drug regimen, based on bedaquiline, linezolid, and the novel drug pretomanid, achieved high rates of treatment success in an observational cohort of people with XDR-TB (Conradie 2020). Early diagnosis and characterization of resistance is a prerequisite for delivery of these new treatment regimens for drug-resistant tuberculosis as quickly as possible to those who could benefit, drawing attention to the need for faster, cheaper, and more easily deployable diagnostic technologies.

Though individualization of MDR-TB treatment regimens according to DST is promoted by guidance, gaps in infrastructure and personnel to support DST based on culture, the conventional method used to detect resistance to first- and second-line tuberculosis drugs, may in part explain why, of an estimated 465,000 new cases of MDR-TB/rifampicin-resistant tuberculosis in 2019, only 44% were detected and notified (WHO Global Tuberculosis Report 2020). The WHO recommends that rapid techniques be used as the initial diagnostic tests to detect tuberculosis and rifampicin resistance in order to minimize delays in starting appropriate treatment (WHO Consolidated Guidelines (Module 3) 2020). The multiplexed nature of these new technologies theoretically permits susceptibility to be detected accurately and comprehensively for a single drug (where variants in multiple genes may cause resistance) and to several different drugs, with their own set of distinct resistance determinants. The flexibility of this technology offers the possibility of simultaneous detection of resistance mutations important for multiple drugs other than rifampicin.

This systematic review will evaluate Xpert MTB/XDR, a newly developed nucleic acid amplification test (NAAT) that detects pulmonary tuberculosis and resistance to tuberculosis drugs other than rifampicin, namely isoniazid, fluoroquinolones, ethionamide, and amikacin.

Target condition being diagnosed

The target conditions are pulmonary tuberculosis and resistance to four tuberculosis drugs: isoniazid, fluoroquinolones, ethionamide, and amikacin.

Pulmonary tuberculosis

Tuberculosis is caused by one of several bacterial species belonging to the *Mycobacterium tuberculosis* complex of which the main human pathogen is *M tuberculosis* (Pai 2016). Tuberculosis most commonly affects the lungs (pulmonary tuberculosis), but may affect any organ or tissue outside of the lungs, such as the brain or spine (extrapulmonary tuberculosis). Signs and symptoms of pulmonary tuberculosis include a persistent cough (for at least two weeks), fever, night sweats, weight loss, haemoptysis (coughing up blood), and fatigue. Tuberculosis is spread from person to person through the air.

Tuberculosis drug resistance

Isoniazid resistance: isoniazid is an important and commonly used first-line drug for tuberculosis. Isoniazid affects mycolic acid (cell wall) synthesis. The drug is taken orally (Curry International Tuberculosis Center 2016; Pai 2016).

Fluoroquinolone resistance: the fluoroquinolones are a class of antibiotics widely used to treat lower respiratory infections. They are second-line drugs for tuberculosis. Ofloxacin is an earlier generation fluoroquinolone and moxifloxacin, levofloxacin, and gatifloxacin are later generation fluoroquinolones. The fluoroquinolones act by relaxing the supercoiling of DNA strands through inhibition of the enzyme DNA gyrase (Chitra 2020). These

drugs are mainly taken orally (Curry International Tuberculosis Center 2016; Pai 2016).

Ethionamide resistance: ethionamide is a second-line drug for tuberculosis in the thioamide drug class. Ethionamide affects mycolic acid synthesis. The drug is taken orally (Curry International Tuberculosis Center 2016; Pai 2016).

Amikacin resistance: amikacin is a second-line drug for tuberculosis in the aminoglycoside drug class, along with kanamycin and capreomycin. These drugs act by inhibiting protein synthesis. Amikacin is mainly administered by intramuscular injection (Curry International Tuberculosis Center 2016; Pai 2016). When a second-line injectable drug is needed in a treatment regimen, amikacin is the preferred drug (WHO Consolidated Guidelines (Module 4) 2020).

In addition to the above drug resistances, Xpert MTB/XDR tests for kanamycin resistance and capreomycin resistance. We are not planning to include these target conditions in this review because kanamycin and capreomycin are less relevant for treating tuberculosis now that an all-oral regimen is recommended (see Index tests).

Index test(s)

The index test is the Xpert MTB/XDR assay (Xpert MTB/XDR, Cepheid, Sunnyvale, USA). Xpert MTB/XDR is a rapid, automated NAAT of low complexity. Low complexity refers to a situation where no special infrastructure is required and basic laboratory skills are suitable to run the test. However, equipment may still be required.

NAATs are molecular systems that can detect small quantities of genetic material (DNA or ribonucleic acid (RNA)) extracted from micro-organisms, such as *M tuberculosis,* by amplifying the quantities to an amount large enough to study in detail. The key advantage of NAATs is that they are rapid diagnostic tests, potentially providing results in a few hours. A variety of molecular amplification methods are available, of which polymerase chain reaction (PCR) is the most common.

Xpert MTB/XDR is a cartridge-based test where almost all processes (such as DNA extraction or PCR procedures (or both)) are performed within the container linked to the diagnostic platform. An initial manual specimen treatment step is needed to add sample reagent to the specimen. Sample reagent helps homogenize the specimen and prepare it for in-cartridge DNA extraction. For homogenization to be effective, there is a 15-minute incubation period with occasional mixing by hand.

Xpert MTB/XDR detects *M tuberculosis* complex DNA and mutations associated with resistance to isoniazid, fluoroquinolones (ofloxacin, moxifloxacin, levofloxacin, gatifloxacin), second-line injectable drugs (amikacin, kanamycin, capreomycin), and ethionamide in a single test. This review will not include detection of resistance to kanamycin and capreomycin because, with the adoption of new treatment regimens using all-oral medicines, the second-line injectable drugs are less relevant (Bainomugisa 2020). However, we will include detection of resistance to amikacin because, when a second-line injectable drug is needed in a treatment regimen, amikacin is the preferred drug (WHO Consolidated Guidelines (Module 4) 2020).

Xpert MTB/XDR is intended for use as a reflex test for a specimen (unprocessed sputum or concentrated sputum sediments) that is determined to be MTB positive (Cepheid package insert 2020). We note that 'MTB' in the Cepheid package insert refers to *M tuberculosis* complex. The term reflex test refers to a diagnostic approach in which an initial test meets predetermined criteria (e.g. outside of the normal range), and a second test is performed automatically, usually without any dedicated request from the healthcare worker. The test could also be performed on culture isolates; however, this is not stated by the manufacturer as an intended use case. Several advantages of the assay have been described by the manufacturer.

- Faster time to result for detection of drug resistance.
- Results in less than 90 minutes.
- Similar easy-to-use process as Xpert MTB/RIF and Xpert Ultra.
- Run on existing GeneXpert platforms equipped with 10-colour modules.

Xpert MTB/RIF (Theron 2016a) and Xpert Ultra (Mishra 2020) have diminished specificity in people with a history of tuberculosis. Importantly, people with previously treated tuberculosis have a higher risk of drug resistance compared to people who are treatment naive (WHO Global Tuberculosis Report 2020), which means that detection of drug resistance is more likely to be performed in such people. Therefore, it is important that in previously treated people, Xpert MTB/XDR accuracy is evaluated for tuberculosis detection as the test may report results for drug resistance in people who are detected as MTB-positive but are culture-negative. Xpert MTB/XDR suppresses the reporting of results for the detection of drug resistance if it fails to detect MTB in the same reaction.

The limit of detection for *M tuberculosis* by Xpert MTB/XDR is 136 colony-forming units (CFU)/mL in unprocessed sputum (Cepheid package insert 2020). This is similar to the limit of detection of Xpert MTB/RIF (112.6 CFU/mL), but higher than that of Xpert Ultra (15.6 CFU/mL) (Chakravorty 2017). The manufacturer states that "Specimens with MTB Trace DETECTED results when tested with the Xpert MTB/RIF Ultra Assay are expected to be below the limit of detection of the MTB/XDR Assay and are not recommended for testing with the Xpert MTB/XDR Assay" (Cepheid package insert 2020). As with Xpert MTB/RIF and Xpert Ultra, Xpert MTB/XDR detects both live and dead bacteria.

The following information comes from the manufacturer's package insert (Cepheid package insert 2020).

- Regarding isoniazid, Xpert MTB/XDR bases detection of resistance on mutations in the *katG* and *fabG1* genes, *oxyR-ahpC* intergenic region, and *inhA* promoter region of the MTB genome.
- Regarding fluoroquinolones, Xpert MTB/XDR bases detection of resistance on mutations in the *gyrA* and *gyrB* quinolone resistance determining regions of the MTB genome.
- Regarding ethionamide, Xpert MTB/XDR bases detection of resistance on mutations in the *inhA* promoter region of the MTB genome. In addition, it is noted that "mutations conferring ethionamide resistance are reported to be present in genomic regions not targeted by the Xpert MTB/XDR assay" (Cepheid package insert 2020). Of interest, Brossier and colleagues found that 22/47 (47%) of ethionamide-resistant clinical isolates had mutations in *ethA*. Hence, the absence of mutations in the *inhA* promoter region does not preclude ethionamide resistance (Brossier 2011). Cepheid acknowledges

that reporting ethionamide resistance based only on the detection of mutations in the *inhA* promoter region is a known limitation that may limit sensitivity, though specificity may be unaffected.

- Regarding amikacin, Xpert MTB/XDR bases detection of resistance on mutations in the *rrs* region of the MTB genome.

Interpretation of results for Xpert MTB/XDR

Xpert MTB/XDR can report results as MTB NOT DETECTED or MTB DETECTED. If results are reported as MTB DETECTED, each drug is reported as resistance DETECTED or NOT DETECTED. If results are reported as MTB NOT DETECTED, or INVALID, ERROR, or NO RESULT, then no drug resistance results are reported (Figure 1).

Figure 1. Possible test results for each target in the Xpert MTB/XDR assay. Copyright © [2020] [Cepheid Inc]: reproduced with permission.
[a]Ethionamide will not provide an indeterminant by assay design.

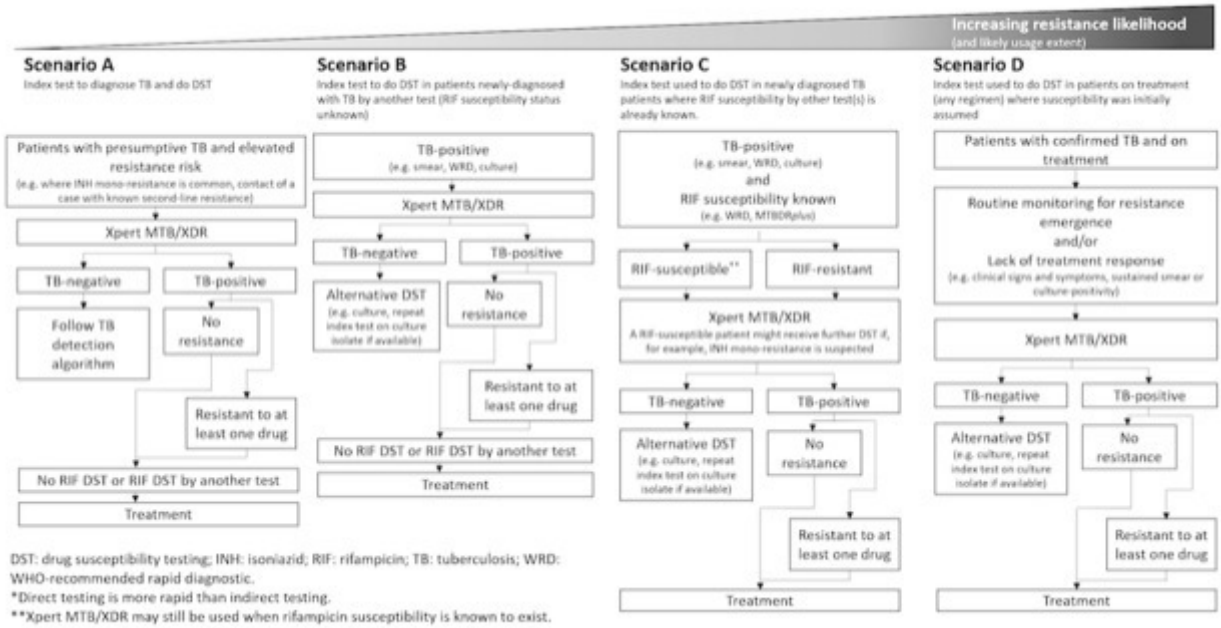
AMK: amikacin; CAP: capreomycin; ETH: ethionamide; FLQ: fluoroquinolone; INH: isoniazid; KAN: kanamycin; MTB: *Mycobacterium tuberculosis*.

Drug Class	Result Call
N/A	INVALID/ERROR/NO RESULT
	MTB DETECTED
	MTB NOT DETECTED
Isoniazid	Low INH Resistance DETECTED
	INH Resistance DETECTED
	INH Resistance NOT DETECTED
	INH Resistance INDETERMINATE
Fluoroquinolone	Low FLQ Resistance DETECTED
	FLQ Resistance DETECTED
	FLQ Resistance NOT DETECTED
	FLQ Resistance INDETERMINATE
Amikacin	AMK Resistance DETECTED
	AMK Resistance NOT DETECTED
	AMK Resistance INDETERMINATE
Kanamycin	KAN Resistance DETECTED
	KAN Resistance NOT DETECTED
	KAN Resistance INDETERMINATE
Capreomycin	CAP Resistance DETECTED
	CAP Resistance NOT DETECTED
	CAP Resistance INDETERMINATE
Ethionamide[a]	ETH Resistance DETECTED
	ETH Resistance NOT DETECTED

Clinical pathway

Figure 2 outlines several scenarios in the clinical pathway for positioning the Xpert MTB/XDR.

Figure 2. Clinical pathway for Xpert MTB/XDR (index test)



- Scenario A. Xpert MTB/XDR used for detection of pulmonary tuberculosis and drug resistance. The role of Xpert MTB/XDR would be replacement for WHO-recommended rapid molecular tests for tuberculosis, such as Xpert MTB/RIF, Xpert MTB/RIF Ultra, and Truenat MTB and MTB Plus assays.
- Scenario B. Xpert MTB/XDR used for detection of drug resistance in people newly diagnosed with pulmonary tuberculosis by another test and whose rifampicin susceptibility is unknown. The role of Xpert MTB/XDR would be replacement for phenotypic culture-based DST (pDST) in people diagnosed with tuberculosis irrespective of rifampicin resistance. pDST is the conventional method used to detect resistance to first- and second-line tuberculosis drugs.
- Scenario C. Xpert MTB/XDR used for detection of drug resistance in people newly diagnosed with pulmonary tuberculosis and rifampicin resistance by other tests (although less likely, it is possible that Xpert MTB/XDR may still be used even when known rifampicin susceptibility exists). The role of Xpert MTB/XDR would be replacement for pDST in people diagnosed with tuberculosis and rifampicin resistance.
- Scenario D. Xpert MTB/XDR used for detection of drug resistance in people being treated for pulmonary tuberculosis. The role of Xpert MTB/XDR would be replacement for existing tests or used in combination with existing tests for treatment monitoring.

For scenarios B and C, although not typical, it is possible that pDST may be used after an Xpert MTB/XDR result. For example, a rifampicin-susceptible patient might receive pDST if isoniazid mono-resistance is still suspected.

For each scenario, we expect direct testing to be favoured over indirect testing; however, indirect testing remains possible if, for example, direct testing initially failed. In addition, we note that the timing of DST for rifampicin can be before, in parallel, or after Xpert MTB/XDR is applied.

The downstream consequences of testing include the following.

- True positive (TP): people would benefit from rapid diagnosis and early initiation of appropriate tuberculosis treatment.
- True negative (TN): people would be spared unnecessary treatment and would benefit from reassurance and pursuit of an alternative diagnosis.
- False positive (FP): people would likely experience anxiety, morbidity from additional testing, possible delays in further diagnostic evaluation, and prolonged and unnecessary treatment with a less effective second-line regimen that may have more adverse effects.
- False negative (FN): people would be at increased risk of morbidity and mortality, and there would be continued risk of community transmission of drug-resistant tuberculosis.

Alternative test(s)

Alternative molecular methods for drug resistance include the commercial line probe assays, a category of genotypic (molecular) tests. These methods have considerable advantages for scaling up programmatic management and surveillance of drug-resistant tuberculosis, offering speed of diagnosis (one or two days), standardized testing, potential for high through-put, and fewer requirements for laboratory biosafety. Drawbacks are that line probe assays require skills and infrastructure only available in intermediate and central laboratories (Unitaid 2017).

Line probe assays for first-line drugs include GenoType MTBDR*plus* assay (MTBDR*plus*, Hain Lifescience, Nehren, Germany), and the Nipro NTM+MDRTB detection kit 2 (Nipro, Tokyo, Japan). These assays detect the presence of mutations associated with drug resistance to isoniazid and rifampicin. MTBDR*plus* is the most widely studied line probe assay. The WHO recommends that for people with a sputum smear-positive specimen or a culture isolate of *M tuberculosis* complex, commercial molecular line probe assays may be used as the initial test instead of pDST to detect resistance to rifampicin and isoniazid (conditional recommendation, moderate certainty in the evidence for the test's accuracy) (WHO Consolidated Guidelines (Module 3) 2020).

Line probe assays for second-line drugs include GenoType MTBDR*sl* assay (MTBDR*sl*, Hain Lifescience, Nehren, Germany). MTBDR*sl* detects specific mutations associated with resistance to fluoroquinolones and second-line injectable drugs. MTBDR*sl* version 2.0 identifies the mutations detected by version 1.0 but does not detect any ethambutol mutations. The test may be performed on a culture isolate or a patient specimen, which eliminates delays associated with culture. Version 1.0 requires a smear-positive specimen, while version 2.0 may use a smear-positive or smear-negative specimen. The WHO recommends that for people with confirmed MDR-TB/rifampicin-resistant tuberculosis, line probe assays for second-line drugs may be used as the initial test, instead of pDST, to detect resistance to fluoroquinolones (conditional recommendation; moderate certainty in the evidence for test accuracy for direct testing of sputum specimens; low certainty in the evidence for test accuracy for indirect testing of *M tuberculosis* cultures). And for people with confirmed MDR-TB/rifampicin-resistant tuberculosis, line probe assays for second-line drugs may be used as the initial test, instead of pDST, to detect resistance to the second-line injectable drugs (conditional recommendation; low certainty in the evidence for test accuracy for direct testing of sputum specimens; very low certainty in the evidence for test accuracy for indirect testing of *M tuberculosis* cultures) (WHO 2016; WHO Consolidated Guidelines (Module 3) 2020).

Rationale

In December 2019, based on new evidence on the management of drug-resistant tuberculosis, the WHO issued recommendations that all people with MDR-TB or rifampicin-resistant tuberculosis, including those who are also resistant to fluoroquinolones, may benefit from effective all-oral treatment regimens, either shorter or longer. People with isoniazid mono-resistant tuberculosis may also benefit from modified regimens that included fluoroquinolones (WHO Consolidated Guidelines (Module 4) 2020). Therefore, in people with tuberculosis and rifampicin-resistant tuberculosis it is critically important to perform additional resistance testing to at least isoniazid and the fluoroquinolones in order to guide treatment decisions. However, to ensure people who start new regimens have a high chance of successful treatment, susceptibilities to as many relevant drugs as possible should be diagnosed early.

The rationale for performing this Cochrane Review is to estimate the diagnostic accuracy of Xpert MTB/XDR, one assay in a new class of diagnostic tests. In 2020, we performed a systematic review to inform updated WHO guidelines on the use of NAATs (including Xpert MTB/XDR) to detect tuberculosis and drug-resistant tuberculosis (WHO Rapid Communication 2021). This Cochrane Review will expand on these efforts.

OBJECTIVES

To estimate the diagnostic accuracy of Xpert MTB/XDR for the detection of pulmonary tuberculosis in people with signs and symptoms of pulmonary tuberculosis.

To estimate the diagnostic accuracy of Xpert MTB/XDR for resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin in people irrespective of rifampicin resistance and people with detected rifampicin resistance. In these populations, pulmonary tuberculosis will have been detected by Xpert MTB/XDR (as it is a reflex test). Such populations typically will have received prior testing verifying tuberculosis with another WHO-approved test.

Secondary objectives

To compare the diagnostic accuracy of Xpert MTB/XDR by direct testing versus indirect testing (whereby Xpert MTB/XDR is performed on a *Mycobacterium tuberculosis* isolate grown from culture).

To investigate the effects of potential sources of heterogeneity on test accuracy.

For pulmonary tuberculosis, potential sources include HIV status, smear status, history of tuberculosis, treatment status (no treatment or currently on treatment), and treatment response status (culture conversion, yes or no).

For drug resistance, potential sources include the type of reference standard and history of tuberculosis treatment. In addition, for fluoroquinolone resistance, a potential source of heterogeneity is the specific drug (e.g. ofloxacin or moxifloxacin) used in the phenotypic culture-based DST (pDST) reference standard. We will also consider whether the WHO-recommended critical drug concentration was used for the pDST reference standard (WHO Critical Concentrations 2018; WHO Critical Concentrations 2021).

Regarding previously treated people, these investigations are important questions for clinical practice. For tuberculosis detection, studies have highlighted the challenges in interpreting Xpert MTB/RIF-positive and Xpert Ultra-positive results in previously treated people (Mishra 2020; Theron 2016a). As mentioned, for detection of drug resistance, previous treatment may increase the likelihood of having drug resistance.

METHODS

Criteria for considering studies for this review

Types of studies

We will include cross-sectional studies and cohort studies that assessed the diagnostic accuracy of Xpert MTB/XDR for both pulmonary tuberculosis and tuberculosis drug resistance, or tuberculosis drug resistance alone. We will include diagnostic accuracy studies in which cases and controls were sampled from a single source population (referred to as a single-gate design). We will exclude case-control studies where cases and controls were sampled from different populations (referred to as a two-gate design). A two-gate design is prone to bias, particularly

when a study enrols participants with severe disease and healthy participants without disease (Rutjes 2005). We will only include studies that reported data comparing Xpert MTB/XDR to an acceptable reference standard (defined below) from which we could extract TP, FP, FN, and TN values.

The PICO format for formulating review questions (Participants, Intervention, Comparator, Outcome) is useful for questions on the impact or effectiveness of testing on patient-important outcomes. However, for diagnostic test accuracy reviews, we will use a modification that better fits test accuracy studies, that is, PIT (Participants, Index test(s), Target condition).

Participants

We will include adults (aged 15 years and older) with presumptive pulmonary tuberculosis. Presumptive tuberculosis refers to "a patient who presents with symptoms or signs suggestive of tuberculosis" (WHO Definitions and Reporting 2020). In addition, we will include people with microbiologically diagnosed pulmonary tuberculosis, meaning people who have received prior testing verifying tuberculosis. Participants with pulmonary tuberculosis will be included whether or not they have documented rifampicin resistance (i.e. irrespective of rifampicin resistance or with detected rifampicin resistance). Regarding detected rifampicin resistance, in this case, people often receive investigation for resistance to isoniazid or any of the second-line tuberculosis drugs for selection of an appropriate drug regimen. Furthermore, DST for drugs other than rifampicin may be important in settings where isoniazid mono-resistance is frequent or a person has known contact with a rifampicin-susceptible case with second-line drug resistance.

We will include HIV-positive and HIV-negative people. Regarding tuberculosis treatment, we will include people who, at enrolment, did not report a history of tuberculosis treatment, reported a history of tuberculosis treatment, or were receiving tuberculosis treatment.

We will include studies that assessed the diagnostic accuracy of Xpert MTB/XDR using sputum (expectorated or induced) consistent with the intended use of the manufacturer, and studies from all types of health facilities and all laboratory levels (peripheral, intermediate, and central) from all countries.

Index tests

The index test is Xpert MTB/XDR. Interpretation of results for Xpert MTB/XDR is shown in Figure 1.

Target conditions

The target conditions are pulmonary tuberculosis and resistance to four tuberculosis drugs: isoniazid, fluoroquinolones, ethionamide, and amikacin.

We have included pulmonary tuberculosis as a target condition because some users may want to do the test to detect pulmonary tuberculosis, in particular, in areas where isoniazid mono-resistance is also likely.

Regarding fluoroquinolone resistance, subcategories of this target condition include ofloxacin resistance, moxifloxacin resistance, levofloxacin resistance, and gatifloxacin resistance.

If we identify a study assessing kanamycin resistance, we will report the results and note this addition in the 'Differences between protocol and review' section. We will not include streptomycin resistance as a target condition because Xpert MTB/XDR does not detect resistance to streptomycin. Of note, streptomycin DST is not routinely performed. Streptomycin is considered a second-line drug for tuberculosis. However, streptomycin is only used as a substitute for amikacin in the following situations: when amikacin is not available; when there is confirmed resistance to amikacin, but confirmed susceptibility to streptomycin; and when an all-oral regimen cannot be constituted (WHO Consolidated Guidelines (Module 4) 2020).

We will report the detection of resistance to individual fluoroquinolone drugs (see Investigations of heterogeneity) when that drug was used for pDST because, although drugs within drug classes often have similar molecular properties, they are not perfectly cross-resistant. Molecular DST, also referred to as genotypic DST (gDST), cannot generally distinguish with high confidence resistance to individual drugs within a class, especially the fluoroquinolones, which have high cross-resistance owing to variants within the *gyrA* hotspot region (Zignol 2016).

Reference standards

Detection of pulmonary tuberculosis

The reference standard is solid or liquid mycobacterial culture or both.

- The presence of pulmonary tuberculosis is defined as a positive *M tuberculosis* culture.
- The absence of pulmonary tuberculosis is defined as a negative *M tuberculosis* culture.

Detection of tuberculosis drug resistance

We include three reference standards, pDST, gDST, and a composite reference standard. These methods are used to determine whether *M tuberculosis* cells are susceptible or resistant to tuberculosis drugs.

- pDST alone.
  - The presence of drug resistance is defined as drug[a] resistance detected by pDST.
  - The absence of drug resistance (referred to as being drug susceptible) is defined as drug[a] resistance not detected by pDST.
- gDST alone.
  - The presence of drug resistance is defined as drug[a] resistance detected by gDST.
  - The absence of drug resistance is defined as drug[a] resistance not detected by gDST.
- Composite reference standard.
  - The presence of drug resistance is defined as drug[a] resistance detected by either pDST or gDST.
  - The absence of drug resistance is defined as drug[a] resistance not detected by pDST and gDST.

[a]Drugs include isoniazid, fluoroquinolones, ethionamide, and amikacin.

Regarding pDST, pDST is performed on *M tuberculosis* cells (isolates) cultured from specimens and is the conventional method used to detect resistance to first- and second-line tuberculosis drugs. We will use pDST as the main reference standard for isoniazid resistance, fluoroquinolone resistance, and amikacin resistance.

Regarding gDST, we will use gDST as the main reference standard for ethionamide resistance because there is considerable overlap in the minimum inhibitory concentrations (MICs) of *M tuberculosis* isolates with and without resistance-causing variants and a pDST reference standard might not correctly classify the target condition.

gDST can be targeted to predefined loci or be genome-wide. Targeted gDST traditionally comprised the Sanger sequencing method, which is still used in research laboratories. However, Sanger sequencing is limited in the number of reads (about 100 reads) that can be attained (depth). Consequently, its ability to detect minority populations of resistant bacilli (which may still be detected by a pDST reference standard) is compromised (Metcalfe 2017). Recent advances in targeted sequencing methods include SMOR (single molecule-overlapping reads; Colman 2015) and Deeplex (Jouet 2021), which are ultra-deep methods that sequence a target more than 1000 times. The deep sequencing methods therefore have greater resolution than the Sanger sequencing method. They also appear robust when performed on DNA extracted directly from a specimen (versus a culture isolate), especially if that specimen is rich in mycobacteria. As with any method that is targeted (limited to a certain number of loci for a drug), targeted gDST will miss phenotypic resistance causing mutations that occur outside of the target, simply because it is not designed to evaluate that region.

Genome-wide gDST typically refers to whole genome sequencing. Importantly, although whole genome sequencing could have been performed, some investigators might only use it in a manner equivalent to targeted sequencing of certain regions. For example, if whole genome sequencing coverage was poor in a region known to be important for resistance, but otherwise adequate in other regions important for resistance, whole genome sequencing will serve in this scenario as a limited form of targeted sequencing.

Importantly, culture, which is often used for pDST or to generate sufficient DNA for some gDST methods (such as whole genome sequencing), involves growing an inoculum in the absence of a drug. This could lead to resistant bacilli present in the original specimen diminishing below the limit of detection of the reference standard method due to competition with the other drug-susceptible bacilli in the inoculum and, potentially, any fitness costs associated with resistance. Fitness costs refer to reduced competitive ability (such as growth rate or virulence) when antibiotics are absent.

Regarding the composite reference standard, the classification rule is based on one of the two reference tests (pDST or gDST) being positive for drug resistance. Consequently, it is not necessary to perform a second reference test once the result of the first reference test is positive (resistant). Hence, the second reference standard is only necessary in people with a negative (susceptible) or failed test result (e.g. indeterminate, contaminated) on the first reference standard test (Rutjes 2005). The composite reference result will be considered drug susceptible when pDST reported drug susceptibility and gDST did not detect a drug-associated resistant mutation.

In QUADAS-2, we consider the reliability of these different reference standards for individual drugs (Heyckendorf 2018).

Search methods for identification of studies

We will attempt to identify all relevant studies regardless of language or publication status (published, unpublished, in press, ongoing).

Electronic searches

We will search the following databases using the search terms and strategy described in Appendix 2. We will limit our searches to 2015 onwards as Xpert MTB/XDR is a newly developed assay launched in July 2020.

- Cochrane Infectious Diseases Group Specialized Register.
- MEDLINE (Ovid).
- Embase (Ovid).
- Science Citation Index – Expanded, Conference Proceedings Citation Index – Science (CPCI-S), and BIOSIS Previews; all three from the Web of Science.
- Scopus (Elsevier).
- Latin American Caribbean Health Sciences Literature (LILACS) (BIREME; lilacs.bvsalud.org/en/).

We will also search ClinicalTrials.gov, the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/trialsearch), and the International Standard Randomized Controlled Trials Number (ISRCTN) registry (www.isrctn.com/) for trials in progress, and ProQuest Dissertations & Theses A&I for dissertations, using terms for tuberculosis and Xpert MTB/XDR.

Searching other resources

We will review reference lists of included articles and any relevant review articles identified through the above methods. We will also contact researchers at the Foundation for Innovative New Diagnostics (FIND), the WHO Global TB Programme, and other experts in the field of tuberculosis diagnostics for information on ongoing and unpublished studies.

Data collection and analysis

Selection of studies

We will use Covidence to manage the selection of studies (Covidence). Two review authors will independently scrutinize titles and abstracts identified from literature searching to identify potentially eligible studies. We will retrieve the article of any citation identified by one of the review authors for full-text review. Then, two review authors will independently assess articles for inclusion using predefined inclusion and exclusion criteria. We will resolve disagreements by discussion with a third review author. We will record all studies excluded after full-text assessment and their reasons for exclusion in the characteristics of excluded studies table. We will illustrate the study selection process in a PRISMA diagram (Page 2021; Salameh 2020). We will collate multiple reports of the same study, so that each study, rather than each report, is the unit of interest in the review.

Data extraction and management

We will develop a standardized data extraction form and pilot the form using two included studies. We have developed a draft data

extraction form based on experience with a previous Cochrane Review (Theron 2016b; Appendix 3). Based upon the pilot, we will finalize the form. Using the finalized form, two review authors will independently extract data from the included studies. We will enter the extracted data into an Excel database on password-protected computers. Data will be secured in the Liverpool School of Tropical Medicine 'Archive' drives of Cochrane Infectious Diseases Group for future review updates.

We will extract the following information for each included study.

- Details of study: first author; publication year; country where testing was performed; specimen country origin; setting (primary care laboratory, hospital laboratory, reference laboratory); study design; manner of participant selection; number of participants enrolled; number of participants for whom results available.
- Characteristics of participants: age; HIV status; smear status; history of tuberculosis; treatment status; treatment conversion status.
- Target conditions.
- Reference standards.
- Details of specimen: type (such as expectorated or induced sputum or culture isolate); condition (fresh or frozen).
- Details of the conduction of the assay, whether performed on a sputum specimen (direct testing) or performed on the culture isolate grown from the patient specimen (indirect testing).
- Details of outcomes: the number of TP, FP, FN, and TN results.
- Whether the WHO-recommended critical drug concentration was used for the pDST reference standard (WHO Critical Concentrations 2018; WHO Critical Concentrations 2021). We will use the currently recommended concentration for each drug to classify studies, not the recommended concentration at the time of the study.
- Inconclusive test results.

We will resolve any discrepancies by discussion with a third review author.

We will classify country income status as low-income, middle-income, or high-income, according to the World Bank List of Economies (World Bank 2020). In addition, we will classify 'country' as being high burden or not high burden for tuberculosis, TB/HIV, or MDR-TB, according to the post-2015 era classification by the WHO (WHO Global Tuberculosis Report 2020). A country may be classified as high burden for one, two, or all three of the high burden categories.

We will follow Cochrane policy, which states that "authors of primary studies will not extract data from their own study or studies. Instead, another author will extract these data, and check the interpretation against the study report and any available study registration details or protocol."

Assessment of methodological quality

We will use the QUADAS-2 tool, tailored to this review, to assess the quality of the included studies (Whiting 2011). QUADAS-2 consists of four domains: patient selection, index test, reference standard, and flow and timing. We will assess all domains for risks of bias and the first three domains for concerns regarding applicability. Two review authors will independently complete QUADAS-2 and resolve disagreements through discussion, if needed, with a third review author. We will present the results of this quality assessment in text, tables, and graphs. We have developed signalling questions based on experience with a previous Cochrane Review (Theron 2016b). The preliminary tool tailored to this review is in Appendix 4.

We will assess studies for conflicts of interest using the Tool for Addressing Conflicts of Interest in Trials (TACIT) if this tool is available while we perform the review (Lundh 2020).

Statistical analysis and data synthesis

We will perform descriptive analyses for the results of the included studies using Stata (Stata), and display key study characteristics in the characteristics of included studies table. We will plot estimates of the studies' observed sensitivities and specificities in forest plots with 95% confidence intervals (CIs) and in receiver operating characteristic (ROC) space using Review Manager 5 (Review Manager 2020).

For pulmonary tuberculosis, where adequate data are available, we will combine data using meta-analysis by fitting a bivariate random-effects model (Chu 2006; Macaskill 2010; Reitsma 2005), using Stata with the metandi and meqrlogit commands (Stata). Heterogeneity is to be expected in results of test accuracy studies; hence, we will use random-effects methods to provide an estimate of the averaged accuracy of Xpert MTB/XDR and to describe the variability in this effect (Macaskill 2010). Specifically, the bivariate random-effects approach allows us to calculate the pooled estimates of sensitivity and specificity while accounting for: variation in sensitivity and specificity estimates within individual studies; correlation between sensitivity and specificity across studies; and variation in sensitivity and specificity between studies.

For drug resistance, for the primary objective (i.e. direct testing of clinical specimens), we will take the following analytical approach. We will create analysis groups by stratifying the analyses by population (irrespective of rifampicin resistance or detected rifampicin resistance); target condition (drug resistance); and type of reference standard (pDST, gDST, and composite reference standard). For some drugs, where the variants associated with resistance are not well understood, pDST is considered a better reference standard against which to measure sensitivity and specificity. Conversely, for other drugs, gDST is considered a better reference standard owing to technical challenges with pDST. Generally, as Xpert MTB/XDR is a DNA-based (genotypic) test, when pDST rather than gDST is used as the reference standard, we expect sensitivity estimates to be reduced and specificity to be increased; however, we will evaluate this while performing the review.

Within each analysis group (e.g. Xpert MTB/XDR, irrespective of rifampicin resistance, isoniazid, pDST), we will plot estimates of the studies' observed sensitivities and specificities in forest plots with 95% CIs and in ROC space, including by type of reference standard, using Review Manager 5 (Review Manager 2020). Where adequate data are available, we will combine data using meta-analysis by fitting a bivariate random-effects model (for the reasons explained above) (Chu 2006; Macaskill 2010; Reitsma 2005), using Stata with the metandi and meqrlogit commands (Stata). In situations with few studies or sparse data, we will perform meta-analysis where appropriate by reducing the bivariate model to two univariate random-effects logistic regression models by assuming no correlation between sensitivity and specificity (Takwoingi 2017).

When we observe little or no heterogeneity on forest plots and summary receiver operating characteristic (SROC) plots, and the analyses consequently do not converge, we will further simplify the models into fixed-effect models by eliminating the random-effects parameters for sensitivity or specificity, or both sensitivity and specificity (Takwoingi 2017). In situations where all studies in a meta-analysis reported a sensitivity of 100% or specificity of 100%, we will use simple pooling by summing the numbers of TPs and total resistant cases to calculate sensitivity or the numbers of TNs and total susceptible cases to calculate specificity, as required. In these situations when needed, we will determine 95% CIs using the Newcombe-Wilson method (Newcombe 1998). We will perform all analyses stratified by population and type of reference standard.

Regarding the fluoroquinolone drug class, we will estimate test accuracy for the drug class as a whole, as well as for the specific drugs (e.g. ofloxacin and moxifloxacin) within the drug class (see Investigations of heterogeneity). For the entire fluoroquinolone drug class, we will define fluoroquinolone-resistant or fluoroquinolone-susceptible against pDST where any fluoroquinolone drug is classified as being resistant or susceptible. We will use this approach because the fluoroquinolones have high cross-resistance owing to variants within the *gyrA* hotspot region (Zignol 2016).

For multicentre studies, we anticipate that there may be variability in terms of how laboratory practices are carried out between different centres. For this reason, in the first instance, we will perform meta-analyses at centre level (i.e. treating each centre as a separate study), if data are available to take this approach. If we decide, based on our assessments of heterogeneity and methodological quality, that it is appropriate to include data from the multiple centres as one study, then we will perform a sensitivity analysis at the study level to investigate the impact of this analysis approach on our overall results.

A secondary objective is to compare the diagnostic accuracy of Xpert MTB/XDR by direct testing versus indirect testing (whereby Xpert MTB/XDR is run on an *M tuberculosis* isolate grown from culture). We will do this by adding a covariate for the type of testing to the model. We will assess the significance of the differences in sensitivity and specificity estimates between studies in which Xpert MTB/XDR was performed by direct testing or indirect testing by a likelihood ratio test comparing models with and without covariate terms. We will only perform comparative analyses for those studies that made direct comparisons between test evaluations with the same participants. Comparative studies are preferred to non-comparative studies when deriving evidence of diagnostic test accuracy (Takwoingi 2013).

We will also extract data on discrepant analysis, where in a given study, gene sequencing was applied only to resolve discordant Xpert MTB/XDR-pDST results. We will analyse these data separately in a narrative summary.

Approach to inconclusive index test results

A test result may be uninterpretable when the main diagnostic feature of the test result is invalid, missing, or obstructed (Shinkins 2013). Invalid inconclusive test results are caused by a property intrinsic to the test. Missing results mean no test result has been recorded though the participant ideally should have had a test result and been included in the study.

For Xpert MTB/XDR, the manufacturer defines two types of invalid inconclusive results, non-determinate and indeterminate.

A non-determinate Xpert MTB/XDR test result is one that results in an Error, Invalid, or No Result and can be due to an operator error, instrument, or cartridge issue (Cepheid package insert 2020). These three options are automatically generated results (despite the one being called a "No Result") and the underlying reason for such a non-determinate is often not specified. The non-determinate Xpert MTB/XDR test results pertain only to the detection of tuberculosis.

An indeterminate Xpert MTB/XDR test result is one that indicates that resistance to a given drug could not definitively be detected based on the test's algorithm (Cepheid package insert 2020). This means that, based on quality control criteria, the test was unable to confidently report this particular result and the software suppressed the reporting of this (there is no conclusive evidence that this failure of quality control criteria is more or less likely to occur in a true resistant or true susceptible sample). The same cartridge can be indeterminate for one drug but not another – for example if the probes binding to *gyrA* for the fluoroquinolone displayed aberrant behaviour (and is hence classified as indeterminate) but the other probes in the reaction for other targets behaved okay. The indeterminate Xpert MTB/XDR test results pertain only to the detection of drug resistance.

For both types of invalid inconclusive result (defined by the manufacturer), we will exclude these from our analyses of diagnostic test accuracy.

In addition, where data are available, we will report when Xpert MTB/XDR does not detect tuberculosis to begin with (missed cases).

We plan to summarize the data so that we can consider the frequency of inconclusive results (before and after a repeat test), and whether there were any imbalances in the frequency of inconclusive results between TPs and TNs. This will allow us to comment at the review stage on the likelihood of bias impacting our results. We will use the following approach to describe these different types of results.

Xpert MTB/XDR MTB NOT DETECTED

Among specimens with pDST (reference standard) results available, we will determine the percentage that were Xpert MTB/XDR MTB NOT DETECTED. Among specimens with results reported as Xpert MTB/XDR MTB NOT DETECTED, we will further determine the percentage that were resistant or susceptible by the reference standard.

Xpert MTB/XDR NON-DETERMINATE

Among specimens initially tested, we will determine the percentage of Xpert MTB/XDR NON-DETERMINATE results and, of these, the number of ERROR, INVALID, and NO RESULT results. We will also determine the percentage of non-determinate results remaining following retesting.

Xpert MTB/XDR INDETERMINATE

Among specimens reporting Xpert MTB/XDR MTB DETECTED, we will determine the percentage that were Xpert MTB/XDR INDETERMINATE (drug resistance is only evaluated when MTB is detected). Among specimens with results reported as Xpert MTB/

XDR INDETERMINATE, we will further determine the percentage that were resistant or susceptible by the reference standard.

Investigations of heterogeneity

For each target condition, we will investigate heterogeneity through visual examination of forest plots of sensitivity and specificity. Then, if sufficient studies are available, we will explore the possible influence of prespecified covariates by adding these covariates to the meta-analysis models described above. We will assess the significance of the difference in test accuracy according to each covariate by performing a likelihood ratio test comparing models with and without covariate terms.

For detection of pulmonary tuberculosis, we will investigate the following.

- HIV status, positive or negative.
- Smear status, positive or negative.
- History of tuberculosis, yes or no.
- Treatment status, no treatment or currently receiving treatment.
- Treatment response status, culture conversion, yes or no.

For detection of drug resistance, we will investigate the following.

- Smear status, positive or negative.
- The specific drug (e.g. ofloxacin or moxifloxacin) used in the pDST reference standard used to determine fluoroquinolone resistance.
- Was the WHO-recommended critical drug concentration used for the pDST reference standard (WHO Critical Concentrations 2018; WHO Critical Concentrations 2021), yes or no? As mentioned, we will use the currently recommended concentration for each drug to classify studies, not the recommended concentration at the time of the study (see Data extraction and management).

All covariates will be categorical.

Sensitivity analyses

For our primary analyses using the pDST reference standard, we will perform sensitivity analyses for QUADAS-2 items to explore whether the accuracy estimates were robust with respect to the methodological quality of the studies. We will include the following signalling questions.

- Was a consecutive or random sample of participants/specimens enrolled?
- Were the reference standard results interpreted without knowledge of the results of the index test results?
- Was the test applied in the manner recommended by the manufacturer (index test domain, low concern about applicability)?

We may also perform sensitivity analyses where we analyse data from multicentre studies as a single study (see Statistical analysis and data synthesis).

Assessment of reporting bias

We will not conduct formal assessment of publication bias using methods such as funnel plots or regression tests, because such techniques have not been helpful for diagnostic test accuracy studies (Macaskill 2010).

Summary of findings and assessment of the certainty of the evidence

We will assess the certainty of evidence using the GRADE approach for diagnostic studies (Balshem 2011; Schünemann 2008; Schünemann 2016). As recommended, we will rate the certainty of evidence as high (not downgraded), moderate (downgraded by one level), low (downgraded by two levels), or very low (downgraded by more than two levels) based on five domains: risk of bias, indirectness, inconsistency, imprecision, and publication bias. For each outcome, the certainty of evidence will start as high when there are high-quality studies (cross-sectional or cohort studies) that enrolled participants with diagnostic uncertainty. If we find a reason for downgrading, we will use our judgement to classify the reason as either serious (downgraded by one level) or very serious (downgraded by two levels). At least two review authors will discuss judgements and apply GRADE using the following methods (GRADEpro GDT; Schünemann 2020a; Schünemann 2020b).

- Risk of bias: we will use QUADAS-2 to assess risk of bias.
- Indirectness: we will assess indirectness in relation to the population (including disease spectrum), setting, intervention (index test), and outcomes (accuracy measures). We will also use prevalence of the target condition as a guide to whether there was indirectness in the population.
- Inconsistency: GRADE recommends downgrading for unexplained inconsistency in sensitivity and specificity estimates. We will carry out prespecified analyses to investigate potential sources of heterogeneity and downgrade when we cannot explain the inconsistency in the accuracy estimates.
- Imprecision: we will consider a precise estimate to be one that would allow a clinically meaningful decision. We will consider the width of the CI and ask ourselves, 'Would we make a different decision if the lower or upper boundary of the CI represented the truth?' In addition, we will determine projected ranges for TP, FN, TN, and FP for the prevalence of resistance to a given drug and make judgements on imprecision from these calculations.
- Publication bias: we will consider the comprehensiveness of the literature search and outreach to researchers in tuberculosis, the presence of only studies that produce precise estimates of high accuracy despite small sample size, and knowledge about studies that were conducted, but are not published.

We will present results in summary of findings tables for each target condition. A summary of findings table allows for presentation of the findings of the review in a clear, transparent, and structured format, as well as key information regarding the certainty of evidence. We will create summary of findings tables using GRADEpro (GRADEpro GDT).

The summary of findings tables will include the following details.

- The review question and its components, population, (prior tests), setting, index test(s), and reference standard: pDST for isoniazid resistance, fluoroquinolone resistance, and amikacin resistance; and gDST for ethionamide resistance.
- Summary estimates of sensitivity and specificity and 95% CIs.
- The number of included studies and participants contributing to the estimates of sensitivity and specificity.

- Prevalences of the target condition with an explanation of why the prevalences have been chosen.
- An assessment of the certainty of the evidence (GRADE).
- Explanations for downgrading, as needed.

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APPENDICES

Appendix 1. Glossary of terms related to drug resistance testing

Amplification

Amplification is replication of a DNA fragment to generate copies. Both the original and the newly synthesized copies can be described as the amplicons.

Codon

A codon is a sequence of three DNA or ribonucleic acid (RNA) bases that corresponds to a specific amino acid or a signal to start or stop transcription or translation. The DNA in coding regions of the genome is read in groups of three bases (A, G, C, T).

Critical concentration

The critical concentration of a tuberculosis agent has been adopted and modified from international convention. The critical concentration is defined as the lowest concentration of a tuberculosis agent in vitro that will inhibit the growth of 99% of phenotypically wild type strains of *M tuberculosis* complex.

Culture isolate

Culture isolate refers to *M tuberculosis* cells from a clinical specimen that have been grown. For tuberculosis diagnosis, a volume of the clinical specimen is processed and incubated under conditions that promote *M tuberculosis* growth. The cells that are grown are referred to a culture isolate.

DNA sequencing

DNA sequencing is a process to determine the nucleotide (A, G, C, T) sequence of fragments of DNA. By comparison of DNA sequences from distinct tuberculosis isolates, variations known as mutations can be identified. Some mutations in *M tuberculosis* are known to be associated with drug resistance.

Drug susceptibility testing

Drug susceptibility tests determine whether *M tuberculosis* cells are susceptible or resistant to antibiotics. Testing may be undertaken using phenotypic or genotypic analyses.

eis promoter

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to second-line injectable drugs, amikacin and kanamycin.

fabG1

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to isoniazid.

Genotypic drug susceptibility testing (gDST)

Genotypic testing involves detecting predetermined mutations in DNA that are known to make the organism resistant to a drug. When mutations causing drug resistance are unknown, genotypic DST is not useful.

gyrA

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to fluoroquinolones.

gyrB

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to fluoroquinolones.

Heteroresistance

Heteroresistance is defined as resistance to certain antibiotics in a subset of a larger microbial population that is generally considered susceptible to these antibiotics according to traditional phenotypic drug susceptibility testing.

Indeterminate test result

An indeterminate Xpert MTB/XDR test result is one that indicates that resistance to a given drug could not definitively be detected based on the test's algorithm.

***inhA* promoter**

Gene target included in the Xpert MTB/XDR test to detect MTB and resistance to isoniazid and ethionamide. Mutations in the *inhA* promoter region of TB are known to confer low-level resistance to isoniazid and high-level cross-resistance to ethionamide.

Intergenic region

Is a region of DNA sequence located between genes and a subset of non-coding DNA. Some intergenic regions act to control coding regions (genes) nearby.

katG

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to isoniazid.

Locus

A locus is the position of a genetic feature in the DNA sequence, like a genetic street address. Loci are standardized between genomes by reference to a common reference genome, such as H37Rv for *M tuberculosis*.

Microbiologically confirmed

Refers to a biological specimen that is positive by culture or a World Health Organization-recommended rapid molecular test, such as Xpert MTB/RIF, Xpert Ultra, or Truenat MTB.

Mutation

A mutation is a change in a DNA sequence. Mutations can result from DNA copying mistakes made during cell division, exposure to ionizing radiation, exposure to chemicals called mutagens, or infection by viruses.

Non-determinate test result

A non-determinate Xpert MTB/XDR test result is one that results in an Error, Invalid, or No Result and can be due to an operator error, instrument, or cartridge issue.

***oxyR-ahpC* intergenic region**

Gene targets included in the Xpert MTB/XDR test to detect mutations that confer resistance to isoniazid.

Phenotypic drug susceptibility testing (pDST)

Phenotypic testing requires growth of *M tuberculosis* in the presence of antibiotics at a specific concentration that will inhibit the growth of a susceptible organism or have no impact on growth of a resistant organism.

Presumptive tuberculosis

Presumptive tuberculosis refers to "a patient who presents with symptoms or signs suggestive of tuberculosis" (WHO Definitions and Reporting 2020).

Promoter region

A promoter region is a sequence of DNA where the transcriptional machinery binds before transcribing the DNA into RNA that may then be translated into an amino acid sequence.

Reflex test

The term reflex test refers to a diagnostic approach in which an initial test meets predetermined criteria (e.g. outside of the normal range), and a second test is performed automatically, usually without a request from the health care worker. For example, a urinalysis may be followed by a culture (reflex test) if in the urine, the presence of nitrites is detected or the number of white blood cells is increased suggesting an infection. In the context of tuberculosis, culture may be used as a reflex test in a person living with HIV who has a Xpert MTB/RIF Ultra-negative result.

Resistance-determining region

A region of the *M tuberculosis* genome where mutations commonly cause resistance to a specific drug.

rrs

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to second-line injectable drugs, amikacin, kanamycin, and capreomycin.

Sanger sequencing

Technique for DNA sequencing based upon the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication, also known as 'the chain termination method.'

Targeted gene sequencing

The process for detecting predetermined mutations in DNA or genomic regions.

Whole genome sequencing (WGS)

The process of determining the complete genome sequence for a given organism at one time through next-generation sequencing methods. This method can determine the order of most nucleotides in a given genome and detect any variations relative to a reference genome using bioinformatics analyses.

Appendix 2. Detailed search strategy

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <2015 to present>

Search strategy:

---

1 Extensively Drug-Resistant Tuberculosis/ or Tuberculosis, Multidrug-Resistant/ or Tuberculosis, Pulmonary/ or Mycobacterium Tuberculosis/

2 (tuberculosis adj3 (lung or pulmonary)).mp. or

3 (tuberculosis adj3 respiratory).mp.

4.(tuberculosis adj3 (drug resistan* or multidrug resistan* or mdr or xdr)).mp.

5 (isoniazid adj3 resistance or isoniazid adj3 resistant).mp.

6 (Ethionamide adj3 resistance) or (ethionamide adj3 resistant).mp

7 (Amikacin adj3 resistance) or (amikacin adj3 resistant).mp

8 ((Fluoroquinolone adj3 resistance) or (Fluoroquinolone adj3 resistant)).mp.

9 (Second-line injectable drug adj3 resistance).mp.

10 (Second-line injectable drug adj3 resistant).mp.

11 ((SLID adj3 resistance) or (SLID adj3 resistant)).mp.

12 (MDR-TB or XDR-TB).mp.

13 ((isoniazid or fluoroquinolone or "second-line injectable drug" or SLID) adj3 (monoresist* or mono-resist*).mp.

14 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13

15 (cartridge adj3 test*).mp.

16 cartridge*.ab. or cartridge*.ti.

17 (Molbio or Truenat or Cepheid or Xpert* or Bioneer or Hain).mp.

18 Genexpert*.mp.

19 exp Point-of-Care Systems/

20 drug susceptibility test*.mp. or drug resistance test*.mp or (rapid adj3 (detect* or test* or diagnos*)).mp. or (poc or poct or "point of care").mp.

21 15 or 16 or 17 or 18 or 19 or 20

22 14 and 21

23 limit 22 to yr="2015 -Current"

This is the preliminary search strategy for MEDLINE (Ovid). We will adapt it for other electronic databases and report all search strategies in full in the final version of the review.

Appendix 3. Data extraction form

Study	
Name of data extractor	1 – SP 2 – KRS 3 – other, specify GT, MdV, GD
First author	
Corresponding author and email	
Was author contacted?	1 – yes 2 – no If yes, dates(s)
Title of paper	
Year (of publication)	
Year (study start date)	

(Continued)

Language	1 – English 2 – other If other, specify:
Was the study conducted without industry sponsorship?	1 – yes 2 – no 9 – unknown/not reported
If industry sponsorship was present, select one item from the list	Answers ordered from least to most industry involvement 1 – donation of test for use in study 2 – test at a special preferred price 3 – receipt of educational support, grants, or speaking fees 4 – financial relationship – author is employee/consultant/stock-holder 5 – involvement in design, analysis, or manuscript production
Study addresses question A (detection of isoniazid only), B (detection of second-line only), (detection of both isoniazid and second-line) C	1 – A 2 – B 3 – C Circle as many options as required
What was the aim of this study in authors' own words?	
Country of laboratory where test was run	
World Bank Classification of laboratory country	1 – low 2 – middle 3 – high 8 – other
Laboratory setting; describe as written in the paper	1 – primary care laboratory 2 – intermediate-level laboratory 3 – central-level laboratory 8 – other, specify 9 – unknown/not reported
Study design	1 – cross-sectional 2 – cohort 3 – single gate diagnostic study 8 – other, specify

(Continued)

		9 – unknown/not reported
Participant selection		1 – consecutive 2 – random 3 – convenience 8 – other, specify 9 – unknown/not reported
Direction of study data collection		1 – prospective 2 – retrospective 3 – both 9 – unknown/not reported
Comments about study design		
Number after screening by exclusion and inclusion criteria		9 – unknown/not reported
Number included in analysis (# screened – # exclusions)		9 – unknown/not reported
Did the study include specimens and/or culture isolates for testing?		1 – specimens 2 – isolates 3 – both 9 – unknown/not reported
Characteristics of participants		
Age	mean SD median IQR range 9 – unknown/not reported	
Gender	male female total # females/total (%) 9 – unknown/not reported	
HIV status	positive negative unknown total # HIV positive/total (%)	

(Continued)	9 – unknown/not reported
Previous tuberculosis	yes no unknown total # previous tuberculosis/total (%) = 9 – unknown/not reported
Type of participants/specimens tested	1 – presumptive tuberculosis 2 – irrespective of rifampicin resistance 3 – with detected rifampicin resistance 8 – other, specify: 9 – unknown/not reported
Reference standards 1 – pDST 2 – gDST 3 – composite The composite reference standard is pDST and gDST, where at least one component test is positive.	
Isoniazid	1 – pDST (specify type and critical concentrations) 2 – sequencing of the *katG, inhA promoter,* and *fabG1* gene 3 – both 1 and 2 in all specimens (specify culture information in 1) 9 -unknown/not reported 1a – MGIT, LJ, other 1b – isoniazid critical concentration MGIT – 0.1 WHO concentration LJ – 0.2 WHO concentration
Fluoroquinolones	1 – pDST (specify type and critical concentrations) 2 – sequencing of the *gyrA* and *gyrB* gene 3 – both 1 and 2 in all specimens (specify culture info in 1) 9 – unknown/not reported 1a – MGIT, LJ, other 1b – drugs used for this class and critical concentration Levofloxacin MGIT – 1.0 WHO concentration

(Continued)

	LJ – 2.0 WHO concentration
	Moxifloxacin (critical concentration)
	MGIT – 0.25 WHO concentration
	LJ – 1.0 WHO concentration
	Moxifloxacin (clinical breakpoint)
	7H10 – 2.0 WHO concentration
	MGIT – 1.0 WHO concentration
Ethionamide	1 – pDST (specify type and critical concentrations)
	2 – sequencing of the *inhA promoter* gene
	3 – both 1 and 2 in all specimens (specify culture information in 1)
	9 – unknown/not reported
	1a – MGIT, LJ, other
	1b – ethionamide critical concentration
	MGIT – 5.0 WHO concentration
	LJ – 40.0 WHO concentration
Amikacin	1 – pDST (specify type and critical concentrations)
	2 – sequencing of the *rrs* gene
	3 – both 1 and 2 in all specimens (specify culture info in 1)
	9 – unknown/not reported
	1a – MGIT, LJ, other
	1b – amikacin critical concentration
	MGIT – 1.0 WHO concentration
	LJ – 30.0 WHO concentration
Test information	
Was microscopy used?	1 – yes
	2 – no
	9 – unknown/not reported
Smear status of specimens (if applicable)	positive
	negative
	unknown
	total
Specimen information	

(Continued)

Type of specimen (may include expectorated sputum) if test performed directly on a specimen	1 – all expectorated 2 – all induced 3 – both types 8 – other 9 – unknown/not reported describe
Were results for Xpert MTB/XDR and culture obtained using the same specimen?	1 – yes 2 – no 3 – not applicable 9 – unknown/not reported
Pretreatment processing procedure if performed for Xpert MTB/XDR specimen	1 – none 2 – NALC-NaOH 3 – NaOH (Petroff) 8 – other 9 – unknown/not reported
For Xpert MTB/XDR specimen, what was the condition of the specimen when tested?	1 – fresh 2 – frozen 3 – both 9 – unknown/not reported
If fresh, specify:	1 – tested after storage at room temperature or refrigerated within 48 hours of collection 2 – tested after storage at room temperature or refrigerated > 48 hours after collection 9 – unknown/not reported
If frozen, specify:	1 – tested after frozen < 1 year of storage 2 – tested frozen ≥ 1 year storage 9 – unknown/not reported
Proportion contaminated cultures, if provided:	= # of contaminated cultures total # cultures performed 9 – unknown/not reported
Proportion inconclusive sequencing results, if provided (does not apply to discrepant analysis)	= # of inconclusive sequencing total # sequencing performed 9 – unknown/not reported

(Continued)

Were patient-important outcomes evaluated?	1 – yes 2 – no 9 – unknown/not reported
Time to diagnosis and Time to report	Isoniazid Fluoroquinolone Ethionamide Amikacin 9 – unknown (45 days (27–122 days) for liquid culture)
Time to treatment initiation	Isoniazid Fluoroquinolone Ethionamide Amikacin 9 – unknown

Tables

TB detection		Culture		
		Yes	No	Total
Xpert MTB/XDR Result	Positive			
	Negative			
	Total			

Isoniazid resistance, direct testing, in people irrespective of rifampicin resistance

Isoniazid, all		pDST		
		Yes	No	Total
Xpert MTB/XDR Result	Positive			
	Negative			
	Total			

Isoniazid, smear positive		pDST		
		Yes	No	Total
Xpert MTB/XDR Result	Positive			
	Negative			
	Total			

Isoniazid, smear negative		pDST		
		Yes	No	Total
Xpert MTB/XDR Result	Positive			
	Negative			
	Total			

Add tables as needed

Abbreviations: gDST: genotypic drug susceptibility testing; IQR: interquartile range; LJ: Löwenstein Jensen; MGIT: Mycobacteria Growth Indicator Tube; pDST: phenotypic drug susceptibility testing; SD: standard deviation; WHO: World Health Organization.

Appendix 4. QUADAS-2 tailored to the review

Domain 1: patient selection

Detection of tuberculosis

Risk of bias: could the selection of patients have introduced bias?

Signalling question 1: was a consecutive or random sample of patients enrolled?

We will answer yes if the study enrolled a consecutive or random sample of eligible participants; no if the study selected participants by convenience; and unclear if the study did not report the manner of participant selection or we could not determine this.

Signalling question 2: was a case-control design avoided?

We will answer yes for all studies.

Signalling question 3: did the study avoid inappropriate exclusions?

We will answer yes if the study included both smear-positive and smear-negative participants; no if the study included primarily or exclusively smear-positive or smear-negative participants; and unclear if we could not determine this. If, at the time of specimen collection, the participant was receiving any type of tuberculosis treatment and if culture reference standard was used, we will answer no because the bactericidal action of antibiotics can cause negative culture and positive polymerase chain reaction (PCR) results.

Applicability: are there concerns that the included participants and setting do not match the review question?

We will answer low concern if participants were evaluated as outpatients (with either expectorated or induced sputum) in local hospitals or primary care centres. We will answer high concern if participants were evaluated exclusively as inpatients in tertiary care centres. We will answer unclear concern if the clinical setting was not reported or there was insufficient information to make a decision. We will also answer

unclear concern if testing was performed at a central-level laboratory and the clinical setting was not reported if, for example, it was difficult to determine whether the laboratory provided services mainly to very sick people or people with a broader clinical spectrum of illness.

Detection of drug resistance

Risk of bias: could the selection of participants have introduced bias?

Signalling question 1: was a consecutive or random sample of participants enrolled?

We will answer the same as for detection of tuberculosis.

Signalling question 2: was a case-control design avoided?

We will answer yes if the study enrolled people with tuberculosis with suspected or sufficiently high pretest probability (per World Health Organization guidelines) for resistance to isoniazid, second-line drugs, or both isoniazid and second-line drugs; no if the study enrolled people with tuberculosis with confirmed previously known resistance to the drug in question; and unclear for all other scenarios or if it was not clearly reported. We consider that accuracy studies may have a cross-sectional design even when the reference standard is performed before the index test if both cases and controls are sampled from a single source population.

Signalling question 3: did the study avoid inappropriate exclusions?

We will answer yes for people who were previously treated for tuberculosis. We will answer no if people who were previously treated were excluded. People previously tested for tuberculosis have a higher risk of having drug resistance and are likely to be the target population for initial use of Xpert MTB/XDR. If people with samples known to be heteroresistant (a mix of susceptible and resistant tuberculosis strains in the specimen) were excluded, which is particularly relevant for the fluoroquinolones, we will answer no. We will answer unclear if we could not determine this.

Applicability: are there concerns that the included participants and setting do not match the review question?

We will judge low concern if the selected clinical specimens or isolates matched the review question, which reflects the way the test will be used in practice. We will judge high concern if the selected specimens or isolates did not represent those for whom the test will be used in practice, such as in people who do not require investigation for resistance to the drugs in question. We will judge unclear concern if we could not determine this.

Domain 2: index test

Detection of tuberculosis

Risk of bias: could the conduct or interpretation of the index test have introduced bias?

Signalling question 1: were the index test results interpreted without knowledge of the results of the reference standard?

We will answer yes for all studies since Xpert MTB/XDR results are automatically generated and the user is provided with printable test results, thus, avoiding subjective interpretation.

Signalling question 2: if a threshold was used, was it prespecified?

We will answer yes for all studies.

Applicability: are there concerns that the index test, its conduct, or its interpretation differ from the review question?

Variations in test technology, execution, or interpretation may affect estimates of the diagnostic accuracy of a test. We will judge the study of low concern for applicability if the test was performed as recommended by the manufacturer. We will judge the study of high concern if the test was applied differently than recommended by the manufacturer, for example, if the test was applied to pooled sputa. We will judge the study of unclear concern if we could not determine this.

Detection of drug resistance

Risk of bias: could the conduct or interpretation of the index test have introduced bias?

Signalling question 1: were the index test results interpreted without knowledge of the results of the reference standard?

We will answer yes for all studies since Xpert MTB/XDR results are automatically generated and the user is provided with printable test results, thus, avoiding subjective interpretation.

Signalling question 2: if a threshold was used, was it prespecified?

We will answer yes for all studies.

Applicability: are there concerns that the index test, its conduct, or its interpretation differ from the review question?

Same judgements as for detection of tuberculosis.

Domain 3: reference standard

Detection of tuberculosis

Risk of bias: could the reference standard, its conduct, or its interpretation have introduced bias?

Signalling question 1: is the reference standard likely to correctly classify the target condition?

We will answer yes for all studies because a microbiological reference standard for *M tuberculosis* is a criterion for inclusion in the review.

Signalling question 2: were the reference standard results interpreted without knowledge of the results of the index test?

We will answer yes if the reference test provided an automated result (e.g. MGIT 960), blinding was explicitly stated, or it was clear that the reference standard was performed at a separate laboratory or performed by different people (or both). We will answer no if the study stated that the reference standard result was interpreted with knowledge of the Xpert MTB/XDR test result. We will answer unclear if we could not determine this.

Applicability: are there concerns that the target condition as defined by the reference standard does not match the question?

We will answer high concern if a type of culture was not used as part of the reference standard, because studies that include only DNA-based tests do not directly measure live *M tuberculosis*. We will answer low concern if culture was performed. We will answer unclear concern if we could not determine this.

Detection of drug resistance

Risk of bias: could the reference standard, its conduct, or its interpretation have introduced bias?

Signalling question 1: is the reference standard likely to correctly classify the target condition?

We will answer these questions for each target condition separately by reference standard as follows.

Drug	pDST	gDST using targeted sequencing	Composite (pDST and gDST using targeted sequencing)	gDST using whole genome sequencing)	Composite (pDST and gDST using whole genome sequencing)
Isoniazid	Yes*	Unclear if few loci were investigated, and yes, if all relevant loci were analysed Loci required for yes: *katG*, *inhA* promoter, *oxyR-ahpC* intergenic region, and *fabG1*	Yes	Unclear if few loci were investigated, and yes, if all relevant loci were analysed Loci required for yes: *katG*, *inhA* promoter, *oxyR-ahpC* intergenic region, and *fabG1*	Yes
Fluoroquinolones	Yes, will depend on critical concentration used for moxifloxacin[a]	Yes Loci required for yes: *gyrA* and *gyrB*	Yes	Yes Loci required for yes: *gyrA* and *gyrB*	Yes
Ethionamide	No, there is considerable overlap in the MICs of *M tuberculosis* isolates with and without re-	Unclear if few loci were investigated, and yes, if all relevant loci were analysed	Unclear	Unclear if few loci were investigated, and yes, if all relevant loci were analysed Loci required for yes: *ethA*, *ethR*, and *inhA* promoter	Unclear

(Continued)

	sistance-causing variants. This means there is considerable overlap in the distribution of MICs for resistant and wild-type isolates	Loci required for yes: *ethA, ethR,* and *inhA* promoter No if only the *inhA* promoter was analysed		No if only the *inhA* promoter was analyzed	
Amikacin	Yes*	Yes, if all relevant loci were analysed Loci required for yes: *rrs* and *eis* promoter	Yes	Yes, if all relevant loci were analysed Loci required for yes: *rrs* and *eis* promoter	Yes

Abbreviations: gDST: genotypic drug susceptibility testing; MIC: minimum inhibitory concentration; pDST: phenotypic drug susceptibility testing.
[a]We will use the currently recommended World Health Organization critical concentrations as a benchmark for judging risk of bias (Appendix 5). For *M tuberculosis*, the antimicrobial susceptibility testing critical concentration is defined as the lowest concentration of an anti-tuberculosis agent in vitro that will inhibit the growth of 99% of phenotypically wild type strains of *M tuberculosis* complex (WHO Critical Concentrations 2018; WHO Critical Concentrations 2021).

Signalling question 2: were the reference standard results interpreted without knowledge of the results of index test?

For pDST, we will answer yes if the reference test provided an automated result (e.g. if liquid culture was used as in MGIT 960 DST), blinding was explicitly stated, or it was clear that the reference test was performed at a separate laboratory, or performed by different people, or both. Of note, pDST on solid media is not automated. We will answer no if the study stated that the reference standard result was interpreted with knowledge of the Xpert MTB/XDR test result. We will answer unclear if we could not determine this. For gDST, we will answer yes for all studies since the results for the reference standard are automated.

We added the following signalling question.

Signalling question 3: were the index test and reference standard performed using the same material (clinical specimen or sediment, or culture isolate)?

Phenotypic DST (pDST) and genotypic DST (gDST) for reference standard testing can be performed on an isolate that has undergone (potentially multiple rounds) of culture in drug-free media. This may lead to the depletion of resistant strains present in the original specimen (which would have been used for the Xpert MTB/XDR testing if direct testing was performed) and cause discrepant results. We think this is an important question as it addresses heteroresistance, which often explains discordance between genotypic and phenotypic results.

For direct testing of a clinical specimen by Xpert MTB/XDR: we will answer yes if the reference test was performed directly on the same clinical specimen; no if the reference standard was performed on a culture isolate; and unclear if we could not determine this. For indirect testing of a culture isolate by Xpert MTB/XDR: we will answer yes if the reference test was performed on the same culture isolate (e.g. indirect sequencing); no if the reference standard was performed on a different culture isolate, or specimen; and unclear if we could not determine this.

Applicability: are there concerns that the target condition as defined by the reference standard does not match the question?

We will judge applicability of low concern for all studies because specimens to be subsequently tested for drug resistance will have already been identified as *M tuberculosis* complex positive.

Domain 4: flow and timing

Detection of tuberculosis

Risk of bias: could the patient flow have introduced bias?

Signalling question 1: was there an appropriate interval between the index test and reference standard?

In most studies, we expect the reference standard to be performed at the same time as Xpert MTB/XDR. However, in some studies, the reference standard may have been performed on a different sample collected at an earlier time. This case applies to some culture isolates,

whose drug susceptibility profile might have been confirmed before Xpert MTB/XDR was available. We will answer yes if Xpert MTB/XDR and the reference standard were performed at the same time or were separated by less than 14 days. We will answer no if Xpert MTB/XDR and the reference standard were not performed at the same time and were separated by 14 days or more. As people suspected of second-line drug resistance are often receiving treatment for tuberculosis, it is possible that variation in the microbial population of specimens collected at different time points may occur. We will answer unclear if we could not determine this.

Signalling question 2: did all patients receive the same reference standard?

We will answer yes if the reference standard was applied to all participants or a random sample of participants, no if the reference standard was only applied to a selective group of participants, and unclear if it was not stated in the paper or if the authors failed to answer this question.

Signalling question 3: were all patients included in the analysis?

We will determine the answer to this question by comparing the number of participants enrolled with the number of participants included in the 2×2 tables. We will note if the study authors reported the number of inconclusive test results. We will answer yes if the number of participants enrolled was clearly stated and corresponded to the number presented in the analysis or if exclusions were adequately described. We will answer no if there were participants missing or excluded from the analysis and there was no explanation given. We will answer unclear if insufficient information was given to assess whether participants were excluded from the analysis.

Detection of drug resistance

We will answer the same as for detection of tuberculosis.

Judgements for risk of bias assessments for a given domain.

- If we answer all signalling questions for a domain yes, then we will judge risk of bias as low.
- If we answer all or most signalling questions for a domain no, then we will judge risk of bias as high.
- If we answer only one signalling question for a domain no, we will discuss further the risk of bias judgement.
- If we answer all or most signalling questions for a domain unclear, then we will judge risk of bias as unclear.
- If we answer only one signalling question for a domain unclear, we will discuss further the risk of bias judgement for the domain.

Appendix 5. Critical concentrations and clinical breakpoints for medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant tuberculosis

Drug groups	Drug	LJ	7H10	7H11	MGIT
First-line drugs	Isoniazid	0.2	0.2	0.2	0.1
Fluoroquinolones	Levofloxacin (CC)	2.0	1.0	—	1.0
	Moxifloxacin (CC)	1.0	0.5	0.5	0.25
	Moxifloxacin (CB)	—	2.0	—	1.0
	Gatifloxacin (CC)	0.5	—	—	0.25
Second-line injectable agents	Amikacin	30.0	2.0	—	1.0
	Capreomycin	40.0	4.0	—	2.5
	Kanamycin	30.0	4.0	—	2.5
Other second-line agents	Ethionamide	40.0	5.0	10	5.0

Table adapted from WHO Critical Concentrations 2018 and WHO Critical Concentrations 2021.

All concentrations are in mg/L and apply to the proportion method with 1% as the critical proportion. Unless otherwise stated, they are critical concentrations (CCs), as opposed to clinical breakpoints (CBs). The clinical breakpoint is used to guide individual clinical decisions in patient treatment.

MGIT is proposed as the reference method for performing DST for second-line tuberculosis agents.

CONTRIBUTIONS OF AUTHORS

SP, GRD, MDV, MC, KRS, and GT drafted the Review protocol.

MC and KRS wrote the statistical analysis section.

All review authors (SP, GRD, MC, MDV, SGS, RW, KRS, and GT) read and approved the final Review protocol draft.

DECLARATIONS OF INTEREST

SP received funding from the World Health Organization (WHO) Global TB Programme, Switzerland.

GRD received funding from the WHO Global TB Programme, Switzerland.

MC received funding from READ-It. READ-It aims to improve the evidence base and ensure its dissemination and helps to ensure healthcare problems relevant to low- and middle-income countries are addressed, and that people living in these countries are part of the process. READ-It (project number 300342-104) is funded by the Foreign, Commonwealth and Development Office (FCDO), UK.

MDV is employed by the Foundation for Innovative New Diagnostics (FIND). FIND has conducted studies and published on Xpert MTB/RIF as part of a collaborative project between FIND, a Swiss non-profit, Cepheid, a US company, and academic partners. The product arising through this partnership was developed under a contract that obligated FIND to pay for development costs and trial costs and Cepheid to make the test available at specified preferential pricing to the public sector in low- and middle-income countries. In addition, FIND conducted studies for the Xpert MTB/RIF Ultra assay, which have also been published.

SGS is employed by FIND. FIND has conducted studies and published on Xpert MTB/XDR and Xpert MTB/RIF as part of a collaborative project between FIND, a Swiss non-profit, Cepheid, a US company, and academic partners. Regarding Xpert MTB/RIF, the product developed through this partnership was developed under a contract that obligated FIND to pay for development costs and trial costs and Cepheid to make the test available at specified preferential pricing to the public sector in low- and middle-income countries. In addition, FIND conducted studies for the Xpert MTB/RIF Ultra assay, which have also been published.

RW none.

KRS received funding from the WHO Global TB Programme, Switzerland. In addition, she has received financial support from Cochrane Infectious Diseases, UK, McGill University, Canada, Baylor College of Medicine, Houston, and the WHO Global TB Programme, Switzerland, for the preparation of related systematic reviews and educational materials, consultancy fees from FIND, Switzerland (for the preparation of systematic reviews and GRADE tables), consultancy fees from Stellenbosch University, Cape Town (for guidance on evidence syntheses), and honoraria, and travel support to attend WHO guideline meetings.

GT received funding from the WHO Global TB Programme, Switzerland. In addition, he has received In-kind research consumable donations provided to employer by Cepheid to work on Xpert MTB/RIF and Xpert MTB/RIF Ultra (not Xpert MTB/XDR) for diagnostic accuracy evaluations for tuberculosis detection. He is the group Principal Investigator for this work. Cepheid has also loaned instruments to conduct these studies. These studies are on different products to those potentially considered for inclusion in this Cochrane Review.

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External sources

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  Registration number 2020/1048818-0; purchase order 202582841

Cochrane Library

Cochrane Database of Systematic Reviews

Xpert MTB/XDR for detection of pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin (Review)

Pillay S, Steingart KR, Davies GR, Chaplin M, De Vos M, Schumacher SG, Warren R, Theron G

Pillay S, Steingart KR, Davies GR, Chaplin M, De Vos M, Schumacher SG, Warren R, Theron G.
Xpert MTB/XDR for detection of pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin.
Cochrane Database of Systematic Reviews 2022, Issue 5. Art. No.: CD014841.
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[Diagnostic Test Accuracy Review]

Xpert MTB/XDR for detection of pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin

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ABSTRACT

Background

The World Health Organization (WHO) End TB Strategy stresses universal access to drug susceptibility testing (DST). DST determines whether *Mycobacterium tuberculosis* bacteria are susceptible or resistant to drugs. Xpert MTB/XDR is a rapid nucleic acid amplification test for detection of tuberculosis and drug resistance in one test suitable for use in peripheral and intermediate level laboratories. In specimens where tuberculosis is detected by Xpert MTB/XDR, Xpert MTB/XDR can also detect resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin.

Objectives

To assess the diagnostic accuracy of Xpert MTB/XDR for pulmonary tuberculosis in people with presumptive pulmonary tuberculosis (having signs and symptoms suggestive of tuberculosis, including cough, fever, weight loss, night sweats).

To assess the diagnostic accuracy of Xpert MTB/XDR for resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin in people with tuberculosis detected by Xpert MTB/XDR, irrespective of rifampicin resistance (whether or not rifampicin resistance status was known) and with known rifampicin resistance.

Search methods

We searched multiple databases to 23 September 2021. We limited searches to 2015 onwards as Xpert MTB/XDR was launched in 2020.

Selection criteria

Diagnostic accuracy studies using sputum in adults with presumptive or confirmed pulmonary tuberculosis. Reference standards were culture (pulmonary tuberculosis detection); phenotypic DST (pDST), genotypic DST (gDST),composite (pDST and gDST) (drug resistance detection).

Data collection and analysis

Two review authors independently reviewed reports for eligibility and extracted data using a standardized form. For multicentre studies, we anticipated variability in the type and frequency of mutations associated with resistance to a given drug at the different centres and considered each centre as an independent study cohort for quality assessment and analysis. We assessed methodological quality with QUADAS-2, judging risk of bias separately for each target condition and reference standard. For pulmonary tuberculosis detection, owing to heterogeneity in participant characteristics and observed specificity estimates, we reported a range of sensitivity and specificity estimates and did not perform a meta-analysis. For drug resistance detection, we performed meta-analyses by reference standard using bivariate random-effects models. Using GRADE, we assessed certainty of evidence of Xpert MTB/XDR accuracy for detection of resistance to isoniazid and fluoroquinolones in people irrespective of rifampicin resistance and to ethionamide and amikacin in people with known rifampicin resistance, reflecting real-world situations. We used pDST, except for ethionamide resistance where we considered gDST a better reference standard.

Main results

We included two multicentre studies from high multidrug-resistant/rifampicin-resistant tuberculosis burden countries, reporting on six independent study cohorts, involving 1228 participants for pulmonary tuberculosis detection and 1141 participants for drug resistance detection. The proportion of participants with rifampicin resistance in the two studies was 47.9% and 80.9%. For tuberculosis detection, we judged high risk of bias for patient selection owing to selective recruitment. For ethionamide resistance detection, we judged high risk of bias for the reference standard, both pDST and gDST, though we considered gDST a better reference standard.

Pulmonary tuberculosis detection

- Xpert MTB/XDR sensitivity range, 98.3% (96.1 to 99.5) to 98.9% (96.2 to 99.9) and specificity range, 22.5% (14.3 to 32.6) to 100.0% (86.3 to 100.0); median prevalence of pulmonary tuberculosis 91.3%, (interquartile range, 89.3% to 91.8%), (2 studies; 1 study reported on 2 cohorts, 1228 participants; very low-certainty evidence, sensitivity and specificity).

Drug resistance detection

People irrespective of rifampicin resistance

- Isoniazid resistance: Xpert MTB/XDR summary sensitivity and specificity (95% confidence interval (CI)) were 94.2% (87.5 to 97.4) and 98.5% (92.6 to 99.7) against pDST, (6 cohorts, 1083 participants, moderate-certainty evidence, sensitivity and specificity).

- Fluoroquinolone resistance: Xpert MTB/XDR summary sensitivity and specificity were 93.2% (88.1 to 96.2) and 98.0% (90.8 to 99.6) against pDST, (6 cohorts, 1021 participants; high-certainty evidence, sensitivity; moderate-certainty evidence, specificity).

People with known rifampicin resistance

- Ethionamide resistance: Xpert MTB/XDR summary sensitivity and specificity were 98.0% (74.2 to 99.9) and 99.7% (83.5 to 100.0) against gDST, (4 cohorts, 434 participants; very low-certainty evidence, sensitivity and specificity).

- Amikacin resistance: Xpert MTB/XDR summary sensitivity and specificity were 86.1% (75.0 to 92.7) and 98.9% (93.0 to 99.8) against pDST, (4 cohorts, 490 participants; low-certainty evidence, sensitivity; high-certainty evidence, specificity).

Of 1000 people with pulmonary tuberculosis, detected as tuberculosis by Xpert MTB/XDR:

- where 50 have isoniazid resistance, 61 would have an Xpert MTB/XDR result indicating isoniazid resistance: of these, 14/61 (23%) would not have isoniazid resistance (FP); 939 (of 1000 people) would have a result indicating the absence of isoniazid resistance: of these, 3/939 (0%) would have isoniazid resistance (FN).

- where 50 have fluoroquinolone resistance, 66 would have an Xpert MTB/XDR result indicating fluoroquinolone resistance: of these, 19/66 (29%) would not have fluoroquinolone resistance (FP); 934 would have a result indicating the absence of fluoroquinolone resistance: of these, 3/934 (0%) would have fluoroquinolone resistance (FN).

- where 300 have ethionamide resistance, 296 would have an Xpert MTB/XDR result indicating ethionamide resistance: of these, 2/296 (1%) would not have ethionamide resistance (FP); 704 would have a result indicating the absence of ethionamide resistance: of these, 6/704 (1%) would have ethionamide resistance (FN).

- where 135 have amikacin resistance, 126 would have an Xpert MTB/XDR result indicating amikacin resistance: of these, 10/126 (8%) would not have amikacin resistance (FP); 874 would have a result indicating the absence of amikacin resistance: of these, 19/874 (2%) would have amikacin resistance (FN).

Authors' conclusions

Review findings suggest that, in people determined by Xpert MTB/XDR to be tuberculosis-positive, Xpert MTB/XDR provides accurate results for detection of isoniazid and fluoroquinolone resistance and can assist with selection of an optimised treatment regimen. Given that Xpert

MTB/XDR targets a limited number of resistance variants in specific genes, the test may perform differently in different settings. Findings in this review should be interpreted with caution. Sensitivity for detection of ethionamide resistance was based only on Xpert MTB/XDR detection of mutations in the *inhA* promoter region, a known limitation. High risk of bias limits our confidence in Xpert MTB/XDR accuracy for pulmonary tuberculosis.

Xpert MTB/XDR's impact will depend on its ability to detect tuberculosis (required for DST), prevalence of resistance to a given drug, health care infrastructure, and access to other tests.

PLAIN LANGUAGE SUMMARY

Xpert MTB/XDR, a rapid test for resistance to tuberculosis drugs

Why is improving the diagnosis of tuberculosis drug resistance important?

Tuberculosis tests, like Xpert MTB/RIF, Xpert MTB/RIF Ultra, and Truenat, only diagnose rifampicin resistance, but do not provide information about resistance to other drugs used to treat tuberculosis. This information is needed to allow for effective treatment to be started quickly.

Not recognizing tuberculosis drug resistance when present (false negative, FN) may result in severe illness and death. An incorrect diagnosis of tuberculosis drug resistance (false positive, FP) may result in stigma and prolonged and unnecessary treatment with less effective drugs that have more side effects.

What is the aim of this review?

How accurate is Xpert MTB/XDR for detecting pulmonary tuberculosis and resistance to tuberculosis drugs (i.e. isoniazid, fluoroquinolones, ethionamide, and amikacin) in adults?

What was studied in the review?

Xpert MTB/XDR is a rapid test for detecting tuberculosis and drug resistance in one test, suitable for laboratories that do not require advanced skills and infrastructure. We assessed Xpert MTB/XDR accuracy against three reference standards.

What are the main results of the review?

We identified two multicentre studies reporting on six separate cohorts (groups of study participants), 1228 participants for pulmonary tuberculosis detection and 1141 participants for drug resistance detection.

For pulmonary tuberculosis detection, we included two studies (one reporting on two separate cohorts). We did not determine an overall summary of Xpert MTB/XDR accuracy.

If Xpert MTB/XDR were to be used in 1000 people with suspected tuberculosis of whom 100 have tuberculosis:

- an estimated 98 to 99 people would have an Xpert MTB/XDR result indicating tuberculosis: of these 1 to 2 (1%) would not have tuberculosis (FP); and 203 to 900 people would have a result indicating the absence of tuberculosis: of these 0 to 697 (0% to 77%) would have tuberculosis (FN).

Drug resistance detection

Of 1000 people detected as tuberculosis positive by Xpert MTB/XDR:

- where 50 have isoniazid resistance, an estimated 61 would have an Xpert MTB/XDR result indicating isoniazid resistance: of these, 14/61 (23%) would not have isoniazid resistance (FP); and 939 (of the 1000 people) would have an Xpert MTB/XDR result indicating the absence of isoniazid resistance: of these, 3/939 (0%) would have isoniazid resistance (FN);

- where 50 have isoniazid resistance, 61 (of 1000 people) would have an Xpert MTB/XDR result indicating isoniazid resistance: of these, 14/61 (23%) would not have isoniazid resistance (FP); and 939 (of 1000 people) would have a result indicating the absence of isoniazid resistance: of these, 3/939 (0%) would have isoniazid resistance (FN);

- where 50 have fluoroquinolone resistance, 66 would have an Xpert MTB/XDR result indicating fluoroquinolone resistance: of these, 19/66 (29%) would not have fluoroquinolone resistance (FP); and 934 would have a result indicating the absence of fluoroquinolone resistance: of these, 3/934 (0%) would have fluoroquinolone resistance (FN);

- where 300 have ethionamide resistance, 296 would have an Xpert MTB/XDR result indicating ethionamide resistance: of these, 2/296 (1%) would not have ethionamide resistance (FP); and 704 would have a result indicating the absence of ethionamide resistance: of these, 6/704 (1%) would have ethionamide resistance (FN);

- where 135 have amikacin resistance, 126 would have an Xpert MTB/XDR result indicating amikacin resistance: of these, 10/126 (8%) would not have amikacin resistance (FP); and 874 would have a result indicating the absence of amikacin resistance: of these, 19/874 (2%) would have amikacin resistance (FN).

How reliable are the results of the studies in this review?

For pulmonary tuberculosis detection, we did not consider the results reliable because around 90% of the participants had Xpert-detected pulmonary tuberculosis to begin with due to the way people were chosen to participate in the studies. For drug resistance detection, we were confident in the results, except for results for ethionamide resistance detection, where the reference standards were not ideal.

Who do the results of this review apply to?

People with suspected pulmonary tuberculosis and tuberculosis drug resistance living in countries with a high burden of tuberculosis drug resistance.

How up-to-date is this review?

We searched for studies up to 23 September 2021. Searches were limited to 2015 onwards as Xpert MTB/XDR was launched in July 2020.

SUMMARY OF FINDINGS

Summary of findings 1. Summary of findings table, Xpert MTB/XDR for pulmonary tuberculosis

Review question: what is the diagnostic accuracy of Xpert MTB/XDR for detection of pulmonary tuberculosis?

Population: people with presumptive pulmonary tuberculosis
Role: an initial test
Index test: Xpert MTB/XDR
Threshold for index test: an automated result is provided
Reference standard: solid or liquid culture
Studies: cross-sectional
Setting: the intended use setting is peripheral and intermediate level laboratories

Limitations: selective recruitment of participants could lead to sensitivity being overestimated; participants may have been on tuberculosis treatment, which could lead to specificity being underestimated. In one study, data were not reported separately for the independent study cohorts. Owing to heterogeneity in both the characteristics of participants and observed specificity values, we did not perform a meta-analysis. We had limited data to assess the number of people with tuberculosis who were missed (not detected as tuberculosis-positive by Xpert MTB/XDR to begin with) and would have drug susceptibility results uncharacterised by Xpert MTB/XDR

Xpert MTB/XDR sensitivity range 98.3% to 98.9%: specificity range 22.5% to 100.0%

Test result	Number of results per 1000 people tested (95% CI)			№ of participants (studies, study cohorts)	Certainty of the evidence (GRADE)
	Prevalence 2.5%	Prevalence 10%	Prevalence 30%		
True positives people with pulmonary tuberculosis	25 to 25	98 to 99	295 to 297	799 (2 studies of which 1 reported on 2 study cohorts)	⊕◯◯◯ VERY LOW[a,b]
False negatives people incorrectly classified as not having pulmonary tuberculosis	0 to 0	1 to 2	3 to 5		
True negatives people without pulmonary tuberculosis	219 to 975	203 to 900	158 to 700	429 (2 studies of which 1 reported on 2 study cohorts)	⊕◯◯◯ VERY LOW[b,c,d]
False positives people incorrectly classified as having pulmonary tuberculosis	0 to 756	0 to 697	0 to 542		

Abbreviations: **CI:** confidence interval; **№:** number.
Prevalence values in the table were suggested by the World Health Organization Global Tuberculosis Programme. The median prevalence of pulmonary tuberculosis was 91.3%, interquartile range, 89.3% to 91.8%.
[a]We downgraded two levels for risk of bias for selective recruitment of participants.

[b]We noted important differences between the review question and the populations studied including prior testing with Xpert MTB/RIF and Xpert Ultra. The median prevalence in the included studies was not within the range of the three prevalence values provided in the Summary of findings table. We downgraded one level for indirectness.
[c]For individual studies, specificity estimates ranged from 22% to 99%. We could in part explain the low specificity in one study by the small number of non-tuberculosis cases and that participants may have been receiving tuberculosis treatment (participants may have tested Xpert MTB/XDR positive and culture (reference standard) negative and be classified as false-positive). We downgraded one level for inconsistency.
[d]We thought the range provided for true negatives and false positives would likely lead to different clinical decisions depending on which values were assumed. We downgraded one level for imprecision.
GRADE certainty of the evidence
High: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.
The results presented in this table should not be interpreted in isolation from the results of individual included studies contributing to each summary test accuracy measure.

Summary of findings 2. Summary of findings table, Xpert MTB/XDR for isoniazid resistance

Review question: what is the diagnostic accuracy of Xpert MTB/XDR for detection of isoniazid resistance?
Population: adults with pulmonary tuberculosis irrespective of rifampicin resistance (i.e. whether or not their rifampicin resistance status was known), detected as tuberculosis positive by Xpert MTB/XDR

Index test: Xpert MTB/XDR

Role: an initial test

Xpert MTB/XDR must first detect tuberculosis (even if the patient is already tuberculosis-positive by another test) before it can detect a resistant or susceptible result
Threshold for index test: an automated result is provided
Prior tests: before receiving Xpert MTB/XDR, people typically will have received testing with another WHO-recommended rapid diagnostic test to confirm tuberculosis

Reference standard: culture-based phenotypic drug susceptibility testing
Studies: cross-sectional
Setting: the intended use setting is peripheral and intermediate level laboratories

Limitations: although the population is adults with pulmonary tuberculosis irrespective of rifampicin resistance, we note that most participants had rifampicin resistance

Xpert MTB/XDR summary sensitivity 94.2% (87.5 to 97.4) and specificity 98.5% (92.6 to 99.7)

Test result	Number of results per 1000 people tested (95% CI)			№ of participants (studies, study cohorts)	Certainty of the evidence (GRADE)
	Prevalence 1%	Prevalence 5%	Prevalence 10%		
True positives people with isoniazid resistance	9 (9 to 10)	47 (44 to 49)	94 (88 to 97)	756 (2 studies reporting on 6 study cohorts)	⊕⊕⊕◯ MODERATE[a,b]
False negatives	1 (0 to 1)	3 (1 to 6)	6 (3 to 12)		

people incorrectly classified as not having isoniazid resistance					
True negatives people without isoniazid resistance	975 (917 to 987)	936 (880 to 947)	887 (833 to 897)	327 (2 studies reporting on 6 study cohorts)	⊕⊕⊕◯ MODERATE[a,b]
False positives people incorrectly classified as having isoniazid resistance	15 (3 to 73)	14 (3 to 70)	13 (3 to 67)		

Abbreviations: **CI:** confidence interval; **№:** number.
Prevalence values in the table were suggested by the World Health Organization Global Tuberculosis Programme. The median prevalence of isoniazid resistance in the six study cohorts was 67.6%, interquartile range, 63.1% to 78.1%,
[a]We had several concerns about whether there was indirectness in the populations studied. First, the median prevalence of isoniazid resistance in this analysis was 67.6%, higher than the three prevalences in the GRADE table. Applicability to settings with a lower prevalence of isoniazid resistance comes with some uncertainty. Second, there are potential differences in the mutations present in isoniazid mono-resistant strains and multidrug-resistant strains. That is, there are studies that suggest that a more diverse set of mutations can be found in mono-resistant strains than multidrug-resistant strains. Third, although the population for this PICO question is 'irrespective of rifampicin resistance,' owing to enrolment criteria, most participants were rifampicin resistant. We downgraded one level for indirectness.
[b]Sensitivity estimates ranged from 81% (New Delhi) to 99% (Mubai and Moldova). Regarding the low sensitivity estimate in New Delhi, heteroresistance and resistance mechanisms outside of those detectable by the Xpert MTB/XDR at this site may in part explain the low sensitivity. We did not downgrade for inconsistency.
GRADE certainty of the evidence
High: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.
The results presented in this table should not be interpreted in isolation from the results of individual included studies contributing to each summary test accuracy measure.

Summary of findings 3. Summary of findings table, Xpert MTB/XDR for fluoroquinolone resistance

Review question: what is the diagnostic accuracy of Xpert MTB/XDR for detection of fluoroquinolone resistance?
Population: adults with pulmonary tuberculosis irrespective of rifampicin resistance (i.e. whether or not their rifampicin resistance status was known), detected as tuberculosis positive by Xpert MTB/XDR

Index test: Xpert MTB/XDR

Role: an initial test

Xpert MTB/XDR must first detect tuberculosis (even if the patient is already tuberculosis-positive by another test) before it can detect a resistant or susceptible result
Threshold for index test: an automated result is provided
Prior tests: before receiving Xpert MTB/XDR, people typically will have received testing with another WHO-recommended rapid diagnostic test to confirm tuberculosis

Reference standard: culture-based phenotypic drug susceptibility testing
Study design: cross-sectional
Setting: the intended use setting is peripheral and intermediate level laboratories

Limitations: Although the population is adults with pulmonary tuberculosis irrespective of rifampicin resistance, we note that most participants had rifampicin resistance
Xpert MTB/XDR sensitivity 93.2% (88.1 to 96.2) and specificity 98.0% (90.8 to 99.6)

Test result	Number of results per 1000 people tested (95% CI)			№ of participants (studies, study cohorts)	Certainty of the evidence (GRADE)
	Prevalence 1%	Prevalence 5%	Prevalence 10%		
True positives people with fluoroquinolone resistance	9 (9 to 10)	47 (44 to 48)	93 (88 to 96)	381 (2 studies reporting on 6 study cohorts)	⊕⊕⊕◯ HIGH[a,b]
False negatives people incorrectly classified as not having fluoroquinolone resistance	1 (0 to 1)	3 (2 to 6)	7 (4 to 12)		
True negatives people without fluoroquinolone resistance	970 (899 to 986)	931 (863 to 946)	882 (817 to 896)	640 (2 studies reporting on 6 study cohorts)	⊕⊕⊕◯ MODERATE[a,c]
False positives people incorrectly classified as having fluoroquinolone resistance	20 (4 to 91)	19 (4 to 87)	18 (4 to 83)		

Abbreviations:**CI:** confidence interval; **№:** number.
Prevalence values in the table were suggested by the World Health Organization Global Tuberculosis Programme. The median prevalence of fluoroquinolone resistance in the six study cohorts was 33.7%, interquartile range, 25.2% to 48.2%.
[a]All study cohorts were conducted in high multidrug-resistant/rifampicin-resistant tuberculosis burden countries. The median prevalence of fluoroquinolone resistance in the study cohorts was higher than the three prevalences listed in the GRADE table. Applicability to settings with lower prevalence of fluoroquinolone resistance comes with some uncertainty. Although the population for this question is 'irrespective of rifampicin resistance', we note that most participants had known rifampicin resistance. We did not downgrade for indirectness. This was a judgement.
[b]Sensitivity estimates ranged from 83% (New Delhi) to 98% (Mumbai). Except for New Delhi, sensitivity was ≥ 91%. Regarding the low sensitivity estimate in New Delhi, heteroresistance and rare mutations at this site may in part explain the low sensitivity. We did not downgrade for inconsistency.
[c]Specificity estimates were inconsistent: 84% (Mumbai), 91% (New Delhi), and ≥ 96% for other study cohorts. We could not explain the heterogeneity in specificity estimates. We downgraded one level inconsistency.
GRADE certainty of the evidence
High: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.
The results presented in this table should not be interpreted in isolation from the results of individual included studies contributing to each summary test accuracy measure.

Summary of findings 4. Summary of findings table, Xpert MTB/XDR for ethionamide resistance

Review question: what is the diagnostic accuracy of Xpert MTB/XDR for detection of ethionamide resistance?
Population: adults with pulmonary tuberculosis with known rifampicin resistance, detected as tuberculosis positive by Xpert MTB/XDR

Role: an initial test
Index test: Xpert MTB/XDR

Xpert MTB/XDR must first detect tuberculosis (even if the patient is already tuberculosis-positive by another test) before it can detect a resistant or susceptible result
Threshold for index test: an automated result is provided
Prior tests: before receiving Xpert MTB/XDR, people typically will have received testing with another WHO-recommended rapid diagnostic test to confirm tuberculosis

Reference standard: genotypic drug susceptibility testing
Study design: cross-sectional
Setting: the intended use setting is peripheral and intermediate level laboratories

Limitations: not all of the loci (i.e. *ethA*, *ethR*, and *inhA* promoter) required for the reference standard to correctly classify the target condition were included
Xpert MTB/XDR sensitivity 98.0% (74.2 to 99.9) and specificity 99.7% (83.5 to 100.0)

Test result	Number of results per 1000 people tested (95% CI)			№ of participants (studies, study cohorts)	Certainty of the evidence (GRADE)
	Prevalence 20%	Prevalence 30%	Prevalence 50%		
True positives people with ethionamide resistance	196 (148 to 200)	294 (223 to 300)	490 (371 to 500)	167 (1 study reporting on 4 study cohorts)	⊕◯◯◯ VERY LOW [a,b,c]
False negatives people incorrectly classified as not having ethionamide resistance	4 (0 to 52)	6 (0 to 77)	10 (0 to 129)		
True negatives people without ethionamide resistance	798 (668 to 800)	698 (584 to 700)	499 (418 to 500)	267 (1 study reporting on 4 study cohorts)	⊕◯◯◯ VERY LOW [a,b,d]
False positives people incorrectly classified as having ethionamide resistance	2 (0 to 132)	2 (0 to 116)	1 (0 to 82)		

Abbreviations: **CI:** confidence interval; **№:** number.
Prevalence values in the table were suggested by the World Health Organization Global Tuberculosis Programme. The median prevalence of ethionamide resistance in the four study cohorts was 39.3%, interquartile range, 25.4% to 52.3%.
[a]We thought there was very serious risk of bias in the reference standard domain because of the absence of several loci (i.e. *ethA*, *ethR*, and *inhA* promoter) required for the reference standard to correctly classify the target condition. Of note, against a phenotypic drug susceptibility reference standard, which does not have this limitation, the summary sensitivity estimate was considerably lower at 51.7% (33.1 to 69.8). We downgraded two levels for risk of bias.
[b]Sensitivity estimates ranged from 78% to 100%. The heterogeneity could be explained in part by the small number of resistant cases in New Delhi and South Africa. We did not downgrade for inconsistency.
[c]The 95% CI was wide. We thought the 95% CI around true positives and false negatives would likely lead to different decisions depending on which confidence limits are assumed. We downgraded one level for imprecision.
[d]The 95% CI was wide. We thought the 95% CI around true negatives and false positives would likely lead to different decisions depending on which confidence limits are assumed. We downgraded one level for imprecision.
GRADE certainty of the evidence

High: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.
The results presented in this table should not be interpreted in isolation from the results of individual included studies contributing to each summary test accuracy measure.

Summary of findings 5. Summary of findings table, Xpert MTB/XDR for amikacin resistance

Review question: what is the diagnostic accuracy of Xpert MTB/XDR for detection of amikacin resistance?
Population: adults with pulmonary tuberculosis with known rifampicin resistance, detected as tuberculosis-positive by Xpert MTB/XDR

Index test: Xpert MTB/XDR

Role: an initial test

Xpert MTB/XDR must first detect tuberculosis (even if the patient is already tuberculosis-positive by another test) before it can detect a resistant or susceptible result
Threshold for index test: an automated result is provided
Prior tests: before receiving Xpert MTB/XDR, people typically will have received testing with another WHO-recommended rapid diagnostic test to confirm tuberculosis

Reference standard: culture-based phenotypic drug susceptibility testing
Studies: cross-sectional
Setting: the intended use setting is peripheral and intermediate level laboratories
Xpert MTB/XDR sensitivity 86.1% (75.0 to 92.7) and specificity 98.9% (93.0 to 99.8)

Test result	Number of results per 1000 people tested (95% CI)			№ of participants (studies, study cohorts)	Certainty of the evidence (GRADE)
	Prevalence 6%	Prevalence 13.5%	Prevalence 20%		
True positives people with amikacin resistance	52 (45 to 56)	116 (101 to 125)	172 (150 to 185)	65 (1 study reporting on 4 study cohorts)	⊕⊕◯◯ LOW[a,b]
False negatives people incorrectly classified as not having amikacin resistance	8 (4 to 15)	19 (10 to 34)	28 (15 to 50)		
True negatives people without amikacin resistance	930 (874 to 938)	855 (804 to 863)	791 (744 to 798)	425 (1 study reporting on 4 study cohorts)	⊕⊕⊕⊕ HIGH
False positives people incorrectly classified as having amikacin resistance	10 (2 to 66)	10 (2 to 61)	9 (2 to 56)		

Abbreviations: **CI:** confidence interval; **№:** number.

Prevalence values in the were table suggested by the World Health Organization Global Tuberculosis Programme. The median prevalence of amikacin resistance in the four study cohorts was 13.5%, interquartile range, 9.6% to 21.0%.

[a]Sensitivity estimates were inconsistent, ranging from 75% (New Delhi) to 95% (South Africa), though the 95% CIs overlapped. The heterogeneity could be explained in part by the small number of resistant cases in New Delhi. We did not downgrade for inconsistency.

[b]The 95% CI was wide. There were few participants with amikacin resistance contributing to this analysis for the observed sensitivity. We downgraded two levels for imprecision.

GRADE certainty of the evidence

High: we are very confident that the true effect lies close to that of the estimate of the effect.

Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

The results presented in this table should not be interpreted in isolation from the results of individual included studies contributing to each summary test accuracy measure.

BACKGROUND

A glossary of terms related to this Cochrane Review is provided in Appendix 1.

Tuberculosis continues to cause great suffering worldwide. Globally, in 2020, tuberculosis ranked second as the cause of death from a single infectious agent after COVID-19; around 10 million people developed tuberculosis disease; and around 1.5 million people died (WHO Global Tuberculosis Report 2021). The COVID-19 pandemic has had a disastrous effect on all aspects of global health, in particular, on tuberculosis services. According to the World Health Organization (WHO), in 2020, case notifications decreased by 18% compared to 2019 and, for the first time in over a decade, annual deaths from tuberculosis increased (Pai 2022; WHO Global Tuberculosis Report 2021). People with tuberculosis are often poor and disadvantaged, have more limited access to health care, and often face stigma and discrimination (WHO Global Tuberculosis Report 2021). Under-nourishment, HIV-coinfection, alcohol use disorders, smoking, and diabetes mellitus are risk factors for the development of tuberculosis. Yet when tuberculosis is detected early and effectively treated, the disease is largely curable.

Drug-resistant tuberculosis is a critical public health problem. Multidrug-resistant tuberculosis (MDR-TB, defined below) and extensively drug-resistant tuberculosis (XDR-TB, defined below) are responsible for almost one third of deaths due to antimicrobial resistance globally (O'Neill 2016). In 2019, approximately 0.5 million people developed multidrug-resistant (MDR)/rifampicin-resistant tuberculosis. Of the 465,000 new cases of rifampicin-resistant tuberculosis in 2019, three countries accounted for around one half of the cases: India (27%), China (14%), and the Russian Federation (8%) (WHO Global Tuberculosis Report 2020).

In addition, drug-resistant tuberculosis is impeding progress towards the WHO's End TB targets (WHO End TB 2015), and those in United Nations Sustainable Development Goal 3 (United Nations Sustainable Development Goals 2030). A vital part of the END TB strategy is early diagnosis through universal access to a WHO-recommended rapid diagnostic test and drug susceptibility testing (DST), which determines whether *Mycobacterium tuberculosis* (*M tuberculosis*) bacteria, the causative agent of tuberculosis, are susceptible or resistant to drugs (WHO End TB 2015). This systematic review assessed the diagnostic accuracy of Xpert MTB/XDR, a newly developed nucleic acid amplification test (NAAT) that detects pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin.

Drug-resistant tuberculosis categories

Five categories are used to classify cases of drug-resistant tuberculosis (WHO Consolidated Guidelines (Module 4) 2020; WHO Extensively Drug-Resistant Tuberculosis 2021).

1. Rifampicin-resistant tuberculosis is caused by *M tuberculosis* strains resistant to rifampicin (resistance caused by mutations in a small region of the *rpoB* gene). These strains may be susceptible or resistant to isoniazid (i.e. MDR-TB), or to other drugs.
2. MDR-TB is tuberculosis caused by resistance to at least rifampicin and isoniazid, two core tuberculosis drugs. A subset of people with rifampicin-resistant tuberculosis will have MDR-TB.
3. Isoniazid-resistant tuberculosis is caused by *M tuberculosis* strains resistant to isoniazid and susceptible to rifampicin.
4. Pre-XDR-TB is caused by *M tuberculosis* that fulfils the definition of MDR-TB or rifampicin-resistant tuberculosis, and which are also resistant to a fluoroquinolone. Fluoroquinolones include levofloxacin and moxifloxacin.
5. XDR-TB is caused by *M tuberculosis* that fulfils the definition of rifampicin-resistant or MDR-TB and which are also resistant to a fluoroquinolone and at least one other additional Group A drug (bedaquiline, linezolid). The present version of Xpert MTB/XDR is not capable of detecting WHO-defined XDR-TB owing to an update in the definition to take into consideration new and repurposed drugs for tuberculosis treatment.

MDR/rifampicin-resistant tuberculosis

Rifampicin resistance is already detected by rapid molecular WHO-recommended diagnostic tests (such as Xpert MTB/RIF, Xpert MTB/RIF Ultra, and Truenat assays) that simultaneously detect tuberculosis and rifampicin resistance. These conditions are combined together in a single test because rifampicin resistance is the most frequent form of tuberculosis resistance. Globally in 2020, 69% of bacteriologically confirmed new tuberculosis cases were tested for rifampicin resistance, though testing coverage varied, for example, 58% in Indonesia and 98% in India (WHO Global Tuberculosis Report 2021). And among people with rifampicin resistance, 77,626/157,842 (49.2%) were tested for resistance to any fluoroquinolone (WHO Global Tuberculosis Report 2021).

Isoniazid mono-resistant tuberculosis

In 2019, 13% of new tuberculosis cases and 17% of previously treated tuberculosis cases had isoniazid resistance (WHO Global Tuberculosis Report 2020), yet DST for isoniazid is often only performed in people who are rifampicin resistant. Although in high MDR-TB settings the presence of rifampicin resistance alone has served as a proxy for MDR-TB and the basis for treatment decisions (Liu 2019; Nasiri 2018), emerging data suggest that in some settings, rifampicin DST has suboptimal specificity for MDR-TB. This means that testing for isoniazid resistance is increasingly important. For example, one study in the eastern Democratic Republic of the Congo found one in five people with rifampicin resistance to be isoniazid susceptible when tested using the GenoType MTBDR *plus*, a line probe assay (Bisimwa 2020). And the most recent South African National Survey of Drug Resistance found hotspots of rifampicin mono-resistance, where the prevalence ratio of such cases exceeded that of MDR-TB by up to 30% (NICD 2016).

Conversely, isoniazid resistance in the presence of rifampicin susceptibility (isoniazid mono-resistance) is also increasingly recognized as another emerging threat as it is associated with a three-fold increased risk of poor treatment outcomes and is an important enabler of MDR-TB (Espinal 2000). However, isoniazid resistance would be missed by molecular WHO-recommended diagnostic tests. DST for isoniazid is more complicated than for rifampicin owing to a greater variety of resistance-associated variants (including large deletions) across several genes (e.g. loci in *katG*, *inhA*, and *ahpC*) (WHO Catalogue of Mutations 2021). Information on these mutations may not be routinely available in lower resource settings.

Treatment of tuberculosis

All forms of tuberculosis require treatment with multiple drugs to which bacteria are susceptible to cure tuberculosis and avoid selection of drug resistance (WHO Consolidated Guidelines (Module 3) 2021). For people with drug-susceptible tuberculosis, a four-month rifapentine-based regimen, with and without moxifloxacin (a fluoroquinolone), is advocated as a possible alternative to the current standard six-month regimen (Dorman 2021; WHO Rapid Communication 2021). For people with isoniazid-resistant rifampicin-susceptible tuberculosis, a six-month regimen that includes levofloxacin (a fluoroquinolone) is recommended (WHO Consolidated Guidelines (Module 4) 2020).

The introduction of new and repurposed drugs (bedaquiline, clofazimine, linezolid, pretomanid, delamanid) has revolutionized options for treating multidrug-resistant tuberculosis and additional drug resistance by improving treatment success, shortening treatment, and dispensing with injectable medications. Fluoroquinolones, however, remain an important component of these newer approaches (Churchyard 2019; Conradie 2020; Conradie 2021; Guglielmetti 2021; Médecins Sans Frontières 2021; WHO Consolidated Guidelines (Module 4) 2020). To promote the uptake of all of these new regimens and allow for prompt initiation of appropriate treatment, rapid DST, in particular for fluoroquinolones, is critical. A rapid communication from the WHO Global Tuberculosis Programme describes key changes to the treatment of drug-resistant tuberculosis, including six-month oral regimens for the treatment of MDR/rifampicin-resistant tuberculosis (with or without resistance to fluoroquinolones) and a nine-month oral regimen for the treatment of MDR/rifampicin-resistant tuberculosis. Updated guidance is expected later in 2022 (WHO Rapid Communication 2022).

Target condition being diagnosed

The target conditions are pulmonary tuberculosis and resistance to four tuberculosis drugs: isoniazid, fluoroquinolones, ethionamide, and amikacin.

Pulmonary tuberculosis

Tuberculosis is caused by one of several bacterial species belonging to the *Mycobacterium tuberculosis* (*M tuberculosis)* complex of which the main human pathogen is *M tuberculosis.* Tuberculosis encompasses a dynamic spectrum, from latent infection to subclinical disease to active disease (Pai 2016). Tuberculosis in this review refers to active disease. Tuberculosis most commonly affects the lungs (pulmonary tuberculosis) but may affect any organ or tissue outside of the lungs, such as the brain or spine (extrapulmonary tuberculosis). Signs and symptoms of pulmonary tuberculosis typically include a persistent cough (for at least two weeks), fever, night sweats, weight loss, haemoptysis (coughing up blood), and fatigue, but may also be asymptomatic for prolonged periods of time (Frascella 2021). Tuberculosis is spread from person to person through the air.

Tuberculosis drug resistance

Isoniazid resistance: isoniazid is an important and commonly used first-line drug for tuberculosis. Isoniazid affects mycolic acid (cell wall) synthesis. The drug is taken orally (Curry International Tuberculosis Center 2016; Pai 2016).

Fluoroquinolone resistance: the fluoroquinolones are a class of drugs widely used to treat lower respiratory infections. They are second-line drugs for tuberculosis. Ofloxacin is an earlier generation fluoroquinolone and moxifloxacin, levofloxacin, and gatifloxacin are later generation fluoroquinolones. The fluoroquinolones act by relaxing the supercoiling of DNA strands through inhibition of the enzyme DNA gyrase (Chitra 2020). These drugs are mainly taken orally (Curry International Tuberculosis Center 2016; Pai 2016).

Ethionamide resistance: ethionamide is a second-line drug for tuberculosis in the thioamide drug class. Ethionamide affects mycolic acid synthesis. The drug is taken orally (Curry International Tuberculosis Center 2016; Pai 2016).

Amikacin resistance: amikacin is a second-line drug for tuberculosis in the aminoglycoside drug class, along with kanamycin and capreomycin. These drugs act by inhibiting protein synthesis. Amikacin is mainly administered by intramuscular injection (Curry International Tuberculosis Center 2016; Pai 2016). When a second-line injectable drug is needed in a treatment regimen, amikacin is the preferred drug (WHO Consolidated Guidelines (Module 4) 2020).

In addition to the above drug resistances, Xpert MTB/XDR tests for kanamycin resistance and capreomycin resistance. Kanamycin and capreomycin are less relevant for treating drug-resistant tuberculosis now that an all-oral regimen is recommended. Also, the WHO recommends 'kanamycin and capreomycin are not to be included in the treatment of MDR/rifampicin-resistant tuberculosis in patients on longer regimens' (WHO Consolidated Guidelines (Module 4) 2020), (see Index tests).

Index test(s)

Xpert MTB/XDR (Cepheid, Sunnyvale, USA) is a rapid, automated NAAT of low complexity. In a single test, Xpert MTB/XDR can detect *M tuberculosis* complex (MTBC) DNA and mutations associated with resistance to isoniazid, fluoroquinolones (ofloxacin, moxifloxacin, levofloxacin, gatifloxacin), second-line injectable drugs (amikacin, kanamycin, capreomycin), and ethionamide (Cepheid package insert 2021). Xpert MTB/XDR was designed as a 'reflex test.' In a reflex test, when an initial test result meets predetermined criteria, a second test is performed automatically. According to the manufacturer, Xpert MTB/XDR can be used on unprocessed sputum, concentrated sputum sediments, or MGIT (Mycobacteria Growth Indicator Tube) culture. The manufacturer reports that Xpert MTB/XDR accuracy in fresh and frozen sputum specimens is similar (Cepheid package insert 2021).

NAATs are molecular systems that can detect small quantities of genetic material DNA or ribonucleic acid (RNA)) extracted from micro-organisms, such as *M tuberculosis,* by amplifying regions of DNA or RNA to an amount large enough to study in detail. The key advantage of NAATs is that they are rapid diagnostic tests, potentially providing results in a few hours. A variety of molecular amplification methods are available, of which polymerase chain reaction (PCR) is the most common.

Low complexity refers to a situation where no special infrastructure is required and basic laboratory skills are suitable to run the test. To run Xpert MTB/XDR, an initial manual specimen treatment step is needed in which sample reagent is added to the specimen. Sample reagent helps homogenize the specimen and prepare it for

in-cartridge DNA extraction. A 15-minute incubation period with occasional mixing by hand is required for homogenisation to be effective. Subsequently, DNA extraction and PCR procedures are performed within the container linked to the diagnostic platform.

Several advantages of the assay have been described by the manufacturer.

- Faster time to result for detection of drug resistance.
- Results in less than 90 minutes.
- Similar easy-to-use process as Xpert MTB/RIF and Xpert MTB/RIF Ultra.
- Run on existing GeneXpert platforms equipped with 10-colour modules.

The following information comes from the manufacturer's package insert (Cepheid package insert 2021). We note that in the package insert, 'MTB' refers to MTBC.

- Regarding isoniazid, Xpert MTB/XDR bases detection of resistance on mutations in the *katG* and *fabG1* genes, *oxyR - ahpC* intergenic region, and *inhA* promoter region of the MTB genome.
- Regarding fluoroquinolones, Xpert MTB/XDR bases detection of resistance on mutations in the *gyrA* and *gyrB* quinolone resistance determining regions of the MTB genome.
- Regarding ethionamide, Xpert MTB/XDR bases detection of resistance on mutations in the *inhA* promoter region of the MTB genome. In addition, it is noted that 'mutations conferring ethionamide resistance are reported to be present in genomic regions not targeted by the Xpert MTB/XDR assay' (Cepheid package insert 2021). Of interest, Brossier and colleagues found that 22/47 (47%) of ethionamide-resistant clinical isolates had mutations in *ethA*. Hence, the absence of mutations in the *inhA* promoter region does not preclude ethionamide resistance (Brossier 2011). (The manufacturer acknowledges that reporting ethionamide resistance based only on the detection of mutations in the *inhA* promoter region is a known limitation that may limit sensitivity, though specificity may be unaffected).
- Regarding amikacin, Xpert MTB/XDR bases detection of resistance on mutations in the *rrs* region of the MTB genome.

When a second-line injectable drug is needed in a treatment regimen, amikacin is the preferred drug (WHO Consolidated Guidelines (Module 4) 2020). Although we prioritised the most important drug resistances to include based on guidance from the WHO, when a study included data for kanamycin or capreomycin resistance, we also reported Xpert MTB/XDR accuracy for detection of resistance to these drugs.

Interpretation of results for Xpert MTB/XDR

Xpert MTB/XDR can report results as MTB NOT DETECTED or MTB DETECTED. If results are reported as MTB DETECTED, each drug is reported as resistance DETECTED or NOT DETECTED. If results are reported as MTB NOT DETECTED, or INVALID, ERROR, or NO RESULT, then no drug resistance results are reported (Figure 1).

Figure 1. Possible test results for each target in the Xpert MTB/XDR assay. [a]Ethionamide will not provide an indeterminant by assay design. Copyright © [2020] [Cepheid Inc]: reproduced with permission.

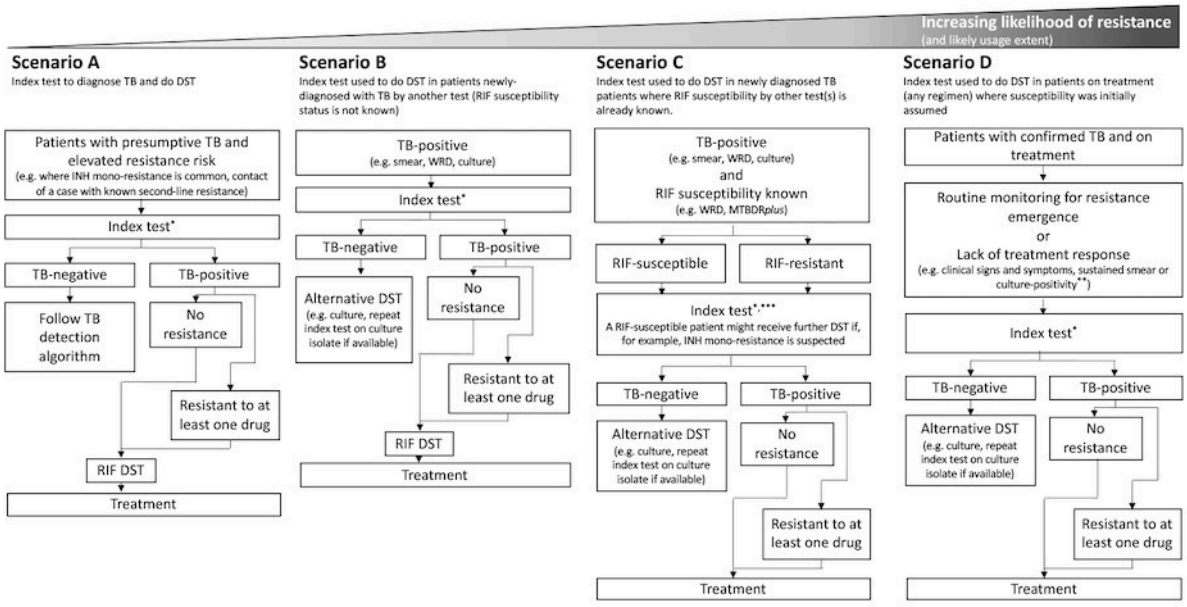
Abbreviations: AMK: amikacin; CAP: capreomycin; ETH: ethionamide; FLQ: fluoroquinolone; INH: isoniazid; KAN: kanamycin; MTB: *Mycobacterium tuberculosis*.

Drug Class	Result Call
N/A	INVALID/ERROR/NO RESULT
	MTB DETECTED
	MTB NOT DETECTED
Isoniazid	Low INH Resistance DETECTED
	INH Resistance DETECTED
	INH Resistance NOT DETECTED
	INH Resistance INDETERMINATE
Fluoroquinolone	Low FLQ Resistance DETECTED
	FLQ Resistance DETECTED
	FLQ Resistance NOT DETECTED
	FLQ Resistance INDETERMINATE
Amikacin	AMK Resistance DETECTED
	AMK Resistance NOT DETECTED
	AMK Resistance INDETERMINATE
Kanamycin	KAN Resistance DETECTED
	KAN Resistance NOT DETECTED
	KAN Resistance INDETERMINATE
Capreomycin	CAP Resistance DETECTED
	CAP Resistance NOT DETECTED
	CAP Resistance INDETERMINATE
Ethionamide[a]	ETH Resistance DETECTED
	ETH Resistance NOT DETECTED

Clinical pathway

Figure 2 outlines several scenarios in the clinical pathway for positioning Xpert MTB/XDR.

Figure 2. Clinical pathway for Xpert MTB/XDR (index test). Abbreviations: DST: drug susceptibility testing; INH: isoniazid; RIF: rifampicin; TB: tuberculosis; WRD: WHO-recommended rapid diagnostic. *Direct testing of sputum is preferred; indirect testing (on cultured isolates) could also be done. **Xpert MTB/XDR may be considered in patients who were Xpert MTB/RIF Ultra rifampicin susceptible prior to treatment and transitioned to Xpert MTB/RIF Ultra rifampicin resistant while on treatment. ***Xpert MTB/XDR may be considered in a rifampicin susceptible patient if INH-mono-resistance is suspected. The composition of a TB treatment regimen will depend on other factors, including RIF susceptibility determined by another test. RIF DST can be done before, in parallel, or after Xpert MTB/XDR. For ease of presentation, TB and MTBC are treated equivalently.



- Scenario A. Xpert MTB/XDR used for detection of pulmonary tuberculosis and drug resistance.
- Scenario B. Xpert MTB/XDR used for detection of drug resistance in people newly diagnosed with pulmonary tuberculosis by another test and whose rifampicin susceptibility is unknown.
- Scenario C. Xpert MTB/XDR used for detection of drug resistance in people newly diagnosed with pulmonary tuberculosis and rifampicin resistance by other tests.
- Scenario D. Xpert MTB/XDR used for detection of drug resistance in people being treated for pulmonary tuberculosis. We did not identify studies that assessed this role.

For each scenario, we expected direct testing (whereby Xpert MTB/XDR is tested directly on a sputum specimen) to be favoured over indirect testing (whereby Xpert MTB/XDR is run on an *M tuberculosis* isolate grown from culture); however, indirect testing remains possible if, for example, direct testing initially failed.

The intended use setting is peripheral and intermediate level laboratories.

The downstream consequences of Xpert MTB/XDR testing include the following.

- TP (true positive): people would benefit from rapid diagnosis and early initiation of effective tuberculosis treatment.
- TN (true negative): people would be spared unnecessary treatment and would benefit from reassurance. For drug resistance detection, in particular, people would be more likely to be treated with more effective drugs with fewer adverse events compared to drugs used to treat drug-resistant tuberculosis.
- False positive (FP): people may experience anxiety and stigma, testing for additional drug resistance and associated diagnostic delays, and treatment with less effective drugs that have serious adverse effects. These consequences are likely more severe in people who have a FP result for drug resistance than in people who have a FP result for pulmonary tuberculosis.
- False negative (FN): if there is a FN result for tuberculosis, there will be no further information about drug susceptibility. If there is FN result for drug resistance, people may be ineligible for some treatment regimens. People would be at increased

risk of morbidity and mortality and there would be continued risk of transmission of tuberculosis and possibly drug-resistant tuberculosis in the community.

Prior test(s)

Before receiving Xpert MTB/XDR, people typically will have received testing with a WHO-recommended rapid diagnostic test to confirm tuberculosis.

Role of index test(s)

The WHO recommends the role of Xpert MTB/XDR as a follow-on test after tuberculosis is confirmed. In this role, Xpert MTB/XDR would be a replacement for line probe assays or culture-based phenotypic DST (pDST). In addition, Xpert MTB/XDR could be used in combination with existing tools that only test for rifampicin resistance, allowing detection of isoniazid-resistant, rifampicin-susceptible tuberculosis (WHO Consolidated Guidelines (Module 3) 2021). Xpert MTB/XDR could also be positioned as an initial test for detection of tuberculosis and drug resistance. We note that the timing of DST for rifampicin and other drugs can be before, in parallel, or after Xpert MTB/XDR is performed, Figure 2,

Alternative test(s)

Here we summarize selective alternative testing methods. The report 'Tuberculosis Diagnostics Pipeline Report: Advancing the Next Generation of Tools' describes additional tuberculosis tests and tests in development (Branigan 2021).

Mycobacterial culture is a method used to grow bacteria on nutrient-rich media. Culture-based DST requires growth of *M tuberculosis* in the presence of drugs at a specific concentration that will inhibit the growth of susceptible bacteria or have no impact on growth of resistant bacteria. Culture is a relatively complex and slow procedure. Solid culture typically takes between four to eight weeks for results, and liquid culture, although more sensitive and rapid than solid culture, requires up to six weeks and is more prone to contamination (Chihota 2010). In addition, culture requires specialized laboratories and highly skilled staff, rarely available in high tuberculosis burden countries. Culture is the reference standard for detection of pulmonary tuberculosis and the basis for pDST.

MeltPro kits (Xiamen Zeesan Biotech Co., Ltd., China) are commercially available, low-complexity tests for detection of mutations associated with resistance to rifampicin, isoniazid, fluoroquinolones, and injectable second-line drugs. Several of the available kits are approved by the China Food and Drug Administration for clinical use. MeltPro testing is designed to detect drug resistance on *M tuberculosis*-positive specimens or cultured isolates. MeltPro testing is performed using an all-in-one machine, Sanity 2.0. Manual pipetting is required for sample preparation, whereas the subsequent processes - nucleic acid extraction, sample loading, detection (i.e. real-time PCR), and interpretation of results - are all fully automatic. The detection of drug resistance is based on multicolor melting curve analysis.

Moderate complexity automated NAATs detect tuberculosis and resistance to rifampicin and isoniazid. Four products have been evaluated and recommenced by the WHO: Abbott RealTime MTB and MTB RIF/INH assays (Abbott Laboratories, Abbott Park, USA); the BD MAX MDR-TB assay (Becton, Dickinson and Company, Franklin Lakes, USA), the Hain FluoroType MTBDR assay (Bruker/Hain Lifescience, Nehren, Germany); and the Roche cobas MTB and MTB-RIF/INH assays (Hoffmann-La Roche, Basel, Switzerland). These tests are faster and simpler to perform than pDST and line-probe assays. Following the initial sample preparation step, these tests are mostly automated. The WHO recommends that 'in people with signs and symptoms of pulmonary tuberculosis, moderate complexity automated NAATs may be used on respiratory samples for the detection of pulmonary tuberculosis, and of rifampicin and isoniazid resistance, rather than culture and pDST (Conditional recommendation; moderate-certainty evidence for diagnostic accuracy)'. Moderate complexity automated NAATs are mainly suited for use in laboratory settings in areas with a high workload (i.e. high population density and high prevalence of tuberculosis). These tests require having a system for referring samples and reporting results (WHO Consolidated Guidelines (Module 3) 2021).

Alternative molecular methods for detection of drug resistance also include the commercial line probe assays, a category of genotypic (molecular) tests. Line probe assays include GenoType MTBDR*plus* assay (Bruker-Hain Lifescience, Nehren, Germany), and the Nipro NTM+MDRTB detection kit 2 (Nipro, Tokyo, Japan) for first-line tuberculosis drugs and GenoType MTBDR*sl* assay (Bruker-Hain Lifescience, Nehren, Germany) for second-line drugs. These methods have considerable advantages over pDST for scaling up programmatic management and surveillance of drug-resistant tuberculosis, offering speed of diagnosis (one or two days), standardized testing, potential for high through-put, and fewer requirements for laboratory biosafety. Drawbacks are that line probe assays require skills and infrastructure only available in intermediate and central laboratories (WHO Operational handbook - diagnosis 2021).

Rationale

Based on new evidence on the management of drug-resistant tuberculosis, the WHO has issued recommendations that all people with MDR/rifampicin-resistant tuberculosis, including those who are also resistant to fluoroquinolones, may benefit from all-oral treatment regimens (WHO Consolidated Guidelines (Module 4) 2020). In people with tuberculosis and rifampicin-resistant tuberculosis it is critically important to perform additional resistance testing to at least isoniazid and the fluoroquinolones in order to guide treatment decisions. People with isoniazid mono-resistant tuberculosis may also benefit from modified regimens that include fluoroquinolones. Information on *inhA* promotor mutations could also guide high-dose isoniazid therapy. Hence, rapid extended profiling of drug resistance could allow for early initiation of appropriate treatment and likely better patient outcomes. Amplification of drug resistance would also be less likely. Extended profiling of drug resistance could also be of importance in considering the use of the four-month fluoroquinolone-containing regimens for drug-susceptible tuberculosis (Dorman 2021). An all-in-one rapid test to detect resistance to rifampicin and other drugs would be ideal; however, this technology is not currently available.

Xpert MTB/XDR is one assay in a new class of diagnostic tests referred to as 'low complexity automated NAATs for detection of resistance to isoniazid and second-line anti-tuberculosis agents' (WHO Consolidated Guidelines (Module 3) 2021). In 2020, we performed a systematic review to inform updated WHO guidelines on the use of NAATs (including Xpert MTB/XDR) to detect

tuberculosis and drug-resistant tuberculosis (WHO Consolidated Guidelines (Module 3) 2021). This Cochrane Review expands on these efforts.

A complementary Cochrane qualitative evidence synthesis addressed the question, 'What are the perspectives and experiences of people providing and receiving low complexity NAATs to diagnose tuberculosis and tuberculosis drug resistance?' In answering this question, the review authors aimed to identify the implications for health equity and effective implementation of the tests (Engel 2022).

OBJECTIVES

- To assess the diagnostic accuracy of Xpert MTB/XDR for pulmonary tuberculosis in people with presumptive pulmonary tuberculosis.
- To assess the diagnostic accuracy of Xpert MTB/XDR for resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin in people with tuberculosis detected by Xpert MTB/XDR, irrespective of rifampicin resistance (whether or not their rifampicin resistance status was known) and with known rifampicin resistance.

Presumptive tuberculosis refers to an individual who presents with symptoms or signs suggestive of tuberculosis (WHO Definitions and Reporting 2020). Symptoms suggestive of tuberculosis include cough, fever, weight loss, and night sweats.

Secondary objectives

As a secondary objective, we planned to compare the diagnostic accuracy of Xpert MTB/XDR by direct testing (whereby Xpert MTB/XDR is tested directly on a sputum specimen) versus indirect testing (whereby Xpert MTB/XDR is performed on a *Mycobacterium tuberculosis* (*M tuberculosis*) isolate grown from culture). However, owing to limited data, we narratively described these analyses and presented results in forest plots.

Investigations of sources of heterogeneity

We planned to investigate the effects of a number of potential sources of heterogeneity as outlined in our protocol, however, our ability to investigate these was limited by the available data. The sources of heterogeneity that we investigated were smear status (pulmonary tuberculosis detection) and type of reference standard, smear status, HIV status, and previous tuberculosis treatment (drug resistance detection).

We note that investigations in people previously treated for tuberculosis are important questions for clinical practice and studies have highlighted the challenges in interpreting the related tests, Xpert MTB/RIF (Theron 2016a) and Xpert MTB/RIF Ultra (Mishra 2020).

METHODS

Criteria for considering studies for this review

Types of studies

We included cross-sectional and cohort studies that assessed the diagnostic accuracy of Xpert MTB/XDR for both pulmonary tuberculosis and tuberculosis drug resistance, or tuberculosis drug resistance alone. We included diagnostic accuracy studies in which people with the target condition and people without the target condition were sampled from a single source population (referred to as a single-gate design) (Rutjes 2005). We only included studies that reported data comparing Xpert MTB/XDR to an acceptable reference standard (defined below) from which we could extract or derive TP, FP, FN, and TN values.

Participants

We included adults 15 years and older with presumptive pulmonary tuberculosis. In addition, we included adults with bacteriologically-confirmed pulmonary tuberculosis irrespective of rifampicin resistance (whether or not their rifampicin resistance status was known) and with known rifampicin resistance. We included HIV-positive and HIV-negative people. We included people who, at study enrolment, did not report previous tuberculosis treatment or reported receiving tuberculosis treatment. We included studies that assessed the diagnostic accuracy of Xpert MTB/XDR using sputum (expectorated or induced) consistent with the intended use of the manufacturer, and studies from all types of health facilities and all laboratory levels (peripheral, intermediate, and central) from all countries.

Index tests

The index test was Xpert MTB/XDR. Xpert MTB/XDR tests for drug resistance after testing has identified the presence of *M tuberculosis* in the specimen. Interpretation of results for Xpert MTB/XDR is shown in Figure 1.

Before receiving Xpert MTB/XDR, people will have typically received testing verifying tuberculosis with another WHO-recommended rapid diagnostic test.

Some people detected as having tuberculosis by another WHO-recommended rapid diagnostic test may not be detected as having tuberculosis by Xpert MTB/XDR, We note that in comparison to related Xpert tests that detected tuberculosis, the limit of detection of Xpert MTB/XDR for *M tuberculosis* was 71.9 colony-forming units (CFU)/mL, similar to the limit of detection of Xpert MTB/RIF (86.9 CFU/mL), but above the limit of detection of Xpert MTB/RIF Ultra (15.6 CFU/mL) (Cao 2021; Chakravorty 2017).

Target conditions

The target conditions were pulmonary tuberculosis and resistance to four tuberculosis drugs: isoniazid, fluoroquinolones, ethionamide, and amikacin.

We included pulmonary tuberculosis as a target condition because some users of the Xpert MTB/XDR assay may want to do the test to detect pulmonary tuberculosis, in particular, in areas where isoniazid mono-resistance is also likely.

Reference standards

Detection of pulmonary tuberculosis

The reference standard for detection of pulmonary tuberculosis was solid or liquid culture or both solid and liquid culture.

- The presence of pulmonary tuberculosis was defined as a positive *M tuberculosis* culture.
- The absence of pulmonary tuberculosis was defined as a negative *M tuberculosis* culture.

Detection of tuberculosis drug resistance

We included three reference standards for detection of drug resistance, pDST, gDST, and a composite reference standard. These methods are used to determine whether *M tuberculosis* bacteria are susceptible or resistant to tuberculosis drugs.

- pDST alone.
  - The presence of drug resistance was defined as drug resistance detected by pDST.
  - The absence of drug resistance for a given drug (referred to as being drug susceptible) was defined as drug resistance not detected by pDST.

We considered pDST to be the most suitable reference standard for detection of resistance to isoniazid, fluoroquinolones, and amikacin. pDST is the conventional method for detecting resistance to first- and second-line tuberculosis drugs.

- gDST alone.
  - The presence of drug resistance was defined as drug resistance detected by gDST.
  - The absence of drug resistance was defined as drug resistance not detected by gDST.

We considered gDST to be the most suitable reference standard for ethionamide resistance because there is considerable overlap in the minimum inhibitory concentrations (MICs) of *M tuberculosis* isolates with and without resistance-causing variants and a pDST reference standard might not correctly classify the target condition.

- Composite reference standard.
  - The presence of drug resistance was defined as drug resistance detected by either pDST or gDST.
  - The absence of drug resistance was defined as drug resistance not detected by both pDST and gDST.

The classification rule for the composite reference standard is based on one of the two reference tests (pDST or gDST) being positive for resistance to a given drug. Consequently, it is not necessary to perform a second reference standard test once the result of the first reference standard test is positive (resistant). Hence, the second reference standard test is only necessary in people with a negative (susceptible) or failed test result (e.g. indeterminate, contaminated) on the first reference standard test (Rutjes 2005). The composite reference standard result was considered drug susceptible when pDST reported drug susceptibility and gDST did not detect a drug-associated resistant mutation.

Search methods for identification of studies

We attempted to identify all relevant studies regardless of language or publication status (published, unpublished, in press, ongoing).

Electronic searches

We searched the following databases up to 23 September 2021, without language restrictions, using the search terms and strategy described in Appendix 2. We limited our searches to 2015 onwards as Xpert MTB/XDR is a newly developed assay, which was launched in July 2020.

- Cochrane Infectious Diseases Group Specialized Register.
- MEDLINE (Ovid).
- Embase (Ovid).
- Science Citation Index – Expanded, Conference Proceedings Citation Index – Science (CPCI-S), and BIOSIS Previews; all three from the Web of Science.
- Scopus (Elsevier).
- Latin American Caribbean Health Sciences Literature (LILACS) (BIREME; lilacs.bvsalud.org/en/).

We also searched ClinicalTrials.gov, the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/trialsearch), and the International Standard Randomized Controlled Trials Number (ISRCTN) registry (www.isrctn.com/) for trials in progress, and ProQuest Dissertations & Theses A&I for dissertations, using terms for tuberculosis and Xpert MTB/XDR.

Searching other resources

We reviewed reference lists of included articles and any relevant review articles identified through the above methods. We also contacted researchers at the Foundation for Innovative New Diagnostics (FIND), the WHO Global Tuberculosis Programme, the manufacturer, and other experts in the field of tuberculosis diagnostics for information on ongoing and unpublished studies. We reviewed data submitted via the WHO public call.

Data collection and analysis

Selection of studies

We used Covidence to manage the selection of studies (Covidence). Two review authors independently screened titles and abstracts identified from literature searching to identify potentially eligible studies. We retrieved the article of any citation identified by one of the review authors for full-text review. Then, two review authors independently assessed articles for inclusion using predefined inclusion and exclusion criteria. We resolved disagreements by discussion with a third review author. We recorded all studies excluded after full-text assessment and their reasons for exclusion in Characteristics of excluded studies. We illustrated the study selection process in a PRISMA diagram (Page 2021; Salameh 2020).

Data extraction and management

We developed a data extraction form based on experience with a previous Cochrane Review (Theron 2016b; Appendix 3). Two review authors independently extracted data on study design, participants, reference standards, and data required to populate a 2x2 contingency table. When possible, we extracted data for each study cohort within a multicentre study (see Statistical analysis and data synthesis). We resolved any discrepancies by discussion with a third review author. We entered the extracted data into an Excel database on password-protected computers. Data will be secured in the Liverpool School of Tropical Medicine 'Archive' drives of Cochrane Infectious Diseases Group for future review updates.

We extracted the following information.

- Details of study: first author; publication year; country where testing was performed; setting (primary care laboratory, hospital laboratory, reference laboratory); study design; manner of participant selection; number of participants enrolled; number of participants for whom results were available.

- Characteristics of participants: age; HIV status; smear status; previous tuberculosis treatment.
- Target conditions.
- Reference standards.
- Details of specimen: type (such as expectorated or induced sputum or cultured isolate); condition (fresh or frozen).
- Details of the conduction of the assay, whether performed on a sputum specimen (direct testing) or performed on the cultured isolate grown from the patient specimen (indirect testing).
- Details of outcomes: the number of TP, FP, FN, and TN results.
- Whether the WHO-recommended critical drug concentration was used for the pDST reference standard (WHO Critical Concentrations 2018; WHO Critical Concentrations 2021). We used the currently recommended concentration for each drug to classify studies, not the recommended concentration at the time of the study.
- Inconclusive test results.
- QUADAS-2 items.
- Details of industry sponsorship, if applicable.

We classified country income status as low-income, middle-income, or high-income, according to the World Bank List of Economies (World Bank 2020). In addition, we classified 'country' as being high burden or not high burden for tuberculosis, HIV-associated tuberculosis, and MDR/rifampicin-resistant tuberculosis based on the WHO classification for the period 2021–2025 (WHO Global Tuberculosis Report 2021). A country may be classified as high burden for one, two, or all three of the high burden categories.

We followed Cochrane policy, which states that, 'Anyone engaged in writing a Cochrane Review, who has had any involvement in the conduct, analysis, and publication of a study that could be included the review, is restricted in what they can do with those data. They CANNOT determine the overall study inclusion and exclusion criteria; and they CANNOT make study eligibility decisions about, extract data from, carry out the risk of bias assessment for, or perform GRADE assessments of that study'.

Assessment of methodological quality

We used QUADAS-2 to assess methodological quality (Whiting 2011). QUADAS-2 consists of four domains: patient selection, index test, reference standard, and flow and timing. We assessed all domains for risk of bias and the first three domains for concerns regarding applicability. Two review authors independently completed QUADAS-2 and resolved disagreements through discussion, if needed, with a third review author. We presented the results of this quality assessment in text and figures. The tool tailored to this review is in Appendix 4.

We appraised methodological quality separately for each study cohort within a multicentre study and separately for each target condition. In addition, for drug resistance detection, in the reference standard domain, we considered risk of bias separately for each drug and each reference standard. This allowed us to assess whether the WHO-recommended critical concentration for the drug was used for the pDST reference standard and whether all relevant loci were included in the gDST reference standard.

Statistical analysis and data synthesis

For multicentre studies, we anticipated that there might have been variability in the frequency and types of mutations associated with resistance to a given drug at the different centres. For this reason, we considered each centre as an independent study cohort. We performed meta-analyses at the study cohort level, if data were available to take this approach.

We displayed key study characteristics in Characteristics of included studies. We plotted estimates of the observed sensitivities and specificities in forest plots with 95% confidence intervals (CIs) using Review Manager 5 (Review Manager 2020).

Detection of pulmonary tuberculosis

For detection of pulmonary tuberculosis, we narratively described the analysis and presented results in forest plots. Owing to heterogeneity in both the participant characteristics and observed specificity values, we did not perform a meta-analysis.

Detection of drug resistance

For detection of drug resistance**,** we took the following analytical approach. We stratified the analyses by type of testing (e.g. directly on sputum); population (irrespective of rifampicin resistance or known rifampicin resistance); target condition; and type of reference standard (pDST, gDST, and composite reference standard).

Within each analysis group (e.g. direct, irrespective of rifampicin resistance, isoniazid resistance, pDST), we plotted estimates of the observed sensitivities and specificities for each study cohort in forest plots with 95% CIs using Review Manager 5 (Review Manager 2020). Where adequate data were available, we combined data using meta-analysis by fitting a bivariate random-effects model (Chu 2006; Macaskill 2010; Reitsma 2005), using Stata (Version 14) with the metandi and meqrlogit commands (Stata). In situations with sparse data, we performed meta-analysis where appropriate by reducing the bivariate model to two univariate random-effects logistic regression models by assuming no correlation between sensitivity and specificity (Takwoingi 2017). When we observed little or no heterogeneity on forest plots, and the analyses consequently did not converge, we further simplified the models into fixed-effect models by eliminating the random-effects parameters for sensitivity or specificity, or both sensitivity and specificity (Takwoingi 2017). In situations where all study cohorts in a meta-analysis reported a sensitivity of 100% or specificity of 100%, we used simple pooling by summing the numbers of TPs and total resistant cases to calculate sensitivity or the numbers of TNs and total susceptible cases to calculate specificity, as required. In these situations when needed, we determined 95% CIs using the Newcombe-Wilson method (Newcombe 1998). We required data from at least four study cohorts for meta-analysis.

Regarding the fluoroquinolone drug class, we estimated test accuracy for the drug class as a whole against pDST, meaning that if there were documented resistance to a given fluoroquinolone, this would be interpreted as resistance to the whole fluoroquinolone class. We used this approach because the fluoroquinolones have high cross-resistance owing to variants within the *gyrA* hotspot region (Zignol 2016).

Inconclusive index test results and missed cases

A test result may be uninterpretable when the main diagnostic feature of the test result is invalid, missing, or obstructed (Shinkins 2013). Invalid inconclusive test results are caused by a property intrinsic to the test. Missing results mean no test result has been recorded though the participant ideally should have had a test result and been included in the study.

For Xpert MTB/XDR, the manufacturer defines two types of invalid inconclusive results, non-determinate and indeterminate.

- A *non-determinate* Xpert MTB/XDR test result is one that results in an Error, Invalid, or No Result and can be due to an operator error, instrument, or cartridge issue (Cepheid package insert 2021). Non-determinate Xpert MTB/XDR test results pertain only to the detection of MTBC, not to the detection of drug resistance.

- An *indeterminate* Xpert MTB/XDR test result is one that indicates that resistance to a given drug could not definitively be detected based on the test's algorithm (Cepheid package insert 2021). This means that, based on quality control criteria, the test was unable to confidently report this particular result and the software suppressed the reporting of this. The same cartridge can be indeterminate for one drug but not another. Indeterminate Xpert MTB/XDR test results pertain only to the detection of drug resistance, not to the detection of MTBC.

We excluded non-determinate and indeterminate results from analyses of diagnostic test accuracy. We performed meta-analyses to estimate the summary proportion of non-determinate and indeterminate results using the metaprop command in Stata (Version 14) (Stata).

- Xpert MTB/XDR MTB NOT DETECTED

When data were available, we reported when the index test did not detect tuberculosis to begin with (missed cases), which could result in resistant cases not receiving a result, Appendix 5.

Investigations of heterogeneity

For each target condition, we investigated heterogeneity through visual examination of forest plots of sensitivity and specificity.

Detection of pulmonary tuberculosis

For Xpert MTB/XDR accuracy by smear status, we narratively described these analyses and presented results in forest plots (see Differences between protocol and review).

Detection of drug resistance

For Xpert MTB/XDR accuracy by smear status, HIV status, and previous tuberculosis treatment, we narratively described these analyses and presented results in forest plots (see Differences between protocol and review).

All covariates were categorical.

- Smear status, positive or negative.
- HIV status, positive or negative.
- Previous tuberculosis treatment or no previous tuberculosis treatment.

Sensitivity analyses

For resistance detection for isoniazid and fluoroquinolones in people irrespective of rifampicin resistance, we performed sensitivity analyses by repeating the meta-analyses and excluding the study (reporting on two study cohorts) sponsored by the manufacturer.

For resistance detection for ethionamide and amikacin in people with known rifampicin resistance, we did not perform sensitivity analyses because the main analyses included only one study (reporting on four study cohorts), which was not sponsored by the manufacturer.

Assessment of reporting bias

We did not conduct formal assessment of publication bias using methods such as funnel plots or regression tests, because such techniques have not been helpful for diagnostic test accuracy studies (Macaskill 2010).

Summary of findings and assessment of the certainty of the evidence

We assessed the certainty of evidence using the GRADE approach for diagnostic studies (Balshem 2011; Schünemann 2008; Schünemann 2016). As recommended, we rated the certainty of evidence as high (not downgraded), moderate (downgraded by one level), low (downgraded by two levels), or very low (downgraded by more than two levels) based on five domains: risk of bias, indirectness, inconsistency, imprecision, and publication bias. For each outcome (i.e. sensitivity and specificity), the certainty of evidence started as high when there were high-quality studies (cross-sectional or cohort studies) that enrolled participants with diagnostic uncertainty. If we found a reason for downgrading, we used our judgement to classify the reason as either serious (downgraded by one level) or very serious (downgraded by two levels). At least two review authors discussed judgements and applied GRADE using the following methods (GRADEpro GDT; Schünemann 2020a; Schünemann 2020b).

Risk of bias: we used QUADAS-2 to assess risk of bias.

Indirectness: we assessed indirectness in relation to the population (including disease spectrum), setting, intervention (index test), and outcomes (accuracy measures). We also use prevalence of the target condition as a guide to whether there was indirectness in the population.

Inconsistency: inconsistency can be caused by clinical heterogeneity or methodological heterogeneity, or it may remain unexplained. GRADE recommends downgrading for unexplained inconsistency in sensitivity and specificity estimates. We had planned to carry out pre-specified analyses to investigate potential sources of heterogeneity and downgrade when we could not explain the inconsistency in the accuracy estimates. However, as mentioned above, data were insufficient to carry out most analyses. We looked at the individual point estimates in the forest plots and judged whether they were more or less the same, as well as the 95% CIs to see if they overlapped.

Imprecision: we considered the width of the 95% CI. In addition, we determined projected ranges for two categories of test results that have the most important consequences for patients, the number of FNs and the number of FPs, and made judgements on imprecision

from these calculations. Imprecision also depends on the number of participants included to determine sensitivity and specificity. We took note of the uncertainty around point estimates along with the number of participants providing those data. We acknowledge the judgement of imprecision is subjective.

Publication bias: we considered the comprehensiveness of the literature search and outreach to researchers in tuberculosis, the presence of only studies that produce precise estimates of high accuracy despite small sample size, and knowledge about studies that were conducted, but were not published.

We used GRADEpro (GRADEpro GDT) to create summary of findings tables for each target condition.

The summary of findings tables include the following details.

- The review question and its components, population, (prior tests), setting, index test(s), and reference standard.
- Summary estimates of sensitivity and specificity and 95% CIs.
- The number of included studies and participants contributing to the estimates of sensitivity and specificity.
- Prevalences of the target condition with an explanation of why the prevalences have been chosen.
- An assessment of the certainty of the evidence (GRADE).
- Explanations for downgrading, as needed.

Using GRADE, we assessed certainty of evidence of Xpert MTB/XDR accuracy for detection of resistance to isoniazid and fluoroquinolones in people irrespective of rifampicin resistance and ethionamide and amikacin in people with known rifampicin resistance, reflecting real world situations. For detection of resistance to isoniazid, flouroquinolones, and amikacin, we used pDST as the reference standard (WHO TPP 2021). For detection of resistance to ethionamide, we used gDST as the reference standard.

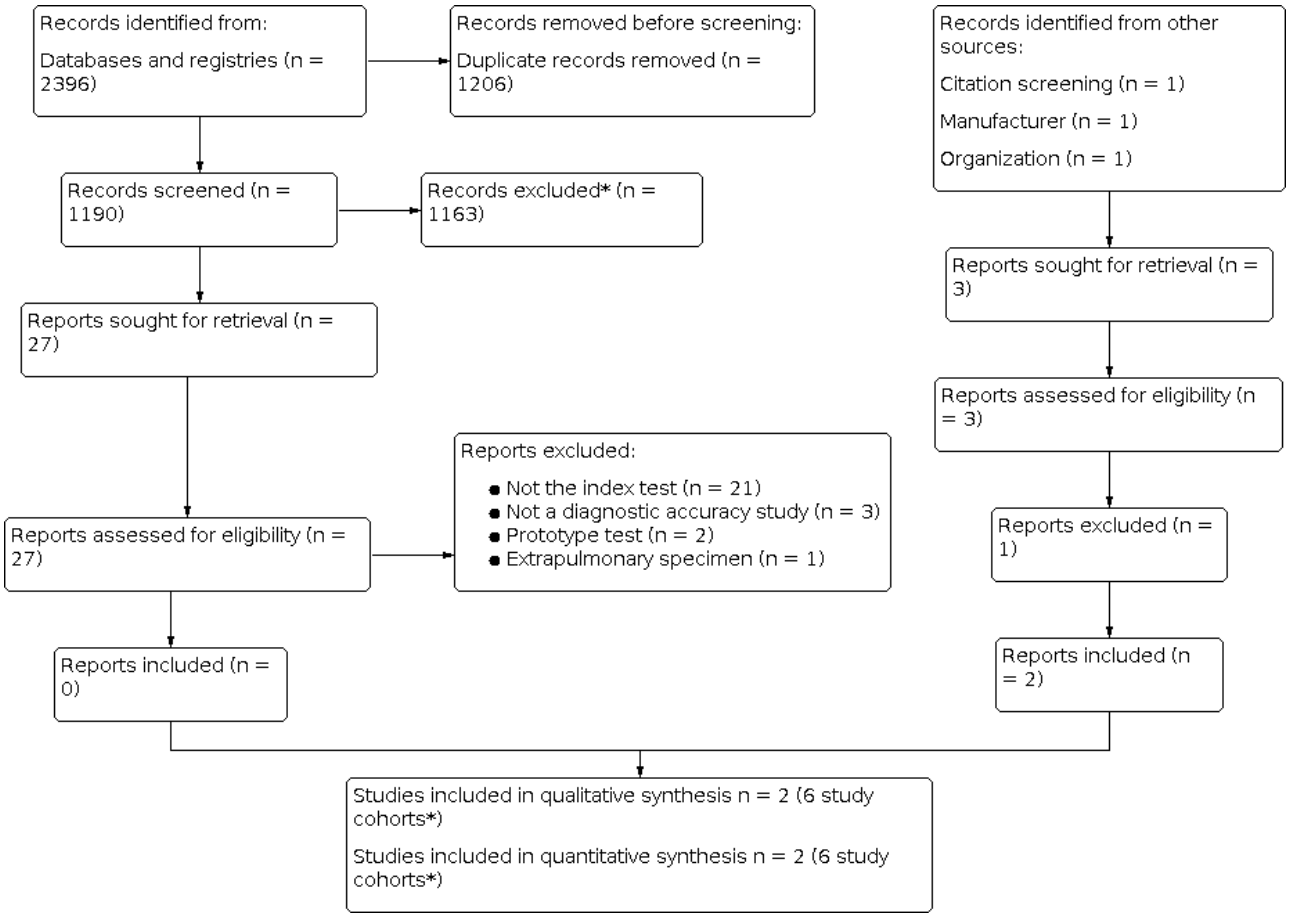
RESULTS

Results of the search

We identified 2396 records from database searching. After removal of 1206 duplicate records, we screened 1190 titles and abstracts for relevance to the review topic. Of these, we excluded 1163 and assessed 27 full-text reports against our inclusion criteria. We excluded all 27 reports for the following reasons: not the index test (n = 21); not a diagnostic accuracy study (n = 3); prototype test (n = 2); and extrapulmonary specimen (n = 1). We identified three records from other sources: one record from the manufacturer (Omar 2020); one record from the Foundation for Innovative New Diagnostics (FIND) (Penn-Nicholson 2021); and one record from additional citation screening (Cao 2021). Following assessment for eligibility, we excluded one report that evaluated Xpert MTB/XDR in both clinical specimens and cultured isolates and the data could not be disaggregated (Cao 2021). Hence, we included two studies reporting on a total of six independent study cohorts. Both studies used a cross-sectional study design. All study cohorts were in high multidrug-resistant/rifampicin-resistant tuberculosis burden countries (Omar 2020; Penn-Nicholson 2021).

Figure 3 shows the PRISMA diagram. We provide a list of excluded studies and reasons for their exclusion in Characteristics of excluded studies.

Figure 3. Study flow diagram. *Two multicentre studies were included, one with two study cohorts and one with four study cohorts. Hence, we included six distinct study cohorts in the review. The following definitions are from Page 2021. Report: a document (paper or electronic) supplying information about a particular study. It could be a journal article, preprint, conference abstract, study register entry, clinical study report, dissertation, unpublished manuscript, government report, or any other document providing relevant information. Record: the title or abstract (or both) of a report indexed in a database or website (such as a title or abstract for an article indexed in Medline). Records that refer to the same report (such as the same journal article) are "duplicates"; however, records that refer to reports that are merely similar (such as a similar abstract submitted to two different conferences) should be considered unique.



Description of the included studies

See Characteristics of included studies and Table 1.

Omar 2020 was a multicentre study that involved two study cohorts at centres in China (Omar 2020 China) and South Africa (Omar 2020 South Africa). The two study cohorts included a total of 530 participants, of whom 487 (91.9%) had tuberculosis verified by culture and 254 (47.9%) had rifampicin resistance. Xpert MTB/XDR and reference standard testing were performed at a central-level laboratory. Both study cohorts used archived raw sputum or concentrated sputum sediment specimens from participants who had been evaluated for pulmonary tuberculosis in inpatient and outpatient settings. Specimens that were culture positive or negative by LJ (Löwenstein–Jensen) medium or MGIT (Mycobacteria Growth Indicator Tube) were included.

Culture positive specimens were included if they met the following criteria:

- at least 1 mL of frozen sputum sediment or 2 mL of raw sputum was available;
- results were available for smear microscopy and culture (MGIT and/or LJ);
- the specimen had results from Xpert MTB/RIF or Xpert MTB/RIF Ultra testing;
- the specimen had pDST results for isoniazid, fluoroquinolones, ethionamide, amikacin, kanamycin, and capreomycin; and
- the specimen had gDST results (loci included in the gDST reference standard are listed below).

Culture negative specimens were included if at least 1 mL of frozen sputum sediment or 2 mL of raw sputum was available. Specimens that had previously thawed were excluded.

Penn-Nicholson 2021 was a multicentre study that involved four study cohorts at centres in Mumbai (Penn-Nicholson 2021 India (Mumbai); Moldova Penn-Nicholson 2021 Moldova); New Delhi

Penn-Nicholson 2021 India (New Delhi); and South Africa (Penn-Nicholson 2021 South Africa). Participants were evaluated for in inpatient and outpatient settings. For detection of pulmonary tuberculosis, of 714 participants initially recruited, 286 (40.1%) reported receiving previous tuberculosis treatment and of 698 participants included in the analysis, 609 (87.2%) had tuberculosis verified by culture. Of 611 participants who had both Xpert MTB/XDR and reference standard results for any drug resistance, 494 (80.9%) had rifampicin resistance. Xpert MTB/XDR and reference standard testing were performed at a central-level laboratory.

The study enrolled participants who had symptoms suggestive of pulmonary tuberculosis (i.e. persistent cough (≥ 2 weeks) or as per local definition of suspected pulmonary tuberculosis) and a risk factor for drug-resistant tuberculosis as follows:

- previously received greater than one month of treatment for a prior tuberculosis episode; or
- failing tuberculosis treatment with positive sputum smear or culture after ≥ three months of a standard tuberculosis treatment; or
- had close contact with a known drug-resistant tuberculosis case; or
- newly diagnosed with MDR-TB within the last 30 days; or
- previously diagnosed with MDR-TB and failed tuberculosis treatment with a positive sputum smear or culture after ≥ three months of a standard MDR-TB treatment regimen.

Participants received prior testing with Xpert MTB/RIF or Xpert MTB/RIF Ultra and those with a positive Xpert MTB/RIF or Xpert MTB/RIF Ultra result and a clear rifampicin result (resistant or susceptible) were included. Culture-positive samples were tested by pDST (MGIT) for resistance to isoniazid, rifampicin, fluoroquinolones, ethionamide, amikacin, kanamycin, and capreomycin. Participants were also required to produce an adequate quantity (3 mL) of sputum.

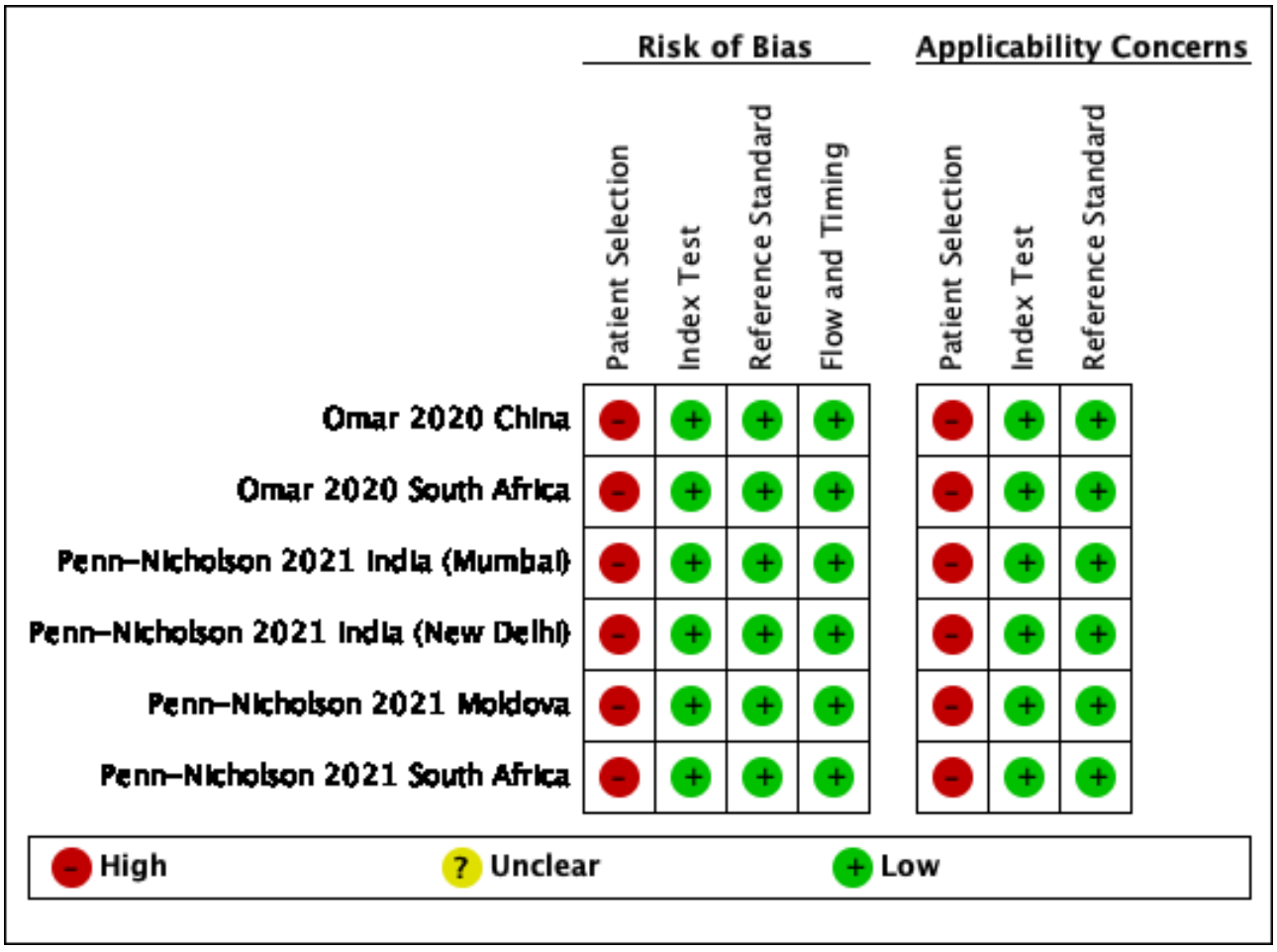
For detection of drug resistance, both multicentre studies evaluated Xpert MTB/XDR against all three reference standards (i.e. pDST, gDST, and composite reference standard). Both multicentre studies included identical loci in the gDST reference standard: *katG*, *inhA* promoter, *fabG1*, a*hpC-oxyR* intergenic region, *gyrA*, *gyrB*, *rrs*, and *eis* promoter.

Methodological quality of included studies

Detection of pulmonary tuberculosis

See Figure 4.

Figure 4. Xpert MTB/XDR for detection of pulmonary tuberculosis. Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study.



In the patient selection domain, we considered all study cohorts (100%) to have high risk of bias. The high proportion of people with tuberculosis (verified by culture), 91.3% in Omar 2020 China, and 92.2% in Omar 2020 South Africasuggested selective recruitment of participants. In Penn-Nicholson 2021 India (Mumbai),Penn-Nicholson 2021 India (New Delhi),Penn-Nicholson 2021 Moldova, and Penn-Nicholson 2021 South Africa), 80.9% of participants had known rifampicin resistance. Regarding applicability for patient selection, we considered all study cohorts to have high concern as the included patients did not match the review question.

In the index test domain, we considered all study cohorts to have low risk of bias and low concern about applicability.

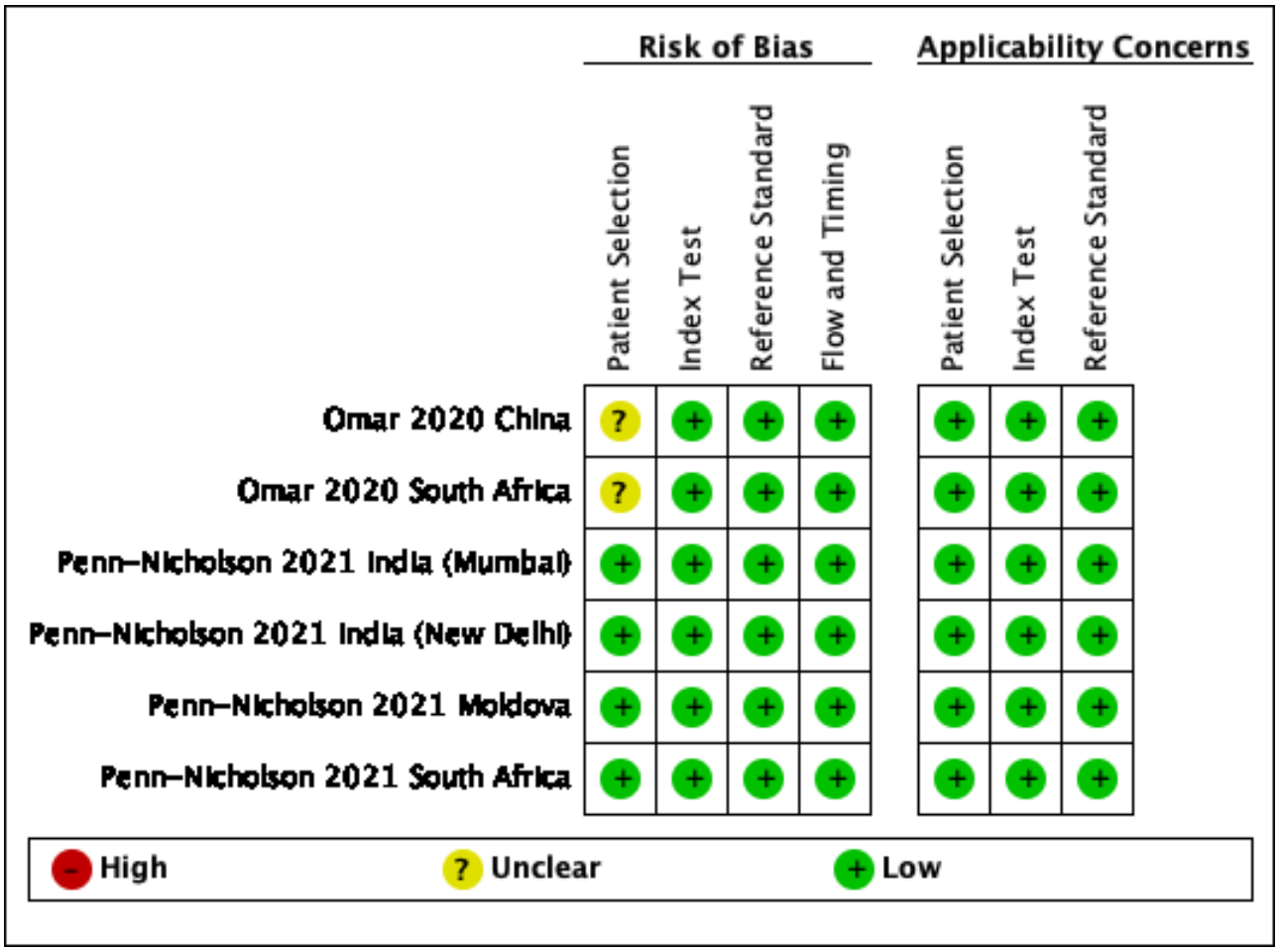
In the reference standard domain, we considered all study cohorts to have low risk of bias and low concern about applicability.

In the flow and timing domain, we considered all study cohorts to have low risk of bias.

Detection of tuberculosis drug resistance

Resitance to isoniazid, fluoroquinolones, and amikacin, Figure 5.

Figure 5. Xpert MTB/XDR for detection of resistance to isoniazid. Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study. Risk of bias and applicability concerns were the same for Xpert MTB/XDR for detection of resistance to fluoroquinolone and amikacin.



In the patient selection domain, we considered four study cohorts (67%) to have low risk of bias (Penn-Nicholson 2021 India (Mumbai); Penn-Nicholson 2021 India (New Delhi); Penn-Nicholson 2021 Moldova; Penn-Nicholson 2021 South Africa), and two study cohorts to have unclear risk of bias because we could not tell if these study cohorts avoided inappropriate exclusions (Omar 2020 China; Omar 2020 South Africa). Regarding applicability for patient selection, we considered all study cohorts to have low concern.

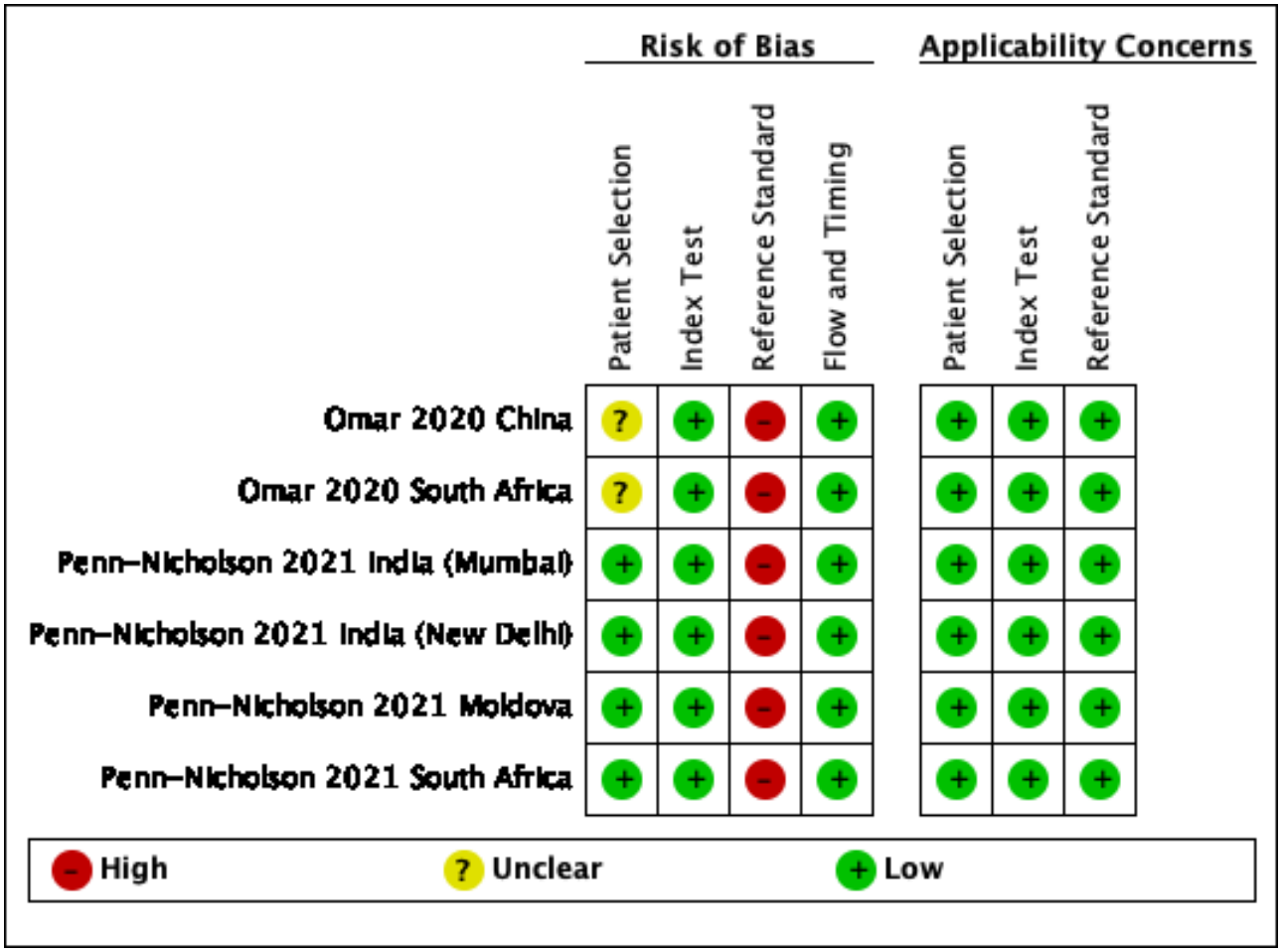
In the index test domain, we considered all study cohorts to have low risk of bias. Regarding applicability, for the index test domain, we considered all study cohorts to have low concern.

In the reference standard domain, for pDST and gDST, we considered all study cohorts have low risk of bias. Regarding applicability, for the reference standard domain, we considered all study cohorts to have low concern.

In the flow and timing domain, we considered all study cohorts to have low risk of bias.

Ethionamide resistance, Figure 6.

Figure 6. Xpert MTB/XDR for detection of resistance to ethionamide. Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study.



For Xpert MTB/XDR for resistance to ethionamide, our assessment of methodological quality was the same as for resistance to the other drugs, except for risk of bias in the reference standard domain. For pDST and gDST, we judged all study cohorts to have high risk of bias. For pDST, this was owing to considerable overlap in the minimum inhibitory concentration (MIC)s of *M tuberculosis* isolates with and without resistance-causing variants. For gDST, this was because no study cohort included all loci required, *ethA*, *ethR*, and *inhA* promoter. We note that Omar 2020 China assessed Xpert MTB/XDR for ethionamide resistance only against the gDST reference standard, and not the pDST reference standard.

Conflicts of interest

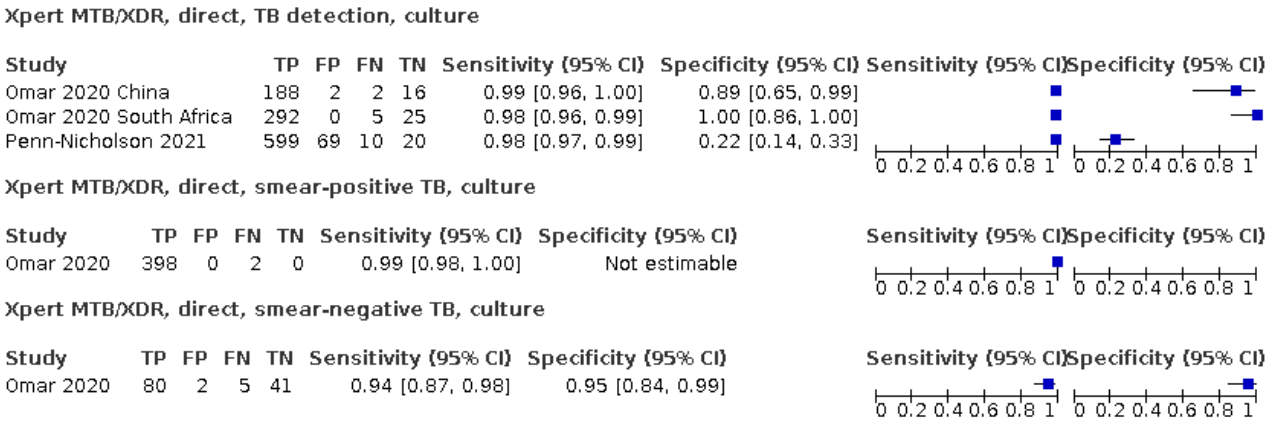
One study reporting on two study cohorts was sponsored by the manufacturer (Omar 2020 China; Omar 2020 South Africa). We performed sensitivity analyses by repeating the meta-analyses and excluding these study cohorts (see Sensitivity analyses).

Findings

Detection of pulmonary tuberculosis

For Xpert MTB/XDR accuracy for detection of pulmonary tuberculosis, we identified two studies. One study reported data for two study cohorts (Omar 2020 China; Omar 2020 South Africa), and one study reported data for the study as a whole (Penn-Nicholson 2021), Figure 7. Xpert MTB/XDR sensitivity ranged from 98.3% (96.1 to 99.5) to 98.9% (96.2 to 99.9) and specificity from 22.5% (14.3 to 32.6) to 100.0% (86.3 to 100.0); the median prevalence of pulmonary tuberculosis was 91.3%, (interquartile range, 89.3% to 91.8%). In Penn-Nicholson 2021; the low specificity (22.5%) may in part be explained by inclusion of participants on tuberculosis treatment (40.1%). Such participants may have tested Xpert MTB/XDR positive and culture (reference standard) negative and been classified as false-positive. We did not perform a meta-analysis owing to heterogeneity in both the characteristics of participants and observed specificity estimates.

Figure 7. Forest plots of Xpert MTB/XDR sensitivity and specificity by direct testing for pulmonary tuberculosis against culture reference standard. TB: tuberculosis; TP = true positive; FP = false positive; FN = false negative; TN = true negative. For detection of pulmonary tuberculosis, only one study reported data for separate study cohorts. For smear-positive and smear-negative TB, data were not reported for separate study cohorts.



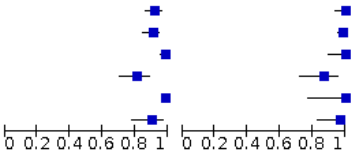
Detection of drug resistance

Forest plots for isoniazid resistance are presented in Figure 8, fluoroquinolone resistance in Figure 9, ethionamide resistance in Figure 10, and amikacin resistance in Figure 11. Xpert MTB/XDR summary sensitivity and specificity estimates for detection of drug resistance are presented in Table 2.

Figure 8. Forest plots of Xpert MTB/XDR sensitivity and specificity by direct testing for isoniazid resistance by population and reference standard. gDST = genotypic drug resistance testing; pDST = phenotypic drug resistance testing; TP = true positive; FP = false positive; FN = false negative; TN = true negative. Study in the forest plots refers to a study cohort within a multicentre study.

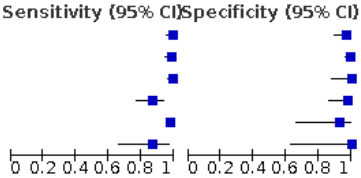
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	117	0	10	60	0.92 [0.86, 0.96]	1.00 [0.94, 1.00]		
Omar 2020 South Africa	127	2	13	149	0.91 [0.85, 0.95]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	143	0	2	33	0.99 [0.95, 1.00]	1.00 [0.89, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	63	5	15	33	0.81 [0.70, 0.89]	0.87 [0.72, 0.96]		
Penn-Nicholson 2021 Moldova	213	0	3	14	0.99 [0.96, 1.00]	1.00 [0.77, 1.00]		
Penn-Nicholson 2021 South Africa	45	1	5	30	0.90 [0.78, 0.97]	0.97 [0.83, 1.00]		



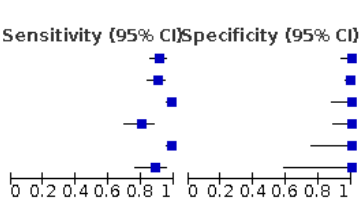
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	113	2	1	64	0.99 [0.95, 1.00]	0.97 [0.89, 1.00]		
Omar 2020 South Africa	128	1	2	160	0.98 [0.95, 1.00]	0.99 [0.97, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	132	0	1	29	0.99 [0.96, 1.00]	1.00 [0.88, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	61	1	9	38	0.87 [0.77, 0.94]	0.97 [0.87, 1.00]		
Penn-Nicholson 2021 Moldova	208	1	4	13	0.98 [0.95, 0.99]	0.93 [0.66, 1.00]		
Penn-Nicholson 2021 South Africa	20	0	3	8	0.87 [0.66, 0.97]	1.00 [0.63, 1.00]		



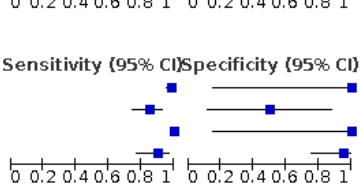
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, composite

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	117	0	11	59	0.91 [0.85, 0.96]	1.00 [0.94, 1.00]		
Omar 2020 South Africa	128	1	14	148	0.90 [0.84, 0.95]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	143	0	2	28	0.99 [0.95, 1.00]	1.00 [0.88, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	68	0	17	31	0.80 [0.70, 0.88]	1.00 [0.89, 1.00]		
Penn-Nicholson 2021 Moldova	213	0	4	13	0.98 [0.95, 0.99]	1.00 [0.75, 1.00]		
Penn-Nicholson 2021 South Africa	45	0	6	7	0.88 [0.76, 0.96]	1.00 [0.59, 1.00]		



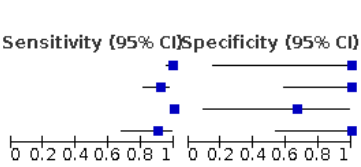
Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	141	0	2	2	0.99 [0.95, 1.00]	1.00 [0.16, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	58	3	10	3	0.85 [0.75, 0.93]	0.50 [0.12, 0.88]		
Penn-Nicholson 2021 Moldova	210	0	0	2	1.00 [0.98, 1.00]	1.00 [0.16, 1.00]		
Penn-Nicholson 2021 South Africa	37	1	4	19	0.90 [0.77, 0.97]	0.95 [0.75, 1.00]		



Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	130	0	1	2	0.99 [0.96, 1.00]	1.00 [0.16, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	54	0	5	7	0.92 [0.81, 0.97]	1.00 [0.59, 1.00]		
Penn-Nicholson 2021 Moldova	206	1	0	2	1.00 [0.98, 1.00]	0.67 [0.09, 0.99]		
Penn-Nicholson 2021 South Africa	18	0	2	6	0.90 [0.68, 0.99]	1.00 [0.54, 1.00]		



Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, composite

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	141	0	2	1	0.99 [0.95, 1.00]	1.00 [0.03, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	61	0	10	3	0.86 [0.76, 0.93]	1.00 [0.29, 1.00]		
Penn-Nicholson 2021 Moldova	210	0	0	2	1.00 [0.98, 1.00]	1.00 [0.16, 1.00]		
Penn-Nicholson 2021 South Africa	37	0	4	5	0.90 [0.77, 0.97]	1.00 [0.48, 1.00]		

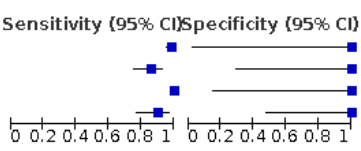
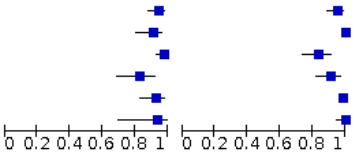


Figure 9. Forest plots of Xpert MTB/XDR sensitivity and specificity by direct testing for fluoroquinolone resistance by population and reference standard. Study in the forest plots refers to a study cohort within a multicentre study. gDST = genotypic drug resistance testing; pDST = phenotypic drug resistance testing; TP = true positive; FP = false positive; FN = false negative; TN = true negative.

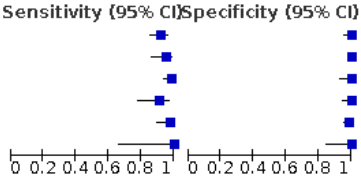
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	90	4	5	87	0.95 [0.88, 0.98]	0.96 [0.89, 0.99]		
Omar 2020 South Africa	58	0	6	167	0.91 [0.81, 0.96]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	102	12	2	62	0.98 [0.93, 1.00]	0.84 [0.73, 0.91]		
Penn-Nicholson 2021 India (New Delhi)	38	6	8	64	0.83 [0.69, 0.92]	0.91 [0.82, 0.97]		
Penn-Nicholson 2021 Moldova	52	2	4	172	0.93 [0.83, 0.98]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 South Africa	15	0	1	64	0.94 [0.70, 1.00]	1.00 [0.94, 1.00]		



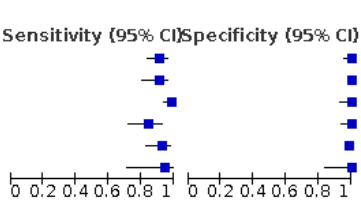
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	94	0	8	78	0.92 [0.85, 0.97]	1.00 [0.95, 1.00]		
Omar 2020 South Africa	58	0	3	228	0.95 [0.86, 0.99]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	107	0	2	53	0.98 [0.94, 1.00]	1.00 [0.93, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	39	0	4	66	0.91 [0.78, 0.97]	1.00 [0.95, 1.00]		
Penn-Nicholson 2021 Moldova	50	3	1	172	0.98 [0.90, 1.00]	0.98 [0.95, 1.00]		
Penn-Nicholson 2021 South Africa	9	0	0	22	1.00 [0.66, 1.00]	1.00 [0.85, 1.00]		



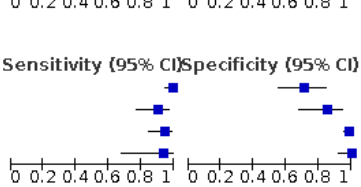
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, composite

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	94	0	9	83	0.91 [0.84, 0.96]	1.00 [0.96, 1.00]		
Omar 2020 South Africa	58	0	6	225	0.91 [0.81, 0.96]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	113	0	2	53	0.98 [0.94, 1.00]	1.00 [0.93, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	44	0	8	61	0.85 [0.72, 0.93]	1.00 [0.94, 1.00]		
Penn-Nicholson 2021 Moldova	52	2	4	169	0.93 [0.83, 0.98]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 South Africa	16	0	1	21	0.94 [0.71, 1.00]	1.00 [0.84, 1.00]		



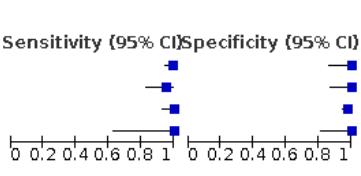
Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	102	12	1	30	0.99 [0.95, 1.00]	0.71 [0.55, 0.84]		
Penn-Nicholson 2021 India (New Delhi)	37	5	4	28	0.90 [0.77, 0.97]	0.85 [0.68, 0.95]		
Penn-Nicholson 2021 Moldova	51	2	3	156	0.94 [0.85, 0.99]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 South Africa	14	0	1	45	0.93 [0.68, 1.00]	1.00 [0.92, 1.00]		



Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	107	0	1	25	0.99 [0.95, 1.00]	1.00 [0.86, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	37	0	2	27	0.95 [0.83, 0.99]	1.00 [0.87, 1.00]		
Penn-Nicholson 2021 Moldova	50	3	0	156	1.00 [0.93, 1.00]	0.98 [0.95, 1.00]		
Penn-Nicholson 2021 South Africa	8	0	0	18	1.00 [0.63, 1.00]	1.00 [0.81, 1.00]		



Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, composite

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	113	0	1	25	0.99 [0.95, 1.00]	1.00 [0.86, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	42	0	4	25	0.91 [0.79, 0.98]	1.00 [0.86, 1.00]		
Penn-Nicholson 2021 Moldova	51	2	3	153	0.94 [0.85, 0.99]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 South Africa	15	0	1	17	0.94 [0.70, 1.00]	1.00 [0.80, 1.00]		

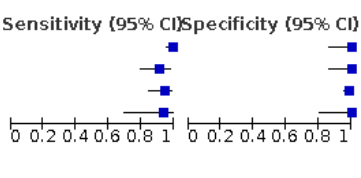
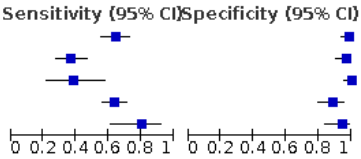


Figure 10. Forest plots of Xpert MTB/XDR sensitivity and specificity by direct testing for ethionamide resistance by population and reference standard. Study in the forest plots refers to a study cohort within a multicentre study. gDST = genotypic drug resistance testing; pDST = phenotypic drug resistance testing; TP = true positive; FP = false positive; FN = false negative; TN = true negative.

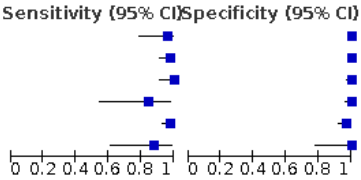
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 South Africa	75	2	41	112	0.65 [0.55, 0.73]	0.98 [0.94, 1.00]
Penn-Nicholson 2021 India (Mumbai)	39	2	66	71	0.37 [0.28, 0.47]	0.97 [0.90, 1.00]
Penn-Nicholson 2021 India (New Delhi)	12	0	19	85	0.39 [0.22, 0.58]	1.00 [0.96, 1.00]
Penn-Nicholson 2021 Moldova	101	8	57	64	0.64 [0.56, 0.71]	0.89 [0.79, 0.95]
Penn-Nicholson 2021 South Africa	24	3	6	48	0.80 [0.61, 0.92]	0.94 [0.84, 0.99]



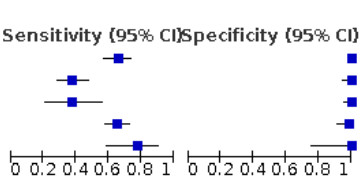
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	23	0	1	157	0.96 [0.79, 1.00]	1.00 [0.98, 1.00]
Omar 2020 South Africa	81	0	2	209	0.98 [0.92, 1.00]	1.00 [0.98, 1.00]
Penn-Nicholson 2021 India (Mumbai)	39	0	0	123	1.00 [0.91, 1.00]	1.00 [0.97, 1.00]
Penn-Nicholson 2021 India (New Delhi)	11	0	2	96	0.85 [0.55, 0.98]	1.00 [0.96, 1.00]
Penn-Nicholson 2021 Moldova	103	4	2	117	0.98 [0.93, 1.00]	0.97 [0.92, 0.99]
Penn-Nicholson 2021 South Africa	14	0	2	15	0.88 [0.62, 0.98]	1.00 [0.78, 1.00]



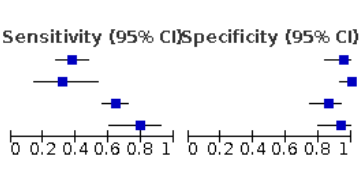
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, composite

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 South Africa	81	0	42	169	0.66 [0.57, 0.74]	1.00 [0.98, 1.00]
Penn-Nicholson 2021 India (Mumbai)	40	0	66	63	0.38 [0.29, 0.48]	1.00 [0.94, 1.00]
Penn-Nicholson 2021 India (New Delhi)	12	0	20	78	0.38 [0.21, 0.56]	1.00 [0.95, 1.00]
Penn-Nicholson 2021 Moldova	108	1	57	62	0.65 [0.58, 0.73]	0.98 [0.91, 1.00]
Penn-Nicholson 2021 South Africa	24	0	7	13	0.77 [0.59, 0.90]	1.00 [0.75, 1.00]



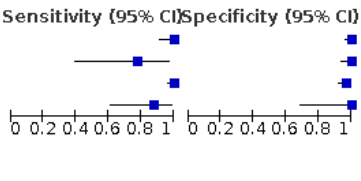
Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	39	2	65	39	0.38 [0.28, 0.48]	0.95 [0.83, 0.99]
Penn-Nicholson 2021 India (New Delhi)	8	0	17	49	0.32 [0.15, 0.54]	1.00 [0.93, 1.00]
Penn-Nicholson 2021 Moldova	100	8	55	49	0.65 [0.56, 0.72]	0.86 [0.74, 0.94]
Penn-Nicholson 2021 South Africa	23	2	6	30	0.79 [0.60, 0.92]	0.94 [0.79, 0.99]



Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	39	0	0	94	1.00 [0.91, 1.00]	1.00 [0.96, 1.00]
Penn-Nicholson 2021 India (New Delhi)	7	0	2	57	0.78 [0.40, 0.97]	1.00 [0.94, 1.00]
Penn-Nicholson 2021 Moldova	103	3	0	103	1.00 [0.96, 1.00]	0.97 [0.92, 0.99]
Penn-Nicholson 2021 South Africa	14	0	2	10	0.88 [0.62, 0.98]	1.00 [0.69, 1.00]



Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, composite

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	40	0	65	35	0.38 [0.29, 0.48]	1.00 [0.90, 1.00]
Penn-Nicholson 2021 India (New Delhi)	8	0	18	42	0.31 [0.14, 0.52]	1.00 [0.92, 1.00]
Penn-Nicholson 2021 Moldova	107	1	55	48	0.66 [0.58, 0.73]	0.98 [0.89, 1.00]
Penn-Nicholson 2021 South Africa	23	0	7	8	0.77 [0.58, 0.90]	1.00 [0.63, 1.00]

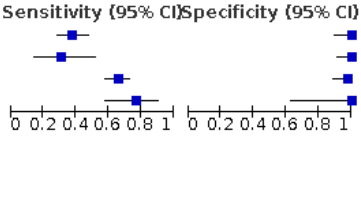
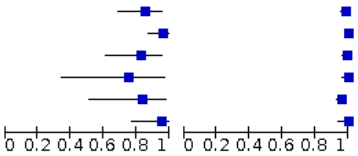


Figure 11. Forest plots of Xpert MTB/XDR sensitivity and specificity by direct testing for amikacin resistance by population and reference standard. Study in the forest plots refers to a study cohort within a multicentre study. gDST = genotypic drug resistance testing; pDST = phenotypic drug resistance testing; TP = true positive; FP = false positive; FN = false negative; TN = true negative.

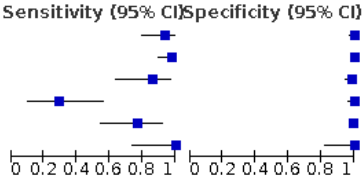
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	29	2	5	141	0.85 [0.69, 0.95]	0.99 [0.95, 1.00]
Omar 2020 South Africa	50	0	2	176	0.96 [0.87, 1.00]	1.00 [0.98, 1.00]
Penn-Nicholson 2021 India (Mumbai)	19	1	4	153	0.83 [0.61, 0.95]	0.99 [0.96, 1.00]
Penn-Nicholson 2021 India (New Delhi)	6	0	2	107	0.75 [0.35, 0.97]	1.00 [0.97, 1.00]
Penn-Nicholson 2021 Moldova	10	8	2	210	0.83 [0.52, 0.98]	0.96 [0.93, 0.98]
Penn-Nicholson 2021 South Africa	21	0	1	59	0.95 [0.77, 1.00]	1.00 [0.94, 1.00]



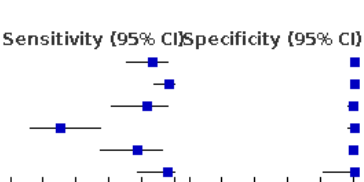
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	31	0	2	144	0.94 [0.80, 0.99]	1.00 [0.97, 1.00]
Omar 2020 South Africa	50	0	1	235	0.98 [0.90, 1.00]	1.00 [0.98, 1.00]
Penn-Nicholson 2021 India (Mumbai)	18	2	3	138	0.86 [0.64, 0.97]	0.99 [0.95, 1.00]
Penn-Nicholson 2021 India (New Delhi)	5	0	12	92	0.29 [0.10, 0.56]	1.00 [0.96, 1.00]
Penn-Nicholson 2021 Moldova	17	1	5	203	0.77 [0.55, 0.92]	1.00 [0.97, 1.00]
Penn-Nicholson 2021 South Africa	12	0	0	19	1.00 [0.74, 1.00]	1.00 [0.82, 1.00]



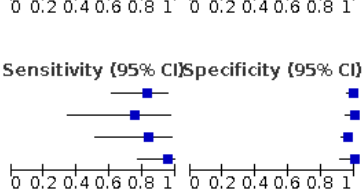
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, composite

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	31	0	5	147	0.86 [0.71, 0.95]	1.00 [0.98, 1.00]
Omar 2020 South Africa	50	0	2	234	0.96 [0.87, 1.00]	1.00 [0.98, 1.00]
Penn-Nicholson 2021 India (Mumbai)	19	1	4	137	0.83 [0.61, 0.95]	0.99 [0.96, 1.00]
Penn-Nicholson 2021 India (New Delhi)	6	0	14	89	0.30 [0.12, 0.54]	1.00 [0.96, 1.00]
Penn-Nicholson 2021 Moldova	17	1	5	203	0.77 [0.55, 0.92]	1.00 [0.97, 1.00]
Penn-Nicholson 2021 South Africa	21	0	1	18	0.95 [0.77, 1.00]	1.00 [0.81, 1.00]



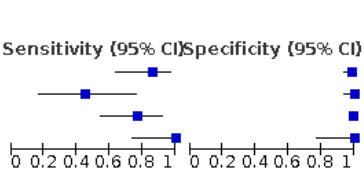
Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	19	1	4	120	0.83 [0.61, 0.95]	0.99 [0.95, 1.00]
Penn-Nicholson 2021 India (New Delhi)	6	0	2	65	0.75 [0.35, 0.97]	1.00 [0.94, 1.00]
Penn-Nicholson 2021 Moldova	10	8	2	192	0.83 [0.52, 0.98]	0.96 [0.92, 0.98]
Penn-Nicholson 2021 South Africa	21	0	1	39	0.95 [0.77, 1.00]	1.00 [0.91, 1.00]



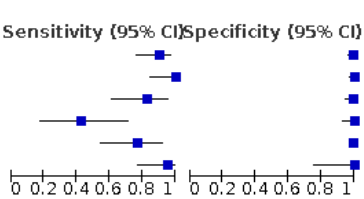
Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	18	2	3	109	0.86 [0.64, 0.97]	0.98 [0.94, 1.00]
Penn-Nicholson 2021 India (New Delhi)	5	0	6	55	0.45 [0.17, 0.77]	1.00 [0.94, 1.00]
Penn-Nicholson 2021 Moldova	17	1	5	186	0.77 [0.55, 0.92]	0.99 [0.97, 1.00]
Penn-Nicholson 2021 South Africa	12	0	0	14	1.00 [0.74, 1.00]	1.00 [0.77, 1.00]



Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, composite

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	36	1	4	136	0.90 [0.76, 0.97]	0.99 [0.96, 1.00]
Omar 2020 South Africa	22	0	0	140	1.00 [0.85, 1.00]	1.00 [0.97, 1.00]
Penn-Nicholson 2021 India (Mumbai)	19	1	4	108	0.83 [0.61, 0.95]	0.99 [0.95, 1.00]
Penn-Nicholson 2021 India (New Delhi)	6	0	8	53	0.43 [0.18, 0.71]	1.00 [0.93, 1.00]
Penn-Nicholson 2021 Moldova	17	1	5	186	0.77 [0.55, 0.92]	0.99 [0.97, 1.00]
Penn-Nicholson 2021 South Africa	21	0	1	13	0.95 [0.77, 1.00]	1.00 [0.75, 1.00]



Xpert MTB/XDR by direct testing for resistance to isoniazid, fluoroquinolones, and amikacin

For detection of resistance to isoniazid and fluoroquinolones, Xpert MTB/XDR summary estimates for sensitivity and specificity were similar when different reference standards were used, both in people irrespective of rifampicin resistance and in people with rifampicin resistance. For detection of resistance to amikacin, Xpert MTB/XDR summary sensitivity estimates against gDST in the different populations were more variable.

We note that Xpert MTB/XDR sensitivity for detection of isoniazid resistance, Figure 8, and amikacin resistance, Figure 11 was lower in New Delhi than in other study cohorts.

Xpert MTB/XDR by direct testing for ethionamide resistance

For detection of ethionamide resistance, Xpert MTB/XDR summary estimates for sensitivity varied when different reference standards were used. Specificity values were more consistent in these analyses. We also note that against both pDST and a composite reference standard, Xpert MTB/XDR sensitivity for detection of

ethionamide resistance was lower in New Delhi and Mumbai than in Moldova and South Africa, Figure 10.

Xpert MTB/XDR by direct testing for resistance to kanamycin and capreomycin

Forest plots of Xpert MTB/XDR sensitivity and specificity estimates for detection of kanamycin and capreomycin resistance are presented in Appendix 6.

For detection of kanamycin resistance, Xpert MTB/XDR summary sensitivity estimates were similar to those for amikacin. For detecting capreomycin resistance, Xpert MTB/XDR summary sensitivity estimates were lower than those for other drugs. Summary specificity estimates were more consistent in these analyses, Table 2.

Comparison Xpert MTB/XDR accuracy by direct testing versus indirect testing

One study compared Xpert MTB/XDR accuracy on sputum (direct testing) with cultured isolates (indirect testing) (Penn-Nicholson 2021). Data were not reported by study cohort. For each drug (isoniazid, fluoroquinolone, ethionamide, and amikacin), Xpert MTB/XDR accuracy for drug resistance by type of testing was similar, Appendix 7.

Inconclusive Xpert MTB/XDR results and missed cases

Data on inconclusive Xpert MTB/XDR results and missed cases are described in Appendix 5.

Non-determinate results

The summary proportion of Xpert MTB/XDR non-determinate results was estimated to be 2.90% (95% CI: 1.97% to 3.84%). The proportion of Xpert MTB/XDR non-determinate results following retesting was 0.2% (1/531) (Omar 2020) and 0.3% (2/709) (Penn-Nicholson 2021).

Xpert XDR/MTB indeterminate results

See Table 3.

One study provided information on retesting following an Xpert MTB/XDR indeterminate result (Penn-Nicholson 2021). No specimens were indeterminate upon retesting for resistance to isoniazid, fluoroquinolone, and ethionamide. Of 657 specimens tested by Xpert MTB/XDR for amikacin resistance, 23 (3.5%) had indeterminate results and 1/23 was indeterminate upon retesting.

Xpert MTB/XDR MTB NOT DETECTED

One study reported information about when Xpert MTB/XDR did not detect tuberculosis to begin with (missed cases) (Omar 2020). Results are summarized in Appendix 5.

Investigations of heterogeneity

Tuberculosis detection

Smear status

One study assessed Xpert MTB/XDR accuracy for pulmonary tuberculosis in smear-positive and smear-negative sputum specimens (Omar 2020), Figure 7. Data were not reported by study cohort. We note that Xpert MTB/XDR sensitivity in smear-negative specimens was higher than expected and may have been overestimated (see Discussion).

Drug resistance detection

Smear status

One study compared Xpert MTB/XDR sensitivity and specificity for drug resistance in smear-positive and smear-negative sputum specimens (Penn-Nicholson 2021). Data were not reported by study cohort. For a given drug (isoniazid, fluoroquinolone, ethionamide, and amikacin), Xpert MTB/XDR accuracy for detection of drug resistance was similar in smear-positive and smear-negative specimens, Appendix 8.

HIV status

One study compared Xpert MTB/XDR sensitivity and specificity for drug resistance in HIV-positive and HIV-negative people (Penn-Nicholson 2021). Data were not reported by study cohort. For resistance to isoniazid and fluoroquinolones, Xpert MTB/XDR sensitivity was similar, while for resistance to ethionamide and amikacin, Xpert MTB/XDR sensitivity was higher in HIV-positive people than in HIV-negative people, Appendix 9. There were few resistant samples in the HIV-positive subgroup compared to the HIV-negative subgroups, which could account for this variability. Xpert MTB/XDR specificity was high and consistent in all analyses.

Previous tuberculosis treatment

One study assessed Xpert MTB/XDR accuracy for detection of drug resistance in people with and without previous tuberculosis treatment (Penn-Nicholson 2021). Data were not reported by study cohort. There were no notable differences in Xpert MTB/XDR sensitivity or specificity for drug resistance in people who reported no previous tuberculosis treatment in the preceding 60 days versus those who reported receiving tuberculosis treatment in the preceding 60 days, Appendix 10.

Sensitivity analyses

Overall, the sensitivity analyses made little difference to the findings, Table 4.

DISCUSSION

This Cochrane Review summarizes the evidence on the diagnostic accuracy of Xpert MTB/XDR, a newly developed nucleic acid amplification test (NAAT) that detects pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin. We identified two multicentre studies reporting on a total of six independent study cohorts and including 1228 participants for pulmonary tuberculosis detection and 1141 participants for drug resistance detection. Both studies took place in high MDR/rifampicin-resistant tuberculosis burden countries. The review had notable limitations. For detection of pulmonary tuberculosis, in the patient selection domain, we judged all studies as having high risk of bias owing to selective participant recruitment. For detection of ethionamide resistance, in the reference standard domain, we judged high risk of bias for both phenotypic drug susceptibility testing (pDST) and genotypic drug susceptibility testing (gDST).

Summary of main results

- For detection of pulmonary tuberculosis, Xpert MTB/XDR sensitivity ranged from 98.3% (96.1 to 99.5) to 98.9% (96.2 to 99.9) and specificity from 22.5% (14.3 to 32.6) to 100.0% (86.3 to 100.0). The median prevalence of pulmonary tuberculosis in this analysis was 91.3%, (interquartile range, 89.3% to 91.8%).
- For resistance to isoniazid, in people irrespective of rifampicin resistance, detected as tuberculosis positive by Xpert MTB/XDR, Xpert MTB/XDR summary sensitivity was 94.2% (87.5 to 97.4) against a reference standard of pDST.
- For resistance to fluoroquinolones, in people irrespective of rifampicin resistance, detected as tuberculosis positive by Xpert MTB/XDR, Xpert MTB/XDR summary sensitivity was 93.2% (88.1 to 96.2) against a reference standard of pDST.
- For resistance to ethionamide, in people with known rifampicin resistance, detected as tuberculosis positive by Xpert MTB/XDR, Xpert MTB/XDR summary sensitivity 98.0% (74.2 to 99.9) against a reference standard of gDST.
- For resistance to amikacin, in people with known rifampicin resistance, detected as tuberculosis positive by Xpert MTB/XDR, Xpert MTB/XDR summary sensitivity was 86.1% (75.0 to 92.7) against a reference standard of pDST.
- Xpert MTB/XDR summary specificity for detection of any drug resistance was > 97.0% in most analyses.
- Overall, for resistance to isoniazid and fluoroquinolones, Xpert MTB/XDR sensitivity estimates for individual studies were consistent against the different reference standards.
- The summary proportion of Xpert MTB/XDR non-determinate results was estimated as 2.90% (95% CI: 1.97% to 3.84%).
- The summary proportion of Xpert MTB/XDR indeterminate results was estimated as 0.34% (0.00 to 0.68) for isoniazid resistance; 1.05% (0.46 to 1.64) for fluoroquinolone resistance; 0.06% (0.00 to 0.34) for ethionamide resistance; and 2.33% (1.46 to 3.20) for amikacin resistance.

For each drug, Xpert MTB/XDR summary sensitivity and specificity estimates were similar in people irrespective of rifampicin resistance and people with rifampicin resistance. However, we note that a high proportion of participants had known rifampicin resistance.

We were unable to perform most pre-specified analyses owing to sparse data.

Xpert MTB/XDR for pulmonary tuberculosis, Summary of findings 1.

In theory, of 1000 people with suspected pulmonary tuberculosis of whom 100 have tuberculosis: an estimated 98 to 99 people would have an Xpert MTB/XDR result indicating tuberculosis, of these 1 to 2 (1%) would be incorrectly classified as having tuberculosis (FP); and an estimated 203 to 900 people would have a result indicating the absence of tuberculosis, of these 0 to 697 (0% to 77%) would have tuberculosis (FN).

Xpert MTB/XDR for isoniazid resistance in people irrespective of rifampicin resistance, detected as tuberculosis positive by Xpert MTB/XDR, Summary of findings 2.

In theory, of 1000 people with pulmonary tuberculosis detected as tuberculosis positive by Xpert MTB/XDR, where 50 have isoniazid resistance, 61 would have an Xpert MTB/XDR result indicating isoniazid resistance: of these, 14/61 (23%) would not have isoniazid resistance (FP); and 939 would have a result indicating the absence of isoniazid resistance: of these, 3/939 (0%) would have isoniazid resistance (FN).

Xpert MTB/XDR for fluoroquinolone resistance in people irrespective of rifampicin resistance, detected as tuberculosis positive by Xpert MTB/XDR, Summary of findings 3

In theory, of 1000 people with pulmonary tuberculosis detected as tuberculosis positive by Xpert MTB/XDR, where 50 have fluoroquinolone resistance, 66 would have an Xpert MTB/XDR result indicating fluoroquinolone resistance: of these, 19/66 (29%) would not have fluoroquinolone resistance (FP) and 934 would have a result indicating the absence of fluoroquinolone resistance: of these, 3/934 (0%) would have fluoroquinolone resistance (FN).

Xpert MTB/XDR for ethionamide resistance in people with known rifampicin resistance, detected as tuberculosis positive by Xpert MTB/XDR, Summary of findings 4.

In theory, of 1000 people with pulmonary tuberculosis detected as tuberculosis positive by Xpert MTB/XDR, where 300 have ethionamide resistance, 296 would have an Xpert MTB/XDR result indicating ethionamide resistance: of these, 2/296 (1%) would not have ethionamide resistance (FP) and 704 would have a result indicating the absence of ethionamide resistance: of these, 6/704 (1%) would have ethionamide resistance (FN).

Xpert MTB/XDR for amikacin resistance in people with known rifampicin resistance, detected as tuberculosis positive by Xpert MTB/XDR, Summary of findings 5.

In theory, of 1000 people with pulmonary tuberculosis detected as tuberculosis positive by Xpert MTB/XDR, where 135 have amikacin resistance, 126 would have an Xpert MTB/XDR result indicating amikacin resistance: of these, 10/126 (8%) would not have amikacin resistance (FP) and 874 would have a result indicating the absence of amikacin resistance: of these, 19/874 (2%) would have amikacin resistance (FN).

We noted that Xpert MTB/XDR sensitivity varied by study cohort. For detection of isoniazid and amikacin resistance, Xpert MTB/XDR sensitivity in New Delhi was considerably lower than in other study cohorts. For detection of ethionamide resistance, against both pDST and a composite reference standard, Xpert MTB/XDR sensitivity was lower in New Delhi and Mumbai than in Moldova and South Africa. Variants outside of those covered by the Xpert MTB/XDR assay may play a role in some settings, which could in part explain this variability.

For detection of capreomycin resistance, Xpert MTB/XDR summary sensitivity estimates were lower than those for resistance to other drugs. A Cochrane Review that assessed the diagnostic accuracy of MTBDR*sl* (a line probe assay) for resistance to second-line tuberculosis drugs showed a similar trend (Theron 2016b).

Xpert MTB/XDR is the first in a class of new technologies referred to as 'low complexity automated NAATs' for second-line drug-resistant tuberculosis. These new technologies are suitable for use in peripheral and intermediate level laboratories. Xpert MTB/XDR detects resistance to drugs other than rifampicin, namely isoniazid, fluoroquinolones, ethionamide, and amikacin (as well as kanamycin and capreomycin, second-line injectable drugs which

are no longer recommended for people with MDR/rifampicin-resistant tuberculosis). However, WHO guidelines stress that the use of a low complexity automated NAAT to detect fluoroquinolone resistance does not eliminate the need for culture-based pDST, required to determine resistance to other tuberculosis drugs (e.g. bedaquiline, delamanid, other drugs) (WHO Consolidated Guidelines (Module 3) 2021).

Xpert MTB/XDR could guide treatment decisions and allow for rapid initiation of effective therapy, especially regarding the use of fluoroquinolones in people with drug-resistant tuberculosis. The use of Xpert MTB/XDR in people with rifampicin-susceptible tuberculosis could also improve the detection of isoniazid resistance. Furthermore, with the exciting advent of new rifapentine-based shortened regimens for drug-susceptible tuberculosis, with and without moxifloxacin (a fluoroquinolone), the potential impact of Xpert MTB/XDR has increased (Dorman 2021).

We found, based on our summary estimates, that Xpert MTB/XDR sensitivity and specificity met the minimal (lowest acceptable) criteria for WHO's target product profile (TPP) for drug susceptibility testing (DST) to be used at peripheral microscopy centres. However, there is considerable uncertainty in the estimates and the lower limits of the 95% CIs lie below the TPP targets (WHO TPP 2021):

- diagnostic sensitivity > 90% for detection of isoniazid and fluoroquinolone resistance and ≥ 80% sensitivity for detection of amikacin resistance when measured against the pDST reference standard;

- analytical specificity ≥ 98% for any tuberculosis drug for which the test is able to identify resistance when compared with gDST as the reference standard.

Nonetheless, several challenges and questions need to be considered.

Xpert MTB/XDR must first detect tuberculosis, even if an individual is already tuberculosis-positive by another test, before it can generate a resistant or susceptible result. Our ability to assess Xpert MTB/XDR accuracy for detection of pulmonary tuberculosis was limited by the available data, which we considered to be at high risk of bias due to selective participant recruitment. As Xpert MTB/XDR is likely to be used as a follow-on test to an initial test that detects tuberculosis and rifampicin resistance (i.e. Xpert MTB/RIF, Xpert MTB/RIF Ultra, Truenat MTB, and Truenat MTB Plus), this approach would miss isoniazid or fluoroquinolone mono-resistant tuberculosis. Furthermore, if a patient has an Xpert MTB/RIF Ultra-trace positive result, they are unlikely to be detected as tuberculosis-positive by Xpert MTB/XDR. Xpert MTB/XDR, unlike Xpert MTB/RIF Ultra, relies on detection of a single rather than multicopy gene and Xpert MTB/RIF Ultra trace results occur only when the multicopy target is detected (Cepheid package insert 2021). As mentioned previously, the limit of detection of Xpert MTB/XDR for *M tuberculosis* is 71.9 colony-forming units (CFU)/mL, not as low as the limit of detection of Xpert MTB/RIF Ultra (15.6 CFU/mL) (Cao 2021; Chakravorty 2017).

Additionally, even if patients are Xpert MTB/RIF Ultra-positive, it is possible that the numbers and ability of bacteria to grow would decrease due to empiric treatment prior to a specimen being sent for Xpert MTB/XDR testing. This could result in a loss of culture-positivity (and preclude downstream pDST testing) even if Xpert MTB/XDR remains positive for tuberculosis due to the presence of MTB DNA. When tuberculosis is detected, the test may still report an indeterminate result for detection of drug resistance, though we found the summary proportion of indeterminate results to be low (≤ 2%). If Xpert MTB/XDR is done on sample reagent-treated sputum initially used for tuberculosis detection using Xpert MTB/RIF Ultra, the sample reagent may have, depending on storage conditions and duration, detrimentally affected DNA in the sputum in a manner that detracts from Xpert MTB/XDR performance (Banada 2010). This is an implementation challenge that requires further study.

The WHO positions Xpert MTB/XDR as a follow-on test for detection of additional drug resistance. However, the WHO has also set as a research priority the evaluation of Xpert MTB/XDR as an initial test for tuberculosis detection in people with signs and symptoms of tuberculosis (WHO Consolidated Guidelines (Module 3) 2021).

Non-actionable results (results which do not allow for clinician decisions) include all kinds of results (Xpert MTB/XDR MTB NOT DETECTED, non-determinate, indeterminate). This issue, which is a problem with MTBDR*sl* (a line probe assay), is becoming increasingly important as we seek to expand rapid DST (direct testing), including to those who are paucibacillary (tuberculosis disease caused by a small number of bacteria) and smear-negative and in whom tuberculosis detection by reflex DST would therefore be challenging. Our review had limited data to assess the number of people with tuberculosis who were missed (not detected as tuberculosis-positive by Xpert MTB/XDR to begin with), and would have drug susceptibility results uncharacterised by Xpert MTB/XDR.

In our review, in people with smear-negative specimens, Xpert MTB/XDR sensitivity (95% CI) for detection of pulmonary tuberculosis was 94% (87% to 98%) (based on one study) and may have been overestimated. We considered this study to have high risk of bias for participant selection. In contrast, a recent Cochrane Review found, in smear-negative (culture-positive) specimens, summary sensitivity of 77.5% (67.6 to 85.6) for Xpert MTB/RIF Ultra and 60.6% (48.4 to 71.7) for Xpert MTB/RIF (7 studies) (Zifodya 2021).

We did not have sufficient data to assess Xpert MTB/XDR accuracy for detection of pulmonary tuberculosis in people with and without previous tuberculosis treatment. This is an important concern as the test may report results for drug resistance in people who are detected as MTB-positive, but are in fact culture-negative. The related tests, Xpert MTB/RIF (Theron 2016a) and Xpert MTB/RIF Ultra (Mishra 2020), have diminished specificity in people with previous tuberculosis treatment. Importantly, since people with a history of tuberculosis have a higher risk of drug resistance compared to people who have not had tuberculosis before (WHO Global Tuberculosis Report 2021), DST is more likely to be done in this group.

Regarding detection of ethionamide resistance, Xpert MTB/XDR accuracy is based only on the detection of mutations in the *inhA* promoter region. Hence this limits the test's value in decision making for ruling-out resistance.

Heteroresistance, the clinical significance of which is uncertain, can be challenging for molecular tests to detect (pDST is generally the best method for detecting minority populations) and may in part explain Xpert MTB/XDR false-negative results. However,

more data are needed on the ability of Xpert MTB/XDR to detect heteroresistance.

Finally, we wish to underscore that an all-in-one test for tuberculosis drug resistance would be highly desirable. However, detecting resistance to additional drugs using Xpert MTB/XDR may not be technologically feasible without great expense or loss of other gene targets.

Strengths and weaknesses of the review

Strengths and weaknesses of the review process

We were unable to perform several analyses as originally intended in the protocol because the paucity of data precluded pre-specified investigations of heterogeneity. When we observed heterogeneity and could not explore potential sources of heterogeneity, we took this into account when deciding whether to downgrade for inconsistency.

Strengths and weaknesses due to methodological quality assessment

For tuberculosis detection, as assessed by QUADAS-2, in the patient selection domain, we considered all study cohorts (100%) to have high risk of bias. The high proportion (> 90%) of participants with tuberculosis suggests that there was selective recruitment. For drug resistance detection, in the reference standard domain, both studies had low risk of bias for resistance to isoniazid, fluoroquinolones, and amikacin, and high risk of bias for resistance to ethionamide (for both pDST and gDST). Both studies used the critical concentrations for pDST currently recommended by the WHO.

Completeness of evidence

The findings in this review were based on comprehensive searching, strict selection criteria, and standardized data extraction. To identify studies, we searched multiple databases up to 23 September 2021 without language restriction. However, we acknowledge that we may have missed studies despite the comprehensive search. We corresponded with primary study authors to obtain additional data and information that was missing from the papers. The small number of studies and small number of participants in several of the analyses affected the precision of the results.

Accuracy of the reference standards used

Detection of pulmonary tuberculosis

Culture is regarded as the best available reference standard for the bacteriological confirmation of pulmonary tuberculosis and was the reference standard for detection of pulmonary tuberculosis in this review. Liquid culture is considered to be more sensitive than solid culture (Lewinsohn 2017). Liquid culture or both solid and liquid culture were the reference standards in these analyses.

Detection of drug resistance

As recommended by the WHO, we used culture-based pDST as the main reference standard for isoniazid resistance, fluoroquinolone resistance, and amikacin resistance (WHO TPP 2021). Culture involves growing an inoculum (the introduction of the bacteria into a culture medium) in the absence of a drug. This could lead to resistant bacteria present in the original specimen diminishing below the limit of detection of the reference standard method due to competition with the other drug-susceptible bacteria in the inoculum.

We used gDST as the main reference standard for ethionamide resistance because there is considerable overlap in the minimum inhibitory concentrations of *M tuberculosis* isolates with and without resistance-causing variants and a pDST reference standard might not correctly classify the target condition. Ethionamide resistance caused by *inhA* mutations is detected by the Xpert MTB/XDR, however, the test may not detect all variants of ethionamide resistance. We note that the gDST reference standard used only included the *inhA* promoter.

Applicability of findings to the review question

For detection of pulmonary tuberculosis, owing to inclusion of participants based on Xpert MTB/RIF- and Xpert MTB/RIF Ultra-positive results, we had high concern about applicability of the findings to the review question. For detection of drug resistance, the two multicentre studies (reporting on six study cohorts) took place at sites located in high MDR/rifampicin-resistant tuberculosis burden countries. However, two study cohorts were in India and two were in South Africa, possibly limiting applicability to other settings.

AUTHORS' CONCLUSIONS

Implications for practice

The review findings suggest that Xpert MTB/XDR provides accurate results for detection of isoniazid and fluoroquinolone resistance and can assist with selection of an optimal treatment regimen. Given that Xpert MTB/XDR targets a limited number of resistance variants in specific genes, the test may perform differently in different settings. Findings in this review should, therefore, be interpreted with caution. Xpert MTB/XDR sensitivity for ethionamide resistance detection was based only on detection of mutations in the *inhA* promoter region by Xpert MTB/XDR, a known limitation. High risk of bias limits our confidence in Xpert MTB/XDR accuracy for pulmonary tuberculosis.

The impact of Xpert MTB/XDR is expected to be affected by the test's ability to detect tuberculosis (required for drug susceptibility testing (DST)), prevalence of resistance to a given drug, health care infrastructure, and access to other tests.

Implications for research

Future studies should assess the accuracy of Xpert MTB/XDR in different population groups, including children and people living with HIV. In addition, studies should assess the accuracy of Xpert MTB/XDR in different geographical settings, in smear-negative specimens, and with different types of clinical specimens. Assessing Xpert MTB/XDR accuracy in people who have previously received tuberculosis treatment is an important research gap and will inform whether confirmatory indirect testing of cultured isolates is feasible. Studies should also evaluate Xpert MTB/XDR as an initial test for tuberculosis detection, in addition to use as a follow-on test in all people with signs and symptoms of tuberculosis. Studies should assess the proportion of people with tuberculosis who are missed (not detected as tuberculosis-positive by Xpert MTB/XDR to begin with), and would have drug susceptibility results uncharacterised by Xpert MTB/XDR. Studies

are needed to understand whether new tuberculosis diagnostics, such as Xpert MTB/XDR, influence mortality and other health outcomes important to people. Such studies may inform the use of this test on both diagnostic and treatment pathways.

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Omar 2020 China *{unpublished data only}*

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

WHO Operational handbook - diagnosis 2021

World Health Organization.WHO operational handbook on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detention, 2021 update. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO. www.who.int/publications/i/item/9789240030589 (accessed 11 November 2021).

WHO Rapid Communication 2021

World Health Organization.Treatment of drug-susceptible tuberculosis: rapid communication. June 2021. who.int/publications/i/item/9789240028678 (accessed 14 February 2022).

WHO Rapid Communication 2022

World Health Organization.Rapid communication: key changes to the treatment of drug-resistant tuberculosis. May 2022. who.int/publications/i/item/WHO-UCN-TB-2022-2 (accessed 2 May 2022).

WHO TPP 2021

World Health Organization 2021.Target product profile for next-generation drug-susceptibility testing at peripheral centres. www.who.int/publications/i/item/9789240032361 (accessed 24 October 2021).

World Bank 2020

World Bank.World Bank List of Economies. datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups (accessed 18 November 2020).

Zifodya 2021

Zifodya JS, Kreniske JS, Schiller I, Kohli M, Dendukuri N, Schumacher SG, et al.Xpert Ultra versus Xpert MTB/RIF for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database of Systematic Reviews* 2021, Issue 2. Art. No: CD009593. [DOI: 10.1002/14651858.CD009593.pub4]

Zignol 2016

Zignol M, Dean AS, Alikhanova N, Andres S, Cabibbe AM, Cirillo DM, et al.Population-based resistance of Mycobacterium tuberculosis isolates to pyrazinamide and fluoroquinolones: results from a multicountry surveillance project. *Lancet Infectious Diseases* 2016;**16**(10):1185-92.

References to other published versions of this review

Pillay 2021

Pillay S, Davies GR, Chaplin M, De Vos M, Schumacher SG, Warren R, et al.Xpert MTB/XDR for detection of pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin. *Cochrane Database of Systematic Reviews* 2021, Issue 6. Art. No: CD014841. [DOI: 10.1002/14651858.CD014841]

Omar 2020

Study characteristics	
Patient Sampling	Cross-sectional, the manner of participant selection was not random or consecutive For drug resistance detection, MTB positive specimens were characterized by pDST and gDST prior to or during the study
Patient characteristics and setting	Presenting signs and symptoms: not reported; archived frozen raw sputum or sputum sediment specimens Exclusions: specimens that had been previously thawed were excluded; < 1 mL of frozen sputum sediment or < 2 mL of raw sputum Prior testing: archived (frozen) specimens confirmed to be MTB positive or negative by culture; Xpert MTB/RIF or Xpert MTB/RIF Ultra Age: ≥ 15 years (range, 13 to > 80 years; one participant was 13 years) in full study Sex, female: 38% HIV infection: China (0%); South Africa not reported Previous TB treatment: not reported Treatment of current episode: 199 (37.5%) study participated were reported to be on treatment, 6 (1.1%) were reported to not be on treatment and treatment status was unknown/not available for 325 study participants Sample size: 530; 254 (47.9%) with known rifampicin resistance Clinical setting: outpatient and inpatient Laboratory level: central Country: China, South Africa World Bank Income Classification: China (middle income) and South Africa (middle income) High TB burden country: China (yes), South Africa (yes) High TB/HIV burden country: China (yes), South Africa (yes) High MDR-TB burden country: China (yes), South Africa (yes)
Index tests	Xpert MTB/XDR
Target condition and reference standard(s)	Pulmonary tuberculosis Culture with MGIT or LJ culture; Xpert MTB/RIF and Xpert MTB/RIF Ultra Resistance to: isoniazid, fluoroquinolones, ethionamide, amikacin, kanamycin, capreomycin pDST, gDST, composite reference standard China: INH High 0.4 mg/L; INH Low 0.1 mg/L; MFX High 2.0 and Low 0.5 mg/L; OFX: 2.0 mg/L; ETO not done; AMK 1.0 mg/L; KAN 2.5 mg/L; CAP not done South Africa: INH High 0.4 mg/L Low 0.1 mg/L; MFX High 1.0 and Low 0.25 mg/L; OFX 2.0 mg/L; LVX 1.0 mg/L; ETO 5.0 mg/L; AMK 1.0 mg/L; KAN 2.5 mg/L; CAP 2.5 mg/L There were 8 gene targets of interest (*katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, gyrA, gyrB, rrs*, *eis* promoter) were reported

Omar 2020 *(Continued)*

Flow and timing	3 patients were excluded due to insufficient volume and 1 patient for non-determinate Xpert MTB/XDR result. For ethionamide, pDST results were not available for 270/530 (50.9%) of participants.
Comparative	
Notes	The composite reference result was considered drug resistant if the pDST was reported as drug resistant or the sequencing results had detected a drug associated resistant mutation. The composite reference result was considered drug susceptible when both pDST reported drug susceptibility and sequencing did not detect a drug associated resistant mutation. Analyses were undertaken where sequencing data associated with the specimen were reviewed to identify the location and type of mutations present for the drug resistance targets or if the specimen was wild type. The intent of the eligibility criteria was that all specimens used for testing would be characterized and have data available prior to enrolment; however, this was not possible as many specimens available at the study sites had MTB culture results, but did not have other data required. Study sites attempted to complete any missing pDST, sequencing, and Xpert MTB/RIF or Xpert MTB/RIF Ultra testing in parallel with Xpert MTB/XDR testing during the study. Sequencing method: China - Sanger Sequencing: targeted genes in supernatant DNA were amplified by designated primers and sent for Sanger sequencing; South Africa – Whole Genome Sequencing using NGS on the Illumina MiSeq using paired end sequencing methodology (2 x 300bp).

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Unclear
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		

Omar 2020 *(Continued)*

Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Omar 2020 China

Study characteristics	
Patient Sampling	Cross-sectional, the manner of participant selection was not random or consecutive For drug resistance detection, MTB positive specimens were characterized by pDST and gDST prior to or during the study

Omar 2020 China *(Continued)*

Patient characteristics and setting	Presenting signs and symptoms: not reported; archived frozen raw sputum or sputum sediment specimens Exclusions: specimens that had been previously thawed were excluded; < 1 mL of frozen sputum sediment or < 2 mL of raw sputum Prior testing: archived (frozen) specimens confirmed to be MTB positive or negative by culture; Xpert MTB/RIF or Xpert MTB/RIF Ultra Age: ≥ 15 years (range, 13 to > 80 years; one participant was 13 years) in full study Sex, female: 38% in full study HIV infection: 0% Previous TB treatment: not reported Treatment of current episode: 199 (37.5%) study participated were reported to be on treatment, 6 (1.1%) were reported to not be on treatment and treatment status was unknown/not available for 325 study participants (parent study) Sample size: 208 Clinical setting: outpatient and inpatient Laboratory level: central Country: China World Bank Income Classification: middle income High TB burden country: yes High TB/HIV burden country: yes High MDR-TB burden country: yes
Index tests	Xpert MTB/XDR
Target condition and reference standard(s)	Pulmonary tuberculosis Culture with MGIT or LJ culture; Xpert MTB/RIF and Xpert MTB/RIF Ultra Resistance to: isoniazid, fluoroquinolones, ethionamide, amikacin, kanamycin, capreomycin (not done) pDST, gDST, composite reference standard INH High 0.4 mg/L; INH Low 0.1 mg/L; MFX High 2.0 and Low 0.5 mg/L; OFX: 2.0 mg/L; ETO not done; AMK 1.0 mg/L; KAN 2.5 mg/L There were 8 gene targets of interest (*katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, gyrA, gyrB, rrs*, *eis* promoter) were reported
Flow and timing	
Comparative	
Notes	The composite reference result was considered drug resistant if the pDST was reported as drug resistant or the sequencing results had detected a drug associated resistant mutation. The composite reference result was considered drug susceptible when both pDST reported drug susceptibility and sequencing did not detect a drug associated resistant mutation.

Omar 2020 China *(Continued)*

	Discrepant analysis was undertaken where sequencing data associated with the specimen were reviewed to identify the location and type of mutations present for the drug resistance targets or if the specimen was wild type. The intent of the eligibility criteria was that all specimens used for testing would be characterized and have data available prior to enrolment; however, this was not possible as many specimens available at the study sites had MTB culture results, but did not have other data required. Study sites attempted to complete any missing pDST, sequencing, and Xpert MTB/RIF or Xpert MTB/RIF Ultra testing in parallel with Xpert MTB/XDR testing during the study Sequencing method: Sanger Sequencing: targeted genes in supernatant DNA were amplified by designated primers and sent for Sanger sequencing

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Unclear
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it prespecified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		

Omar 2020 China *(Continued)*

Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Omar 2020 South Africa

Study characteristics	
Patient Sampling	Cross-sectional, the manner of participant selection was not random or consecutive For drug resistance detection, MTB positive specimens were characterized by pDST and gDST prior to or during the study
Patient characteristics and setting	Presenting signs and symptoms: not reported; archived frozen raw sputum or sputum sediment specimens Exclusions: specimens that had been previously thawed were excluded; < 1 mL of frozen sputum sediment or < 2 mL of raw sputum Prior testing: archived (frozen) specimens confirmed to be MTB positive or negative by culture; Xpert MTB/RIF or Xpert MTB/RIF Ultra Age: ≥ 15 years (range, 13 to > 80 years; one participant was 13 years) in full study Sex, female: 38% in full study HIV infection: not reported Previous TB treatment: not reported Treatment of current episode: 199 (37.5%) study participated were reported to be on treatment, 6 (1.1%) were reported to not be on treatment and treatment status was unknown/not available for 325 study participants (parent study)

Omar 2020 South Africa *(Continued)*

	Sample size: 322 Clinical setting: outpatient and inpatient Laboratory level: central Country: South Africa World Bank Income Classification: middle income High TB burden country: yes High TB/HIV burden country: yes High MDR-TB burden country: yes
Index tests	Xpert MTB/XDR
Target condition and reference standard(s)	Pulmonary tuberculosis Culture with MGIT or LJ culture; Xpert MTB/RIF and Xpert MTB/RIF Ultra Resistance to: isoniazid, fluoroquinolones, ethionamide, amikacin, kanamycin, capreomycin pDST, gDST, composite reference standard INH High 0.4 mg/L Low 0.1 mg/L; MFX High 1.0 and Low 0.25 mg/L; OFX 2.0 mg/L; LVX 1.0 mg/L; ETO 5.0 mg/L; AMK 1.0 mg/L; KAN 2.5 mg/L; CAP 2.5 mg/L There were 8 gene targets of interest (*katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, gyrA, gyrB, rrs*, *eis* promoter)
Flow and timing	
Comparative	
Notes	The composite reference result was considered drug resistant if the pDST was reported as drug resistant or the sequencing results had detected a drug associated resistant mutation. The composite reference result was considered drug susceptible when both pDST reported drug susceptibility and sequencing did not detect a drug associated resistant mutation. Discrepant analysis was undertaken where sequencing data associated with the specimen were reviewed to identify the location and type of mutations present for the drug resistance targets or if the specimen was wild type. The intent of the eligibility criteria was that all specimens used for testing would be characterized and have data available prior to enrolment; however, this was not possible as many specimens available at the study sites had MTB culture results, but did not have other data required. Study sites attempted to complete any missing pDST, sequencing, and Xpert MTB/RIF or Xpert MTB/RIF Ultra testing in parallel with Xpert MTB/XDR testing during the study. Sequencing method: South Africa – Whole Genome Sequencing using NGS on the Illumina MiSeq using paired end sequencing methodology (2 x 300bp)

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			

Omar 2020 South Africa *(Continued)*

Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Unclear
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it prespecified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		

Omar 2020 South Africa *(Continued)*

Did all patients receive the same reference standard?	Yes	
Were all patients included in the analysis?	Yes	
Could the patient flow have introduced bias?		Low risk

Penn-Nicholson 2021

Study characteristics	
Patient Sampling	Cross-sectional, consecutive, prospective Participants were prescreened for pulmonary tuberculosis symptoms and the presence of at least one risk factor for drug-resistant tuberculosis. Participants who met screening criteria received prior testing with Xpert MTB/RIF or Xpert MTB/RIF Ultra and those found to be Xpert MTB/RIF MTBC-positive or Xpert MTB/RIF Ultra MTBC-positive were enrolled. More than half of the population was also preselected for rifampicin resistance (and not just pulmonary tuberculosis). Screening was random and enrolment was consecutive and sequential for the two phases
Patient characteristics and setting	Presenting signs and symptoms: symptoms suggestive of pulmonary tuberculosis, i.e. persistent cough (≥ 2 weeks) or as per local definition of suspected pulmonary tuberculosis), and at least one of the following. - Previously received > 1 month of treatment for a prior tuberculosis 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 Exclusions for enrolment: sputum volume < 3 mL Age: ≥ 18 years; median 37 years (range 18 to 77) Sex, female: 214/611 (35%) HIV infection: 69/425 (16%) Previous TB treatment: 286 participants had received > 1 month of treatment for a previous tuberculosis episode Sample size: 698 for tuberculosis detection; 611 for resistance detection; 494/611 (80.9%) with known rifampicin resistance Clinical setting: outpatient and inpatient Laboratory level: central Country: India (Mumbai), India (New Delhi), Moldova, South Africa World Bank Income Classification: Moldova (middle income), India (middle income), South Africa (middle income)

Penn-Nicholson 2021 *(Continued)*

	High TB burden country: Moldova (no), India (yes), South Africa (yes) High TB/HIV burden country: Moldova (no), India (yes), South Africa (yes) High MDR-TB burden country: Moldova (yes), India (yes), South Africa (yes)
Index tests	Xpert MTB/XDR
Target condition and reference standard(s)	Pulmonary tuberculosis Xpert MTB/RIF and Xpert MTB/RIF Ultra Resistance to isoniazid, fluoroquinolones, moxifloxacin, levofloxacin, ethionamide, amikacin, kanamycin, capreomycin INH 0.1 mg/L; MFX High 1.0 mg/L and Low 0.25 mg/L; LFX 1.0 mg/L; ETO 5.0 mg/; AMK 1.0 mg/L; KAN 2.5 mg/L; CAP 2.5 mg/L pDST (MGIT960), gDST (whole genome sequencing), composite Genetic loci required for resistance testing criteria satisfied for isoniazid, fluoroquinolones, and amikacin gene targets: *katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, rpoB, gyrA, gyrB, rrs, eis* promoter
Flow and timing	
Comparative	
Notes	99/710 participants (13.9%) were excluded and accounted for owing to the following. • Culture negative: 89/99 (89.9%) • Culture positive but MTBC not identified: 3 • Culture contaminated: 5 • Culture result missing (but Xpert XDR available): 1 • No valid Xpert XDR results: 1 There was 1 indeterminate result for amikacin resistance in a specimen that was amikacin resistant by pDST. This specimen was gDST susceptible.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	

Penn-Nicholson 2021 *(Continued)*

Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it prespecified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Penn-Nicholson 2021 India (Mumbai)

Study characteristics	
Patient Sampling	Cross-sectional, consecutive, prospective Participants were prescreened for pulmonary tuberculosis symptoms and the presence of at least one risk factor for drug-resistant tuberculosis. Participants who met screening criteria received prior testing with Xpert MTB/RIF or Xpert MTB/RIF Ultra and those found to be Xpert MTB/RIF MTBC-positive or Xpert MTB/RIF Ultra MTBC-positive were enrolled. More than half of the population was also preselected for rifampicin resistance (and not just pulmonary tuberculosis). Screening was random and enrolment was consecutive and sequential for the two phases
Patient characteristics and setting	Presenting signs and symptoms: symptoms suggestive of pulmonary tuberculosis, i.e. persistent cough (≥ 2 weeks) or as per local definition of suspected pulmonary tuberculosis), and at least one of the following. - Previously received > 1 month of treatment for a prior tuberculosis 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 Exclusions for enrolment: sputum volume < 3 mL Age: ≥ 18 years; median 31 years (range 18 to 77) Sex, female: 88/179 (49%) HIV infection: 1/42 (2%) Previous TB treatment: 286 participants had received >1 month of treatment for a previous tuberculosis episode (in the full study) Sample size: 179; 146/179 (82%) with known rifampicin resistance Clinical setting: outpatient and inpatient in the full study Laboratory level: central Country: India (Mumbai) World Bank Income Classification: middle income High TB burden country: yes High TB/HIV burden country: yes High MDR-TB burden country: yes
Index tests	Xpert MTB/XDR
Target condition and reference standard(s)	Pulmonary tuberculosis Xpert MTB/RIF and Xpert Ultra

Penn-Nicholson 2021 India (Mumbai) *(Continued)*

	Resistance to isoniazid, fluoroquinolones, moxifloxacin, levofloxacin, ethionamide, amikacin, kanamycin, capreomycin INH 0.1 mg/L; MFX High 1.0 mg/L and Low 0.25 mg/L; LFX 1.0 mg/; ETO 5.0 mg/; AMK 1.0 mg/L; KAN 2.5 mg/L; CAP 2.5 mg/L pDST (MGIT960), gDST (whole genome sequencing), composite Genetic loci required for resistance testing criteria satisfied for isoniazid, fluoroquinolones, and amikacin gene targets: *katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, rpoB, gyrA, gyrB, rrs, eis* promoter
Flow and timing	
Comparative	
Notes	The study was designed to assess the diagnostic accuracy of Xpert MTB/XDR as a reflex test to detect tuberculosis drug resistance, and not detection of pulmonary tuberculosis. The study population was previously positive for tuberculosis by Xpert MTB/RIF or Xpert MTB/RIF Ultra testing

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it prespecified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	

Penn-Nicholson 2021 India (Mumbai) *(Continued)*

Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Penn-Nicholson 2021 India (New Delhi)

Study characteristics	
Patient Sampling	Cross-sectional, consecutive, prospective Participants were prescreened for pulmonary tuberculosis symptoms and the presence of at least one risk factor for drug-resistant tuberculosis. Participants who met screening criteria received prior testing with Xpert MTB/RIF or Xpert MTB/RIF Ultra and those found to be Xpert MTB/RIF MTBC-positive or Xpert MTB/RIF Ultra MTBC-positive were enrolled. More than half of the population was also preselected for rifampicin resistance (and not just pulmonary tuberculosis). Screening was random and enrolment was consecutive and sequential for the two phases
Patient characteristics and setting	Presenting signs and symptoms: symptoms suggestive of pulmonary tuberculosis, i.e. persistent cough (≥ 2 weeks) or as per local definition of suspected pulmonary tuberculosis), and at least one of the following. - Previously received > 1 month of treatment for a prior tuberculosis episode or

Penn-Nicholson 2021 India (New Delhi) *(Continued)*

	- 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 Exclusions for enrolment: sputum volume < 3 mL Age: ≥ 18 years; median 30 years (range 18 to 72) Sex, female: 52/120 (43%) HIV infection: 0% Previous TB treatment: 286 participants had received >1 month of treatment for a previous tuberculosis episode (in the full study) Sample size: 120; 75/120 (63%) with known rifampicin resistance Clinical setting: outpatient and inpatient in the full study Laboratory level: central Country: India (Delhi) World Bank Income Classification: middle income High TB burden country: yes High TB/HIV burden country: yes High MDR-TB burden country: yes
Index tests	Xpert MTB/XDR
Target condition and reference standard(s)	Pulmonary tuberculosis Xpert MTB/RIF and Xpert Ultra Resistance to isoniazid, fluoroquinolones, moxifloxacin, levofloxacin, ethionamide, amikacin, kanamycin, capreomycin INH 0.1 mg/L; MFX High 1.0 mg/L and Low 0.25 mg/L; LFX 1.0 mg/L; ETO 5.0 mg/L; AMK 1.0 mg/L; KAN 2.5 mg/L; CAP 2.5 mg/L pDST (MGIT960), gDST (whole genome sequencing), composite Genetic loci required for resistance testing criteria satisfied for isoniazid, fluoroquinolones, and amikacin gene targets: *katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, rpoB, gyrA, gyrB, rrs, eis* promoter
Flow and timing	
Comparative	
Notes	The study was designed to assess the diagnostic accuracy of Xpert MTB/XDR as a reflex test to detect tuberculosis drug resistance, and not detection of pulmonary tuberculosis. The

Penn-Nicholson 2021 India (New Delhi) *(Continued)*

	study population was previously positive for tuberculosis by Xpert MTB/RIF or Xpert MTB/RIF Ultra testing

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it prespecified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern

Penn-Nicholson 2021 India (New Delhi) *(Continued)*

DOMAIN 4: Flow and Timing		
Was there an appropriate interval between index test and reference standard?	Yes	
Did all patients receive the same reference standard?	Yes	
Were all patients included in the analysis?	Yes	
Could the patient flow have introduced bias?		Low risk

Penn-Nicholson 2021 Moldova

Study characteristics	
Patient Sampling	Cross-sectional, consecutive, prospective Participants were prescreened for pulmonary tuberculosis symptoms and the presence of at least one risk factor for drug-resistant tuberculosis. Participants who met screening criteria received prior testing with Xpert MTB/RIF or Xpert MTB/RIF Ultra and those found to be Xpert MTB/RIF MTBC-positive or Xpert MTB/RIF Ultra MTBC-positive were enrolled. More than half of the population was also preselected for rifampicin resistance (and not just pulmonary tuberculosis). Screening was random and enrolment was consecutive and sequential for the two phases
Patient characteristics and setting	Presenting signs and symptoms: symptoms suggestive of pulmonary tuberculosis, i.e. persistent cough (≥ 2 weeks) or as per local definition of suspected pulmonary tuberculosis), and at least one of the following. - Previously received > 1 month of treatment for a prior tuberculosis 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 Exclusions for enrolment: sputum volume < 3 mL Age: ≥ 18 years; median 43 years (range 18 to 70) Sex, female: 45/230 (20%) HIV infection: 27/230 (12%) Previous TB treatment: 286 participants had received >1 month of treatment for a previous tuberculosis episode (in the full study) Sample size: 230; 212/230 (92%) with known rifampicin resistance Clinical setting: outpatient and inpatient in full study

Penn-Nicholson 2021 Moldova *(Continued)*

	Laboratory level: central Country: Republic of Moldova World Bank Income Classification: middle income High TB burden country: no High TB/HIV burden country: no High MDR-TB burden country: yes
Index tests	Xpert MTB/XDR
Target condition and reference standard(s)	Pulmonary tuberculosis Xpert MTB/RIF and Xpert Ultra Resistance to isoniazid, fluoroquinolones, moxifloxacin, levofloxacin, ethionamide, amikacin, kanamycin, capreomycin INH 0.1 mg/L; MFX High 1.0 mg/L and Low 0.25 mg/L; LFX 1.0 mg/L; ETO 5.0 mg/; AMK 1.0 mg/L; KAN 2.5 mg/L; CAP 2.5 mg/L pDST (MGIT960), gDST (whole genome sequencing), composite Genetic loci required for resistance testing criteria satisfied for isoniazid, fluoroquinolones, and amikacin gene targets: *katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, rpoB, gyrA, gyrB, rrs, eis* promoter
Flow and timing	
Comparative	
Notes	The study was designed to assess the diagnostic accuracy of Xpert MTB/XDR as a reflex test to detect tuberculosis drug resistance, and not detection of pulmonary tuberculosis. The study population was previously positive for tuberculosis by Xpert MTB/RIF or Xpert MTB/RIF Ultra testing.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	

Penn-Nicholson 2021 Moldova *(Continued)*

Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it prespecified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Penn-Nicholson 2021 South Africa

Study characteristics	
Patient Sampling	Cross-sectional, consecutive, prospective Participants were prescreened for pulmonary tuberculosis symptoms and the presence of at least one risk factor for drug-resistant tuberculosis. Participants who met screening criteria received prior testing with Xpert MTB/RIF or Xpert MTB/RIF Ultra and those found to be Xpert MTB/RIF MTBC-positive or Xpert MTB/RIF Ultra MTBC-positive were enrolled. More than half of the population was also preselected for rifampicin resistance (and not just pulmonary tuberculosis). Screening was random and enrolment was consecutive and sequential for the two phases
Patient characteristics and setting	Presenting signs and symptoms: symptoms suggestive of pulmonary tuberculosis, i.e. persistent cough (≥ 2 weeks) or as per local definition of suspected pulmonary tuberculosis), and at least one of the following. - Previously received > 1 month of treatment for a prior tuberculosis 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 Exclusions for enrolment: sputum volume < 3 mL Age: ≥ 18 years; median 36 years (range 18 to 64) Sex, female: 29/82 (35%) HIV infection: 41/47 (87%) Previous TB treatment: 286 participants had received >1 month of treatment for a previous tuberculosis episode (in the full study) Sample size: 82; 61/82 (74%) with known rifampicin resistance Clinical setting: outpatient and inpatient in full study Laboratory level: central Country: South Africa World Bank Income Classification: middle income High TB burden country: yes High TB/HIV burden country: yes High MDR-TB burden country: yes
Index tests	Xpert MTB/XDR
Target condition and reference standard(s)	Pulmonary tuberculosis Xpert MTB/RIF and Xpert Ultra Resistance to isoniazid, fluoroquinolones, moxifloxacin, levofloxacin, amikacin, kanamycin, capreomycin, ethionamide

Penn-Nicholson 2021 South Africa *(Continued)*

	INH 0.1 mg/L; MFX High 1.0 mg/L and Low 0.25 mg/L; LFX 1.0 mg/L; ETO 5.0 mg/L; AMK 1.0 mg/L; KAN 2.5 mg/L; CAP 2.5 mg/L pDST (MGIT 960), gDST (whole genome sequencing), composite Genetic loci required for resistance testing criteria satisfied for isoniazid, fluoroquinolones, and amikacin gene targets: *katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, rpoB, gyrA, gyrB, rrs, eis* promoter
Flow and timing	
Comparative	
Notes	The study was designed to assess the diagnostic accuracy of Xpert MTB/XDR as a reflex test to detect tuberculosis drug resistance, and not detection of pulmonary tuberculosis. The study population was previously positive for tuberculosis by Xpert MTB/RIF or Xpert MTB/RIF Ultra testing

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it prespecified?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern

Penn-Nicholson 2021 South Africa *(Continued)*

DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Abbreviations: **AMK:** amikacin; **CAP:** capreomycin; **ETO:** ethionamide; **gDST:** genotypic drug susceptibility testing; **INH:** isoniazid; **KAN:** kanamycin; **LJ:** Löwenstein–Jensen medium;**LFX:** levofloxacin; **MDR-TB:** multidrug-resistant tuberculosis; **MFX:** moxifloxacin; **MGIT:** Mycobacteria Growth Indicator Tube;**MTB:** *Mycobacterium tuberculosis;* **NGS:** next-generation sequencing ; **OFX:** ofloxacin; **pDST:** phenotypic drug susceptibility testing; **RIF:** rifampicin;**TB:** tuberculosis**; XDR:** extensively drug-resistant.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Andreevskaya 2020	Not the index test
Beutler 2020	Not a diagnostic accuracy study
Bisognin 2020	Not the index test
Broda 2018	Not the index test
Cao 2021	Combined clinical specimens and cultured isolates
Chakravorty 2017	Prototype test
Chang 2020	Not the index test

Study	Reason for exclusion
Chumpa 2020	Not the index test
Ciesielczuk 2020	Not the index test
Foongladda 2016	Not the index test
Galarza 2016	Not the index test
Georghiou 2021	Not a diagnotic study; analytical study
Han 2019	Extrapulmonary specimens
Havlicek 2018	Not the index test
Huang 2015	Not the index test
Kim 2019	Not the index test
Klotoe 2018	Not the index test
Law 2018	Not the index test
Lee 2015	Not the index test
Li 2017	Not the index test
Mokaddas 2019	Not the index test
Murray 2019	Not a diagnostic accuracy study
Pang 2016	Not the index test
Santos 2017	Not the index test
Shah 2019	Not the index test
Strydom 2015	Not the index test
Wang 2018	Not the index test
Xie 2017	Prototype test

Characteristics of ongoing studies *[ordered by study ID]*

NCT03303963

Study name	DIAgnostics for Multidrug Resistant Tuberculosis in Africa (DIAMA)
Target condition and reference standard(s)	Tuberculosis, Multidrug-Resistant
Index and comparator tests	Diagnostic Test: Deeplex test, MolBio TrueNat for 2nd line, GeneXpert 2nd line Diagnostic Test: Fluorescein DiAcetate (FDA) Microscopy, GeneXpert Ct value, pre-rRNA synthesis

NCT03303963 *(Continued)*

Starting date	4 May 2017
Contact information	affolabi_dissou@yahoo.fr
Notes	

DATA

Presented below are all the data for all of the tests entered into the review.

Table Tests. Data tables by test

Test	No. of studies	No. of participants
1 Xpert MTB/XDR, direct, TB detection, culture	3	1228
2 Xpert MTB/XDR, direct, smear-positive TB, culture	1	400
3 Xpert MTB/XDR, direct, smear-negative TB, culture	1	128
4 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, pDST	6	1083
5 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, gDST	6	999
6 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, composite	6	1055
7 Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, pDST	4	492
8 Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, gDST	4	434
9 Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, composite	4	476
10 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, pDST	6	1021
11 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, gDST	6	997
12 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, composite	6	1021
13 Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, pDST	4	491
14 Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, gDST	4	434
15 Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, composite	4	452

Test	No. of studies	No. of participants
16 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, pDST	5	835
17 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, gDST	6	1001
18 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, composite	5	843
19 Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, pDST	4	492
20 Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, gDST	4	434
21 Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, composite	4	457
22 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, pDST	6	1008
23 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, gDST	6	990
24 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, composite	6	1005
25 Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, pDST	4	490
26 Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, gDST	4	433
27 Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, composite	6	782
28 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, pDST	6	947
29 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, gDST	6	990
30 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, composite	6	1008
31 Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, pDST	4	491
32 Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, gDST	4	433
33 Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, composite	4	446
34 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, pDST	5	771
35 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, gDST	6	991
36 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, composite	5	823

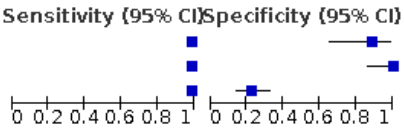
Test	No. of studies	No. of participants
37 Xpert MTB/XDR, direct, with known rifampicin resistance, capreomycin, pDST	4	491
38 Xpert MTB/XDR, direct, with known rifampicin resistance, capreomycin, gDST	4	434
39 Xpert MTB/XDR, direct, with known rifampicin resistance, capreomycin, composite	4	444
40 Xpert MTB/XDR, direct, isoniazid, composite, direct comparison	1	564
41 Xpert MTB/XDR, indirect, isoniazid, composite, direct comparison	1	564
42 Xpert MTB/XDR, direct, fluoroquinolone, composite, direct comparison	1	530
43 Xpert MTB/XDR, indirect, fluoroquinolone, composite, direct comparison	1	530
44 Xpert MTB/XDR, direct, ethionamide, composite, direct comparison	1	541
45 Xpert MTB/XDR, indirect, ethionamide, composite, direct comparison	1	541
46 Xpert MTB/XDR, direct, amikacin, composite, direct comparison	1	509
47 Xpert MTB/XDR, indirect, amikacin, composite, direct comparison	1	509
48 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, isoniazid, composite	1	438
49 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, isoniazid, composite	1	137
50 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, fluoroquinolone, composite	1	410
51 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, fluoroquinolone, composite	1	134
52 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, ethionamide, composite	1	417
53 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, ethionamide, composite	1	132
54 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, amikacin, composite	1	404
55 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, amikacin, composite	1	130
56 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, isoniazid, composite	1	60
57 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, isoniazid, composite	1	340

Test	No. of studies	No. of participants
58 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, fluoroquinolone, composite	1	45
59 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, fluoroquinolone, composite	1	333
60 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, ethionamide, composite	1	53
61 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, ethionamide, composite	1	332
62 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, amikacin, composite	1	44
63 Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, amikacin, composite	1	317
64 Xpert MTB/XDR, direct, no previous treatment, isoniazid, composite	1	418
65 Xpert MTB/XDR, direct, previous treatment, isoniazid, composite	1	105
66 Xpert MTB/XDR, direct, no previous treatment, fluoroquinolone, composite	1	391
67 Xpert MTB/XDR, direct, previous treatment, fluoroquinolone, composite	1	100
68 Xpert MTB/XDR, direct, no previous treatment, ethionamide, composite	1	398
69 Xpert MTB/XDR, direct, previous treatment, ethionamide, composite	1	102
70 Xpert MTB/XDR, direct, no previous treatment, amikacin, composite	1	378
71 Xpert MTB/XDR, direct, previous treatment, amikacin, composite	1	94

Test 1. Xpert MTB/XDR, direct, TB detection, culture

Xpert MTB/XDR, direct, TB detection, culture

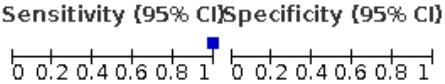
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	188	2	2	16	0.99 [0.96, 1.00]	0.89 [0.65, 0.99]		
Omar 2020 South Africa	292	0	5	25	0.98 [0.96, 0.99]	1.00 [0.86, 1.00]		
Penn-Nicholson 2021	599	69	10	20	0.98 [0.97, 0.99]	0.22 [0.14, 0.33]		



Test 2. Xpert MTB/XDR, direct, smear-positive TB, culture

Xpert MTB/XDR, direct, smear-positive TB, culture

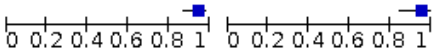
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020	398	0	2	0	0.99 [0.98, 1.00]	Not estimable		



Test 3. Xpert MTB/XDR, direct, smear-negative TB, culture

Xpert MTB/XDR, direct, smear-negative TB, culture

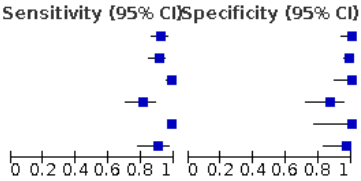
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020	80	2	5	41	0.94 [0.87, 0.98]	0.95 [0.84, 0.99]		



Test 4. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, pDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, pDST

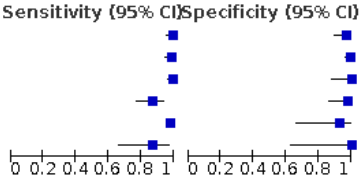
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	117	0	10	60	0.92 [0.86, 0.96]	1.00 [0.94, 1.00]		
Omar 2020 South Africa	127	2	13	149	0.91 [0.85, 0.95]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	143	0	2	33	0.99 [0.95, 1.00]	1.00 [0.89, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	63	5	15	33	0.81 [0.70, 0.89]	0.87 [0.72, 0.96]		
Penn-Nicholson 2021 Moldova	213	0	3	14	0.99 [0.96, 1.00]	1.00 [0.77, 1.00]		
Penn-Nicholson 2021 South Africa	45	1	5	30	0.90 [0.78, 0.97]	0.97 [0.83, 1.00]		



Test 5. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, gDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, gDST

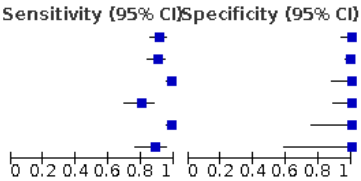
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	113	2	1	64	0.99 [0.95, 1.00]	0.97 [0.89, 1.00]		
Omar 2020 South Africa	128	1	2	160	0.98 [0.95, 1.00]	0.99 [0.97, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	132	0	1	29	0.99 [0.96, 1.00]	1.00 [0.88, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	61	1	9	38	0.87 [0.77, 0.94]	0.97 [0.87, 1.00]		
Penn-Nicholson 2021 Moldova	208	1	4	13	0.98 [0.95, 0.99]	0.93 [0.66, 1.00]		
Penn-Nicholson 2021 South Africa	20	0	3	8	0.87 [0.66, 0.97]	1.00 [0.63, 1.00]		



Test 6. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, isoniazid, composite

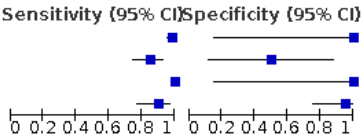
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	117	0	11	59	0.91 [0.85, 0.96]	1.00 [0.94, 1.00]		
Omar 2020 South Africa	128	1	14	148	0.90 [0.84, 0.95]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	143	0	2	28	0.99 [0.95, 1.00]	1.00 [0.88, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	68	0	17	31	0.80 [0.70, 0.88]	1.00 [0.89, 1.00]		
Penn-Nicholson 2021 Moldova	213	0	4	13	0.98 [0.95, 0.99]	1.00 [0.75, 1.00]		
Penn-Nicholson 2021 South Africa	45	0	6	7	0.88 [0.76, 0.96]	1.00 [0.59, 1.00]		



Test 7. Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, pDST

Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	141	0	2	2	0.99 [0.95, 1.00]	1.00 [0.16, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	58	3	10	3	0.85 [0.75, 0.93]	0.50 [0.12, 0.88]		
Penn-Nicholson 2021 Moldova	210	0	0	2	1.00 [0.98, 1.00]	1.00 [0.16, 1.00]		
Penn-Nicholson 2021 South Africa	37	1	4	19	0.90 [0.77, 0.97]	0.95 [0.75, 1.00]		



Test 8. Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, gDST

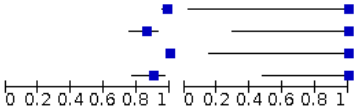
Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	130	0	1	2	0.99 [0.96, 1.00]	1.00 [0.16, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	54	0	5	7	0.92 [0.81, 0.97]	1.00 [0.59, 1.00]		
Penn-Nicholson 2021 Moldova	206	1	0	2	1.00 [0.98, 1.00]	0.67 [0.09, 0.99]		
Penn-Nicholson 2021 South Africa	18	0	2	6	0.90 [0.68, 0.99]	1.00 [0.54, 1.00]		

Test 9. Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, composite

Xpert MTB/XDR, direct, with known rifampicin resistance, isoniazid, composite

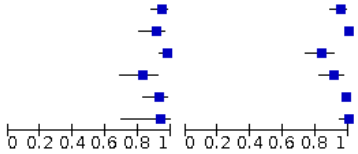
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	141	0	2	1	0.99 [0.95, 1.00]	1.00 [0.03, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	61	0	10	3	0.86 [0.76, 0.93]	1.00 [0.29, 1.00]		
Penn-Nicholson 2021 Moldova	210	0	0	2	1.00 [0.98, 1.00]	1.00 [0.16, 1.00]		
Penn-Nicholson 2021 South Africa	37	0	4	5	0.90 [0.77, 0.97]	1.00 [0.48, 1.00]		



Test 10. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, pDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, pDST

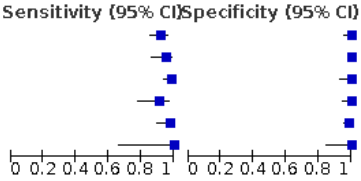
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	90	4	5	87	0.95 [0.88, 0.98]	0.96 [0.89, 0.99]		
Omar 2020 South Africa	58	0	6	167	0.91 [0.81, 0.96]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	102	12	2	62	0.98 [0.93, 1.00]	0.84 [0.73, 0.91]		
Penn-Nicholson 2021 India (New Delhi)	38	6	8	64	0.83 [0.69, 0.92]	0.91 [0.82, 0.97]		
Penn-Nicholson 2021 Moldova	52	2	4	172	0.93 [0.83, 0.98]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 South Africa	15	0	1	64	0.94 [0.70, 1.00]	1.00 [0.94, 1.00]		



Test 11. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, gDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, gDST

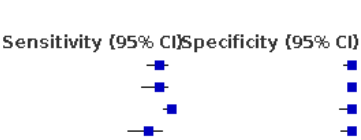
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	94	0	8	78	0.92 [0.85, 0.97]	1.00 [0.95, 1.00]		
Omar 2020 South Africa	58	0	3	228	0.95 [0.86, 0.99]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	107	0	2	53	0.98 [0.94, 1.00]	1.00 [0.93, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	39	0	4	66	0.91 [0.78, 0.97]	1.00 [0.95, 1.00]		
Penn-Nicholson 2021 Moldova	50	3	1	172	0.98 [0.90, 1.00]	0.98 [0.95, 1.00]		
Penn-Nicholson 2021 South Africa	9	0	0	22	1.00 [0.66, 1.00]	1.00 [0.85, 1.00]		



Test 12. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, fluoroquinolone, composite

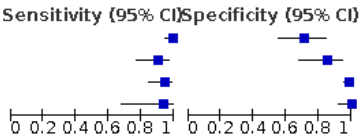
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	94	0	9	83	0.91 [0.84, 0.96]	1.00 [0.96, 1.00]		
Omar 2020 South Africa	58	0	6	225	0.91 [0.81, 0.96]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	113	0	2	53	0.98 [0.94, 1.00]	1.00 [0.93, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	44	0	8	61	0.85 [0.72, 0.93]	1.00 [0.94, 1.00]		
Penn-Nicholson 2021 Moldova	52	2	4	169	0.93 [0.83, 0.98]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 South Africa	16	0	1	21	0.94 [0.71, 1.00]	1.00 [0.84, 1.00]		



Test 13. Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, pDST

Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, pDST

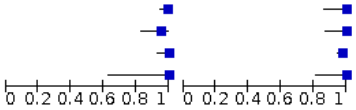
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	102	12	1	30	0.99 [0.95, 1.00]	0.71 [0.55, 0.84]		
Penn-Nicholson 2021 India (New Delhi)	37	5	4	28	0.90 [0.77, 0.97]	0.85 [0.68, 0.95]		
Penn-Nicholson 2021 Moldova	51	2	3	156	0.94 [0.85, 0.99]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 South Africa	14	0	1	45	0.93 [0.68, 1.00]	1.00 [0.92, 1.00]		



Test 14. Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, gDST

Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, gDST

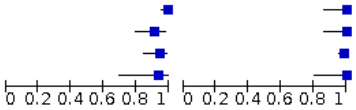
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	107	0	1	25	0.99 [0.95, 1.00]	1.00 [0.86, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	37	0	2	27	0.95 [0.83, 0.99]	1.00 [0.87, 1.00]		
Penn-Nicholson 2021 Moldova	50	3	0	156	1.00 [0.93, 1.00]	0.98 [0.95, 1.00]		
Penn-Nicholson 2021 South Africa	8	0	0	18	1.00 [0.63, 1.00]	1.00 [0.81, 1.00]		



Test 15. Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, composite

Xpert MTB/XDR, direct, with known rifampicin resistance, fluoroquinolone, composite

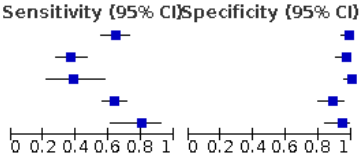
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	113	0	1	25	0.99 [0.95, 1.00]	1.00 [0.86, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	42	0	4	25	0.91 [0.79, 0.98]	1.00 [0.86, 1.00]		
Penn-Nicholson 2021 Moldova	51	2	3	153	0.94 [0.85, 0.99]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 South Africa	15	0	1	17	0.94 [0.70, 1.00]	1.00 [0.80, 1.00]		



Test 16. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, pDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, pDST

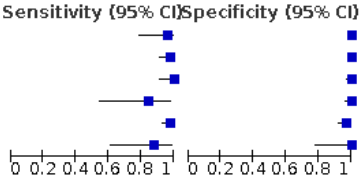
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 South Africa	75	2	41	112	0.65 [0.55, 0.73]	0.98 [0.94, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	39	2	66	71	0.37 [0.28, 0.47]	0.97 [0.90, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	12	0	19	85	0.39 [0.22, 0.58]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 Moldova	101	8	57	64	0.64 [0.56, 0.71]	0.89 [0.79, 0.95]		
Penn-Nicholson 2021 South Africa	24	3	6	48	0.80 [0.61, 0.92]	0.94 [0.84, 0.99]		



Test 17. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, gDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, gDST

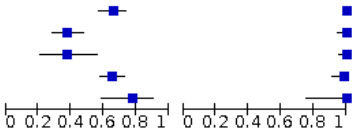
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	23	0	1	157	0.96 [0.79, 1.00]	1.00 [0.98, 1.00]		
Omar 2020 South Africa	81	0	2	209	0.98 [0.92, 1.00]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	39	0	0	123	1.00 [0.91, 1.00]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	11	0	2	96	0.85 [0.55, 0.98]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 Moldova	103	4	2	117	0.98 [0.93, 1.00]	0.97 [0.92, 0.99]		
Penn-Nicholson 2021 South Africa	14	0	2	15	0.88 [0.62, 0.98]	1.00 [0.78, 1.00]		



Test 18. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, ethionamide, composite

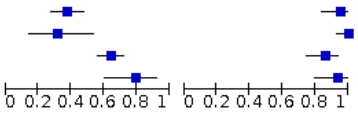
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 South Africa	81	0	42	169	0.66 [0.57, 0.74]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	40	0	66	63	0.38 [0.29, 0.48]	1.00 [0.94, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	12	0	20	78	0.38 [0.21, 0.56]	1.00 [0.95, 1.00]		
Penn-Nicholson 2021 Moldova	108	1	57	62	0.65 [0.58, 0.73]	0.98 [0.91, 1.00]		
Penn-Nicholson 2021 South Africa	24	0	7	13	0.77 [0.59, 0.90]	1.00 [0.75, 1.00]		



Test 19. Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, pDST

Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, pDST

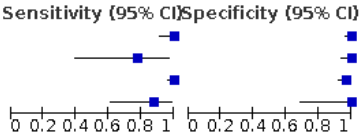
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	39	2	65	39	0.38 [0.28, 0.48]	0.95 [0.83, 0.99]		
Penn-Nicholson 2021 India (New Delhi)	8	0	17	49	0.32 [0.15, 0.54]	1.00 [0.93, 1.00]		
Penn-Nicholson 2021 Moldova	100	8	55	49	0.65 [0.56, 0.72]	0.86 [0.74, 0.94]		
Penn-Nicholson 2021 South Africa	23	2	6	30	0.79 [0.60, 0.92]	0.94 [0.79, 0.99]		



Test 20. Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, gDST

Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, gDST

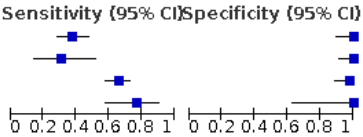
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	39	0	0	94	1.00 [0.91, 1.00]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	7	0	2	57	0.78 [0.40, 0.97]	1.00 [0.94, 1.00]		
Penn-Nicholson 2021 Moldova	103	3	0	103	1.00 [0.96, 1.00]	0.97 [0.92, 0.99]		
Penn-Nicholson 2021 South Africa	14	0	2	10	0.88 [0.62, 0.98]	1.00 [0.69, 1.00]		



Test 21. Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, composite

Xpert MTB/XDR, direct, with known rifampicin resistance, ethionamide, composite

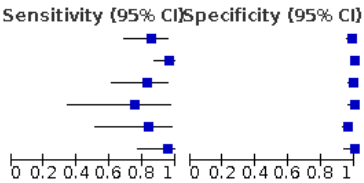
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	40	0	65	35	0.38 [0.29, 0.48]	1.00 [0.90, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	8	0	18	42	0.31 [0.14, 0.52]	1.00 [0.92, 1.00]		
Penn-Nicholson 2021 Moldova	107	1	55	48	0.66 [0.58, 0.73]	0.98 [0.89, 1.00]		
Penn-Nicholson 2021 South Africa	23	0	7	8	0.77 [0.58, 0.90]	1.00 [0.63, 1.00]		



Test 22. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, pDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, pDST

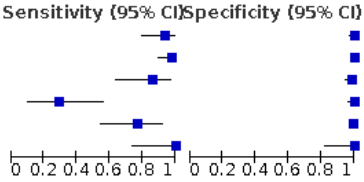
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	29	2	5	141	0.85 [0.69, 0.95]	0.99 [0.95, 1.00]		
Omar 2020 South Africa	50	0	2	176	0.96 [0.87, 1.00]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	19	1	4	153	0.83 [0.61, 0.95]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	6	0	2	107	0.75 [0.35, 0.97]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 Moldova	10	8	2	210	0.83 [0.52, 0.98]	0.96 [0.93, 0.98]		
Penn-Nicholson 2021 South Africa	21	0	1	59	0.95 [0.77, 1.00]	1.00 [0.94, 1.00]		



Test 23. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, gDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, gDST

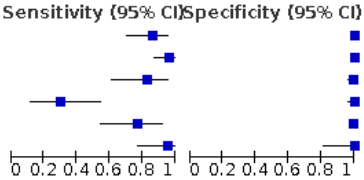
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	31	0	2	144	0.94 [0.80, 0.99]	1.00 [0.97, 1.00]		
Omar 2020 South Africa	50	0	1	235	0.98 [0.90, 1.00]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	18	2	3	138	0.86 [0.64, 0.97]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	5	0	12	92	0.29 [0.10, 0.56]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 Moldova	17	1	5	203	0.77 [0.55, 0.92]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	12	0	0	19	1.00 [0.74, 1.00]	1.00 [0.82, 1.00]		



Test 24. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, amikacin, composite

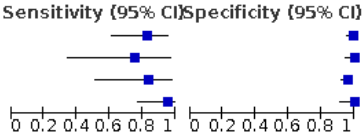
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	31	0	5	147	0.86 [0.71, 0.95]	1.00 [0.98, 1.00]		
Omar 2020 South Africa	50	0	2	234	0.96 [0.87, 1.00]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	19	1	4	137	0.83 [0.61, 0.95]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	6	0	14	89	0.30 [0.12, 0.54]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 Moldova	17	1	5	203	0.77 [0.55, 0.92]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	21	0	1	18	0.95 [0.77, 1.00]	1.00 [0.81, 1.00]		



Test 25. Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, pDST

Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, pDST

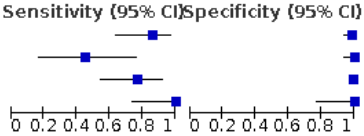
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	19	1	4	120	0.83 [0.61, 0.95]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	6	0	2	65	0.75 [0.35, 0.97]	1.00 [0.94, 1.00]		
Penn-Nicholson 2021 Moldova	10	8	2	192	0.83 [0.52, 0.98]	0.96 [0.92, 0.98]		
Penn-Nicholson 2021 South Africa	21	0	1	39	0.95 [0.77, 1.00]	1.00 [0.91, 1.00]		



Test 26. Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, gDST

Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, gDST

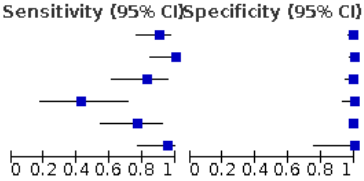
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	18	2	3	109	0.86 [0.64, 0.97]	0.98 [0.94, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	5	0	6	55	0.45 [0.17, 0.77]	1.00 [0.94, 1.00]		
Penn-Nicholson 2021 Moldova	17	1	5	186	0.77 [0.55, 0.92]	0.99 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	12	0	0	14	1.00 [0.74, 1.00]	1.00 [0.77, 1.00]		



Test 27. Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, composite

Xpert MTB/XDR, direct, with known rifampicin resistance, amikacin, composite

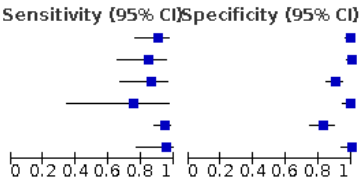
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	36	1	4	136	0.90 [0.76, 0.97]	0.99 [0.96, 1.00]		
Omar 2020 South Africa	22	0	0	140	1.00 [0.85, 1.00]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	19	1	4	108	0.83 [0.61, 0.95]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	6	0	8	53	0.43 [0.18, 0.71]	1.00 [0.93, 1.00]		
Penn-Nicholson 2021 Moldova	17	1	5	186	0.77 [0.55, 0.92]	0.99 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	21	0	1	13	0.95 [0.77, 1.00]	1.00 [0.75, 1.00]		



Test 28. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, pDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, pDST

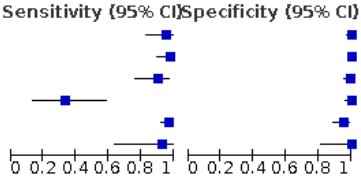
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	36	1	4	136	0.90 [0.76, 0.97]	0.99 [0.96, 1.00]		
Omar 2020 South Africa	22	0	4	140	0.85 [0.65, 0.96]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	24	14	4	135	0.86 [0.67, 0.96]	0.91 [0.85, 0.95]		
Penn-Nicholson 2021 India (New Delhi)	6	1	2	107	0.75 [0.35, 0.97]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 Moldova	111	19	7	93	0.94 [0.88, 0.98]	0.83 [0.75, 0.89]		
Penn-Nicholson 2021 South Africa	21	0	1	59	0.95 [0.77, 1.00]	1.00 [0.94, 1.00]		



Test 29. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, gDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, gDST

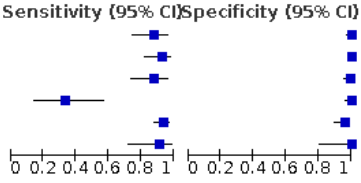
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	37	0	2	138	0.95 [0.83, 0.99]	1.00 [0.97, 1.00]		
Omar 2020 South Africa	51	0	1	234	0.98 [0.90, 1.00]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	36	1	4	120	0.90 [0.76, 0.97]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	6	0	12	91	0.33 [0.13, 0.59]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 Moldova	123	5	4	94	0.97 [0.92, 0.99]	0.95 [0.89, 0.98]		
Penn-Nicholson 2021 South Africa	12	0	1	18	0.92 [0.64, 1.00]	1.00 [0.81, 1.00]		



Test 30. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, composite

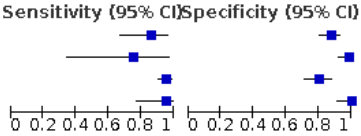
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	37	0	5	141	0.88 [0.74, 0.96]	1.00 [0.97, 1.00]		
Omar 2020 South Africa	51	0	4	231	0.93 [0.82, 0.98]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	36	1	5	119	0.88 [0.74, 0.96]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	7	0	14	89	0.33 [0.15, 0.57]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 Moldova	126	4	9	89	0.93 [0.88, 0.97]	0.96 [0.89, 0.99]		
Penn-Nicholson 2021 South Africa	21	0	2	17	0.91 [0.72, 0.99]	1.00 [0.80, 1.00]		



Test 31. Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, pDST

Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, pDST

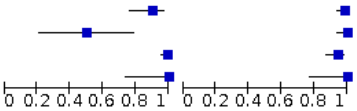
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	24	14	4	102	0.86 [0.67, 0.96]	0.88 [0.81, 0.93]		
Penn-Nicholson 2021 India (New Delhi)	6	1	2	65	0.75 [0.35, 0.97]	0.98 [0.92, 1.00]		
Penn-Nicholson 2021 Moldova	109	19	5	79	0.96 [0.90, 0.99]	0.81 [0.71, 0.88]		
Penn-Nicholson 2021 South Africa	21	0	1	39	0.95 [0.77, 1.00]	1.00 [0.91, 1.00]		



Test 32. Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, gDST

Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, gDST

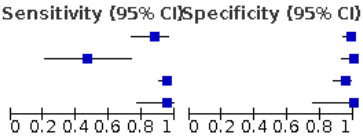
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	36	1	4	91	0.90 [0.76, 0.97]	0.99 [0.94, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	6	0	6	54	0.50 [0.21, 0.79]	1.00 [0.93, 1.00]		
Penn-Nicholson 2021 Moldova	121	5	1	82	0.99 [0.96, 1.00]	0.94 [0.87, 0.98]		
Penn-Nicholson 2021 South Africa	12	0	0	14	1.00 [0.74, 1.00]	1.00 [0.77, 1.00]		



Test 33. Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, composite

Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, composite

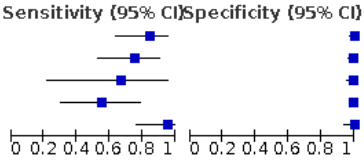
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	36	1	5	90	0.88 [0.74, 0.96]	0.99 [0.94, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	7	0	8	53	0.47 [0.21, 0.73]	1.00 [0.93, 1.00]		
Penn-Nicholson 2021 Moldova	124	4	6	77	0.95 [0.90, 0.98]	0.95 [0.88, 0.99]		
Penn-Nicholson 2021 South Africa	21	0	1	13	0.95 [0.77, 1.00]	1.00 [0.75, 1.00]		



Test 34. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, pDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, pDST

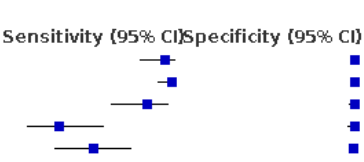
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 South Africa	21	0	4	142	0.84 [0.64, 0.95]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	18	1	6	153	0.75 [0.53, 0.90]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	4	1	2	109	0.67 [0.22, 0.96]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 Moldova	10	1	8	211	0.56 [0.31, 0.78]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	20	0	1	59	0.95 [0.76, 1.00]	1.00 [0.94, 1.00]		



Test 35. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, gDST

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, gDST

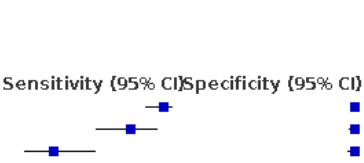
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	29	0	2	146	0.94 [0.79, 0.99]	1.00 [0.98, 1.00]		
Omar 2020 South Africa	49	0	1	236	0.98 [0.89, 1.00]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	19	0	4	139	0.83 [0.61, 0.95]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	5	0	12	92	0.29 [0.10, 0.56]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 Moldova	10	1	10	205	0.50 [0.27, 0.73]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	12	0	0	19	1.00 [0.74, 1.00]	1.00 [0.82, 1.00]		



Test 36. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, composite

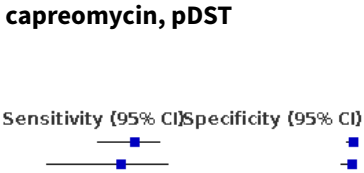
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 South Africa	49	0	4	233	0.92 [0.82, 0.98]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	19	0	7	136	0.73 [0.52, 0.88]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	5	0	14	90	0.26 [0.09, 0.51]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 Moldova	10	1	15	200	0.40 [0.21, 0.61]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	21	0	1	18	0.95 [0.77, 1.00]	1.00 [0.81, 1.00]		



Test 37. Xpert MTB/XDR, direct, with known rifampicin resistance, capreomycin, pDST

Xpert MTB/XDR, direct, with known rifampicin resistance, capreomycin, pDST

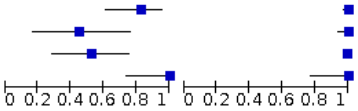
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	18	1	6	120	0.75 [0.53, 0.90]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	4	1	2	67	0.67 [0.22, 0.96]	0.99 [0.92, 1.00]		
Penn-Nicholson 2021 Moldova	10	1	8	193	0.56 [0.31, 0.78]	0.99 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	20	0	1	39	0.95 [0.76, 1.00]	1.00 [0.91, 1.00]		



Test 38. Xpert MTB/XDR, direct, with known rifampicin resistance, capreomycin, gDST

Xpert MTB/XDR, direct, with known rifampicin resistance, capreomycin, gDST

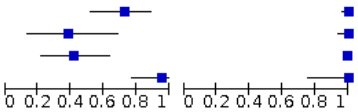
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	19	0	4	110	0.83 [0.61, 0.95]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	5	0	6	55	0.45 [0.17, 0.77]	1.00 [0.94, 1.00]		
Penn-Nicholson 2021 Moldova	10	1	9	189	0.53 [0.29, 0.76]	0.99 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	12	0	0	14	1.00 [0.74, 1.00]	1.00 [0.77, 1.00]		



Test 39. Xpert MTB/XDR, direct, with known rifampicin resistance, capreomycin, composite

Xpert MTB/XDR, direct, with known rifampicin resistance, capreomycin, composite

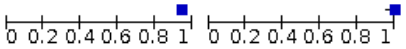
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	19	0	7	107	0.73 [0.52, 0.88]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	5	0	8	54	0.38 [0.14, 0.68]	1.00 [0.93, 1.00]		
Penn-Nicholson 2021 Moldova	10	1	14	184	0.42 [0.22, 0.63]	0.99 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	21	0	1	13	0.95 [0.77, 1.00]	1.00 [0.75, 1.00]		



Test 40. Xpert MTB/XDR, direct, isoniazid, composite, direct comparison

Xpert MTB/XDR, direct, isoniazid, composite, direct comparison

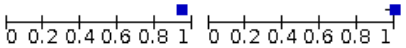
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	459	0	28	77	0.94 [0.92, 0.96]	1.00 [0.95, 1.00]		



Test 41. Xpert MTB/XDR, indirect, isoniazid, composite, direct comparison

Xpert MTB/XDR, indirect, isoniazid, composite, direct comparison

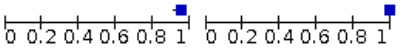
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	461	0	26	77	0.95 [0.92, 0.96]	1.00 [0.95, 1.00]		



Test 42. Xpert MTB/XDR, direct, fluoroquinolone, composite, direct comparison

Xpert MTB/XDR, direct, fluoroquinolone, composite, direct comparison

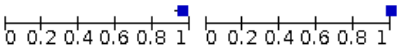
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	222	2	12	294	0.95 [0.91, 0.97]	0.99 [0.98, 1.00]		



Test 43. Xpert MTB/XDR, indirect, fluoroquinolone, composite, direct comparison

Xpert MTB/XDR, indirect, fluoroquinolone, composite, direct comparison

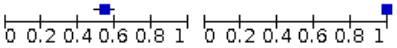
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	224	0	10	296	0.96 [0.92, 0.98]	1.00 [0.99, 1.00]		



Test 44. Xpert MTB/XDR, direct, ethionamide, composite, direct comparison

Xpert MTB/XDR, direct, ethionamide, composite, direct comparison

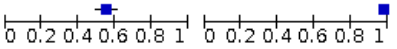
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	178	1	150	212	0.54 [0.49, 0.60]	1.00 [0.97, 1.00]		



Test 45. Xpert MTB/XDR, indirect, ethionamide, composite, direct comparison

Xpert MTB/XDR, indirect, ethionamide, composite, direct comparison

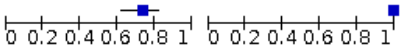
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	182	4	146	209	0.55 [0.50, 0.61]	0.98 [0.95, 0.99]		



Test 46. Xpert MTB/XDR, direct, amikacin, composite, direct comparison

Xpert MTB/XDR, direct, amikacin, composite, direct comparison

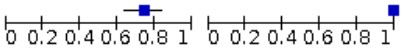
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	60	2	22	425	0.73 [0.62, 0.82]	1.00 [0.98, 1.00]		



Test 47. Xpert MTB/XDR, indirect, amikacin, composite, direct comparison

Xpert MTB/XDR, indirect, amikacin, composite, direct comparison

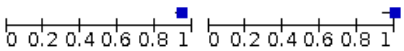
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	61	1	21	426	0.74 [0.64, 0.83]	1.00 [0.99, 1.00]		



Test 48. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, isoniazid, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, isoniazid, composite

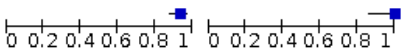
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	362	0	22	54	0.94 [0.91, 0.96]	1.00 [0.93, 1.00]		



Test 49. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, isoniazid, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, isoniazid, composite

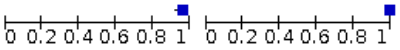
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	105	0	7	25	0.94 [0.88, 0.97]	1.00 [0.86, 1.00]		



Test 50. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, fluoroquinolone, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, fluoroquinolone, composite

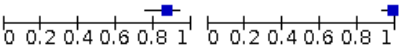
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	171	1	7	231	0.96 [0.92, 0.98]	1.00 [0.98, 1.00]		



Test 51. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, fluoroquinolone, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, fluoroquinolone, composite

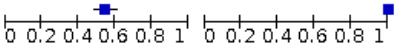
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	52	1	8	73	0.87 [0.75, 0.94]	0.99 [0.93, 1.00]		



Test 52. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, ethionamide, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, ethionamide, composite

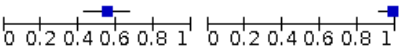
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	143	0	118	156	0.55 [0.49, 0.61]	1.00 [0.98, 1.00]		



Test 53. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, ethionamide, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, ethionamide, composite

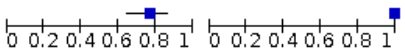
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	39	1	32	60	0.55 [0.43, 0.67]	0.98 [0.91, 1.00]		



Test 54. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, amikacin, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-positive, amikacin, composite

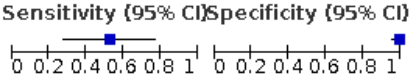
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	52	1	16	335	0.76 [0.65, 0.86]	1.00 [0.98, 1.00]		



Test 55. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, amikacin, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, smear-negative, amikacin, composite

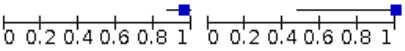
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	9	1	8	112	0.53 [0.28, 0.77]	0.99 [0.95, 1.00]		



Test 56. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, isoniazid, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, isoniazid, composite

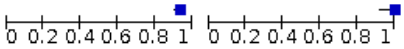
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	53	0	2	5	0.96 [0.87, 1.00]	1.00 [0.48, 1.00]		



Test 57. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, isoniazid, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, isoniazid, composite

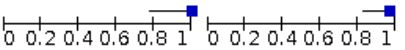
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	275	0	19	46	0.94 [0.90, 0.96]	1.00 [0.92, 1.00]		



Test 58. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, fluoroquinolone, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, fluoroquinolone, composite

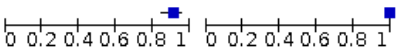
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	15	1	0	29	1.00 [0.78, 1.00]	0.97 [0.83, 1.00]		



Test 59. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, fluoroquinolone, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, fluoroquinolone, composite

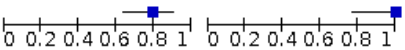
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	111	1	11	210	0.91 [0.84, 0.95]	1.00 [0.97, 1.00]		



Test 60. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, ethionamide, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, ethionamide, composite

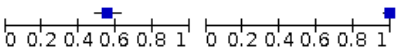
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	31	0	8	14	0.79 [0.64, 0.91]	1.00 [0.77, 1.00]		



Test 61. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, ethionamide, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, ethionamide, composite

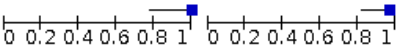
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	109	1	87	135	0.56 [0.48, 0.63]	0.99 [0.96, 1.00]		



Test 62. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, amikacin, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-positive, amikacin, composite

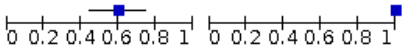
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	15	1	0	28	1.00 [0.78, 1.00]	0.97 [0.82, 1.00]		



Test 63. Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, amikacin, composite

Xpert MTB/XDR, direct, irrespective of rifampicin resistance, HIV-negative, amikacin, composite

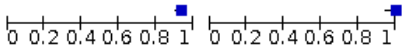
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	27	0	18	272	0.60 [0.44, 0.74]	1.00 [0.99, 1.00]		



Test 64. Xpert MTB/XDR, direct, no previous treatment, isoniazid, composite

Xpert MTB/XDR, direct, no previous treatment, isoniazid, composite

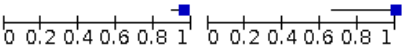
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	333	0	23	62	0.94 [0.90, 0.96]	1.00 [0.94, 1.00]		



Test 65. Xpert MTB/XDR, direct, previous treatment, isoniazid, composite

Xpert MTB/XDR, direct, previous treatment, isoniazid, composite

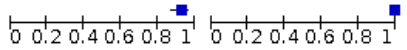
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	92	0	4	9	0.96 [0.90, 0.99]	1.00 [0.66, 1.00]		



Test 66. Xpert MTB/XDR, direct, no previous treatment, fluoroquinolone, composite

Xpert MTB/XDR, direct, no previous treatment, fluoroquinolone, composite

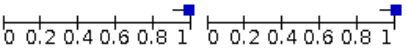
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	141	2	11	237	0.93 [0.87, 0.96]	0.99 [0.97, 1.00]		



Test 67. Xpert MTB/XDR, direct, previous treatment, fluoroquinolone, composite

Xpert MTB/XDR, direct, previous treatment, fluoroquinolone, composite

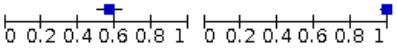
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	53	0	1	46	0.98 [0.90, 1.00]	1.00 [0.92, 1.00]		



Test 68. Xpert MTB/XDR, direct, no previous treatment, ethionamide, composite

Xpert MTB/XDR, direct, no previous treatment, ethionamide, composite

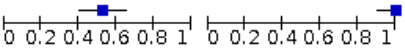
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	136	1	102	159	0.57 [0.51, 0.64]	0.99 [0.97, 1.00]		



Test 69. Xpert MTB/XDR, direct, previous treatment, ethionamide, composite

Xpert MTB/XDR, direct, previous treatment, ethionamide, composite

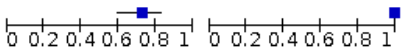
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	35	0	31	36	0.53 [0.40, 0.65]	1.00 [0.90, 1.00]		



Test 70. Xpert MTB/XDR, direct, no previous treatment, amikacin, composite

Xpert MTB/XDR, direct, no previous treatment, amikacin, composite

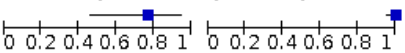
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	45	2	17	314	0.73 [0.60, 0.83]	0.99 [0.98, 1.00]		



Test 71. Xpert MTB/XDR, direct, previous treatment, amikacin, composite

Xpert MTB/XDR, direct, previous treatment, amikacin, composite

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021	10	0	3	81	0.77 [0.46, 0.95]	1.00 [0.96, 1.00]		



ADDITIONAL TABLES

Table 1. Selected characteristics of included studies

Study year	Study cohorts (high MDR burden country?)	Study design	Laboratory level	№ of participants for analyses of drug resistance detection (% with rifampicin resistance)	Median age (range)	PLHIV	Reference standard for drug resistance	Loci included in gDST reference standard
Omar 2020 [a,b]	China (yes) South Africa (yes)	Cross-sectional	Central	530 (47.9%)	(13 to > 80 years)[b]	NR	pDST, gDST, composite	*katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, gyrA, gyrB, rrs*, *eis* promoter
Penn-Nicholson 2021 [a]	Moldova (yes); Mumbai (yes); New Delhi) (yes); South Africa (yes)	Cross-sectional	Central	611 (80.9%)	37 years (18 to 77 years)	16%	pDST, gDST, composite	*katG, inhA* promoter, *oxyR-ahpC* intergenic region, *fabG1, gyrA, gyrB, rrs, eis* promoter

Abbreviations: **gDST:** genotypic drug susceptibility testing; **MDR:** multidrug-resistant tuberculosis; **№:** number;**NR:** not reported; **pDST:** phenotypic drug susceptibility testing; **PLHIV:** people living with HIV.

[a]Characteristics of the individual study centres are provided in Characteristics of included studies.

[b]One participant was 13 years old; all other participants were 15 years and older.

Table 2. Xpert MTB/XDR summary sensitivity and specificity for resistance to tuberculosis drugs

Analysis group	Reference standard	Number of studies; number of study cohorts (participants)	№(%) with drug resistance	Summary sensitivity % (95% CI)	Summary specificity % (95% CI)	Positive predictive value % (95% CI)*	Negative predictive value % (95% CI)*
Irrespective of rifampicin resistance							
Isoniazid	pDST	2 studies; 6 study cohorts (1083)	756 (69.8)	94.2 (87.5 to 97.4)	98.5 (92.6 to 99.7)	76.9 (38.8 to 94.6)	99.7 (99.4 to 99.9)
Isoniazid	gDST	2 studies; 6 study cohorts (999)	682 (68.3)	97.3 (92.8 to 99.0)	98.4 (95.9 to 99.3)	75.6 (55.4 to 88.6)	99.9 (99.6 to 100)
Isoniazid	Composite	2 studies; 6 study cohorts (1055)	768 (72.8)	93.5 (86.5 to 97.0)	99.7 (96.6 to 100.0)	94.2 (58.6 to 99.5)	99.7 (99.3 to 99.8)
With rifampicin resistance							
Isoniazid	pDST	1 study; 4 study cohorts (492)	462 (93.9)	97.6 (84.4 to 99.7)	89.0 (50.2 to 98.5)	79.2 (34.2 to 96.5)	99.2 (94.5 to 99.9)

Table 2. Xpert MTB/XDR summary sensitivity and specificity for resistance to tuberculosis drugs *(Continued)*

Isoniazid	gDST	1 study; 4 study cohorts (434)	416 (95.9)	98.4 (88.9 to 99.8)	97.5 (27.1 to 100.0)	94.5 (15.4 to 99.9)	99.5 (96.6 to 99.9)
Isoniazid	Composite	1 study; 4 study cohorts (476)	465 (97.7)	97.6 (84.7 to 99.7)	100.0 (NE to 100.0)	100.0 (0.0 to NE)	99.3 (95.2 to 99.9)
Irrespective of rifampicin resistance							
Fluoro-quinolones	pDST	2 studies; 6 study cohorts (1021)	381 (37.3)	93.2 (88.1 to 96.2)	98.0 (90.8 to 99.6)	70.6 (34.0 to 91.8)	99.7 (99.4 to 99.8)
Fluoro-quinolones	gDST	2 studies; 6 study cohorts (997)	375 (37.6)	95.7 (91.8 to 97.7)	99.9 (92.0 to 100.0)	97.5 (36.9 to 100.0)	99.8 (99.6 to 99.9)
Fluoro-quinolones	Composite	2 studies; 6 study cohorts (1021)	407 (39.9)	92.8 (88.1 to 95.8)	99.8 (96.0 to 100.0)	95.5 (54.4 to 99.7)	99.6 (99.4 to 99.8)
With rifampicin resistance							
Fluoro-quinolones	pDST	1 study; 4 study cohorts (491)	213 (43.4)	95.4 (89.4 to 98.1)	95.3 (75.3 to 99.3)	89.7 (59.2 to 98.1)	98.6 (96.8 to 99.4)
Fluoro-quinolones	gDST	1 study; 4 study cohorts (434)	205 (47.2)	98.6 (94.3 to 99.7)	98.8 (94.7 to 99.7)	97.2 (88.6 to 99.4)	99.6 (98.2 to 99.9)
Fluoro-quinolones	Composite	1 study; 4 study cohorts (452)	230 (50.9)	96.0 (90.6 to 98.4)	99.1 (96.2 to 99.8)	97.9 (91.3 to 99.5)	98.8 (97.2 to 99.5)
Irrespective of rifampicin resistance							
Ethionamide	pDST	2 studies; 6 study cohorts (835)	440 (52.7)	56.6 (41.8 to 70.3)	97.1 (91.9 to 99.0)	50.9 (28.6 to 72.8)	97.8 (97.0 to 98.4)
Ethionamide	gDST	2 studies; 6 study cohorts (1001)	280 (28.0)	96.4 (92.2 to 98.3)	100.0 (82.5 to 100.0)	99.6 (19.5 to 100.0)	96.5 (92.7 to 98.4)
Ethionamide	Composite	2 studies; 6 study cohorts (843)	481 (47.0)	57.1 (42.8 to 70.2)	99.8 (95.3 to 100.0)	94.7 (39.9 to 99.8)	97.9 (97.1 to 98.5)
With rifampicin resistance							
Ethionamide	pDST	1 study; 4 study cohorts (492)	313 (63.6)	51.7 (33.1 to 69.8)	94.8 (84.8 to 98.3)	81.0 (62.2 to 91.7)	86.7 (81.9 to 90.4)

Table 2. Xpert MTB/XDR summary sensitivity and specificity for resistance to tuberculosis drugs *(Continued)*

Ethionamide	gDST	1 study; 4 study cohorts (434)	167 (38.5)	98.0 (74.2 to 99.9)	99.7 (83.5 to 100.0)	99.3 (68.6 to 100.0)	99.4 (91.2 to 100.0)
Ethionamide	Composite	1 study; 4 study cohorts (457)	323 (70.7)	53.1 (34.7 to 70.7)	99.5 (87.0 to 100.0)	98.0 (63.9 to 99.9)	87.6 (82.6 to 91.3)
Irrespective of rifampicin resistance							
Amikacin	pDST	2 studies; 6 study cohorts (1008)	151 (15.0)	89.1 (80.8 to 94.1)	99.5 (96.9 to 99.9)	90.1 (59.0 to 98.3)	99.5 (99.0 to 99.7)
Amikacin	gDST	2 studies; 6 study cohorts (990)	156 (15.8)	89.5 (64.5 to 97.6)	99.7 (98.4 to 99.9)	93.3 (73.9 to 98.6)	99.5 (97.9 to 99.9)
Amikacin	Composite	2 studies; 6 study cohorts (1005)	175 (17.4)	84.1 (63.0 to 94.3)	99.8 (99.0 to 99.9)	94.9 (81.1 to 98.8)	99.2 (98.0 to 99.7)
With rifampicin resistance							
Amikacin	pDST	1 study; 4 study cohorts (490)	65 (13.3)	86.1 (75.0 to 92.7)	98.9 (93.0 to 99.8)	97.2 (83.4 to 99.6)	95.9 (92.7 to 97.8)
Amikacin	gDST	1 study; 4 study cohorts (433)	66 (15.2)	81.1 (56.0 to 93.6)	99.2 (96.9 to 99.8)	97.8 (92.4 to 99.4)	94.6 (86.8 to 97.9)
Amikacin	Composite	1 study; 4 study cohorts (443)	81 (18.3)	79.0 (55.4 to 91.9)	99.5 (97.6 to 99.9)	98.4 (93.7 to 99.6)	94.0 (86.8 to 97.4)
Irrespective of rifampicin resistance							
Kanamycin	pDST	2 studies; 6 study cohorts (947)	40 (4.22)	90.0 (84.5 to 93.7)	98.6 (91.7 to 99.8)	77.5 (35.7 to 95.5)	99.5 (99.2 to 99.7)
Kanamycin	gDST	2 studies; 6 study cohorts (990)	39 (3.94)	91.7 (74.8 to 97.6)	99.8 (95.8 to 100.0)	96.1 (53.1 to 99.8)	99.6 (98.6 to 99.9)
Kanamycin	Composite	2 studies; 6 study cohorts (1008)	42 (4.17)	85.6 (70.3 to 93.7)	99.9 (93.2 to 100.0)	98.0 (40.0 to 100.0)	99.3 (98.4 to 99.7)
With rifampicin resistance							
Kanamycin	pDST	1 study; 4 study cohorts (491)	28 (5.70)	91.5 (83.1 to 96.0)	94.5 (79.5 to 98.7)	87.7 (63.9 to 96.7)	97.4 (94.8 to 98.7)
Kanamycin	gDST	1 study; 4 study cohorts (433)	40 (9.24)	93.8 (66.5 to 99.1)	98.6 (91.9 to 99.8)	96.7 (83.6 to 99.4)	98.1 (88.9 to 99.7)
Kanamycin	Composite	1 study; 4 study cohorts (446)	41 (9.19)	87.4 (66.0 to 96.1)	98.8 (91.2 to 99.9)	97.0 (81.6 to 99.6)	96.3 (89.7 to 98.7)
Irrespective of rifampicin resistance							

Table 2. Xpert MTB/XDR summary sensitivity and specificity for resistance to tuberculosis drugs *(Continued)*

Capreomycin	pDST	2 studies; 5 study cohorts (771)	25 (3.24)	78.2 (62.4 to 88.6)	99.6 (98.5 to 99.9)	91.4 (72.1 to 97.8)	98.9 (98.0 to 99.4)
Capreomycin	gDST	2 studies; 6 study cohorts (991)	31 (3.13)	86.5 (55.2 to 97.1)	99.9 (99.2 to 100.0)	99.5 (82.0 to 100.0)	93.1 (82.7 to 97.5)
Capreomycin	Composite	2 studies; 5 study cohorts (823)	53 (6.44)	73.1 (39.8 to 91.7)	99.9 (96.6 to 100.0)	98.2 (48.8 to 100.0)	98.7 (96.4 to 98.7)
With rifampicin resistance							
Capreomycin	pDST	1 study; 4 study cohorts (491)	24 (4.89)	76.5 (55.7 to 89.4)	99.3 (97.6 to 99.8)	97.9 (92.9 to 99.4)	93.4 (87.2 to 96.7)
Capreomycin	gDST	1 study; 4 study cohorts (434)	23 (5.30)	75.4 (43.6 to 92.4)	99.9 (93.9 to 100.0)	99.5 (82.0 to 100)	93.1 (82.7 to 97.5)
Capreomycin	Composite	1 study; 4 study cohorts (444)	26 (5.86)	67.2 (35.9 to 88.2)	99.7 (98.1 to 100.0)	99.0 (93.4 to 99.9)	91.0 (80.9 to 96.0)

Abbreviations: **CI:** confidence interval; **gDST:** genotypic drug susceptibility testing; **NE:** not estimable; **№**: number; **pDST:** phenotypic drug susceptibility testing.
Study cohorts were treated as distinct units in the meta-analyses.
*Prevalence for calculating predictive values: 5% in people irrespective of rifampicin resistance and 30% in people with known rifampicin resistance.

Table 3. Summary proportion of Xpert XDR/MTB indeterminate results by drug

Drug	Study	Total	№ indeterminate	Summary proportion (95% CI)
Isoniazid	Omar 2020	498	2	0.34% (0.00 to 0.68)
	Penn-Nicholson 2021	657	2	
Fluoro-quinolones	Omar 2020	498	4	1.05% (0.46 to 1.64)
	Penn-Nicholson 2021	657	9	
Ethionamide	Omar 2020	498	0	0.06% (0.00 to 0.34)
	Penn-Nicholson 2021	657	1	
Amikacin	Omar 2020	498	8	2.33% (1.46 to 3.20)
	Penn-Nicholson 2021	657	23	

Abbreviations: **CI:** confidence interval; **№**: number.

Table 4. Xpert MTB/XDR summary sensitivity and specificity for resistance to isoniazid and fluoroquinolones, sensitivity analyses

Analysis group	Number of studies and number of study cohorts (participants)	№ (%) with drug resistance	Summary sensitivity % (95% CI)	Summary specificity % (95% CI)	Positive predictive value % (95% CI)*	Negative predictive value % (95% CI)*
Isoniazid	2 studies reporting on 6 study cohorts (1083)	756 (69.8)	94.2 (87.5 to 97.4)	98.5 (92.6 to 99.7)	76.9 (38.8 to 94.6)	99.7 (99.4 to 99.9)
Isoniazid	**1 study reporting on 4 study cohorts (605)**	**489 (80.8)**	**95.5 (85.2 to 98.7)**	**97.1 (82.4 to 99.6)**	**63.5 (19.5 to 92.6)**	**99.8 (99.2 to 99.9)**
Fluoro-quinolones	2 studies **reporting on** 6 study cohorts (1021)	381 (37.3)	93.2 (88.1 to 96.2)	98.0 (90.8 to 99.6)	70.6 (34 to 91.8)	99.7 (99.4 to 99.8)
Fluoro-quinolones	**1 study reporting on 4 study cohorts (604)**	**222 (36.8)**	**93.4 (84.3 to 97.4)**	**96.7 (85.3 to 99.3)**	**59.7 (23.8 to 87.5)**	**99.7 (99.2 to 99.9**)

Abbreviations: **CI:** confidence interval; **№**: number.
Results from the sensitivity analyses (**in bold**) in which the manufacturer sponsored study was excluded. The population is people irrespective of rifampicin resistance and the reference standard is phenotypic drug susceptibility testing.
Study cohorts were treated as distinct units in the meta-analyses.
*Prevalence of drug resistance for calculating predictive values was 5%.

APPENDICES

Appendix 1. Glossary of terms related to drug resistance testing

Amplification

Amplification is replication of a deoxyribonucleic acid (DNA) fragment to generate copies. Both the original and the newly synthesized copies can be described as the amplicons.

Bacteriologically confirmed

Refers to a biological specimen that is positive for tuberculosis by smear, culture, or Xpert MTB/RIF, Xpert MTB/RIF Ultra, Truenat MTB or another WHO-recommended rapid diagnostic test (see also **Microbiological reference standard**).

Codon

A codon is a sequence of three nucleotides (building blocks) in a DNA or ribonucleic acid (RNA) molecule that may encode, among other things, a specific amino acid.

Critical concentration

The critical concentration of a tuberculosis agent (drug) has been adopted and modified from international convention. The critical concentration is defined as the lowest concentration of a tuberculosis agent in vitro that will inhibit the growth of 99% of phenotypically wild type strains of *Mycobacterium tuberculosis (M tuberculosis)* complex.

Cultured isolate

Cultured isolate refers to *M tuberculosis* bacteria from a clinical specimen that have been grown. For tuberculosis diagnosis, a volume of the clinical specimen is processed and incubated under conditions that promote *M tuberculosis* growth. The bacteria that are grown are referred to a cultured isolate.

DNA sequencing

DNA sequencing is a process to determine the nucleotide (adenine (A), cytosine (C), guanine (G), and thymine (T)) sequence of fragments of DNA. By comparison of DNA sequences from distinct tuberculosis isolates, variations known as mutations can be identified. Some mutations in *M tuberculosis* are known to be associated with drug resistance.

Drug susceptibility testing

Drug susceptibility tests determine whether *M tuberculosis* bacteria are susceptible or resistant to drugs. Testing may be undertaken using phenotypic or genotypic analyses.

eis promoter

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to second-line injectable drugs, amikacin and kanamycin.

fabG1

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to isoniazid.

Genotypic drug susceptibility testing (gDST)

Genotypic drug susceptibility testing (gDST) involves detecting predetermined mutations in DNA that are known to make the bacteria resistant to a drug. When mutations causing drug resistance are unknown, gDST is not useful.

gDST can be targeted (limited to a certain number of loci for a drug) or genome-wide. Sanger sequencing, a targeted sequencing method, is limited in its depth (x1 vs. x100 for whole genome sequencing). Deep sequencing methods have greater resolution than the Sanger sequencing method. They also appear robust when performed on DNA extracted directly from a specimen (versus a cultured isolate), especially if that specimen is rich in mycobacteria. As with any method that is targeted, targeted gDST will miss phenotypic resistance causing mutations that occur outside of the target, simply because it is not designed to evaluate that region.

Genome-wide gDST typically refers to whole genome sequencing. Importantly, although whole genome sequencing could have been performed, some investigators might only use it in a manner equivalent to targeted sequencing of certain regions. For example, if whole genome sequencing coverage was poor in a region known to be important for resistance, but otherwise adequate in other regions important for resistance, whole genome sequencing will serve in this case as a limited form of targeted sequencing. In other words, even though most of the genome may be sequenced, we may not know where to look for resistance associated variants.

gyrA

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to fluoroquinolones.

gyrB

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to fluoroquinolones.

Heteroresistance

Heteroresistance is defined as resistance to certain drugs in a subset of a larger microbial population that is generally considered susceptible to these drugs according to traditional phenotypic drug susceptibility testing.

Indeterminate test result

An indeterminate Xpert MTB/XDR test result is one that indicates that resistance to a given drug could not definitively be detected based on the test's algorithm.

***inhA* promoter**

Gene target included in the Xpert MTB/XDR test to detect tuberculosis and resistance to isoniazid and ethionamide. Mutations in the *inhA* promoter region of tuberculosis are known to confer low-level resistance to isoniazid and high-level cross-resistance to ethionamide.

Intergenic region

Is a region of DNA sequence located between genes and a subset of non-coding DNA. Some intergenic regions act to control coding regions (genes) nearby.

katG

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to isoniazid.

Locus

A locus is the position of a genetic feature in the DNA sequence, like a genetic street address. Loci are standardized between genomes by reference to a common reference genome, such as H37Rv for *Mycobacterium tuberculosis*.

Microbiological reference standard

Refers to a biological specimen that is positive for tuberculosis by smear, culture, or a WHO-recommended rapid diagnostic test, such as Xpert MTB/RIF, Xpert MTB/RIF Ultra, Truenat MTB, or other WHO-recommended rapid diagnostic test (also see B**acteriologically confirmed**). Recently, the term 'microbiological reference standard' has come into use; particularly in WHO evaluations of new diagnostic tests.

Mutation

A mutation is a change in a DNA sequence. Mutations can result from DNA copying mistakes made during cell division, exposure to ionizing radiation, exposure to chemicals called mutagens, or infection by viruses.

Non-determinate test result

A non-determinate Xpert MTB/XDR test result is one that results in an Error, Invalid, or No Result and can be due to an operator error, instrument, or cartridge issue.

***oxyR-ahpC* intergenic region**

Gene targets included in the Xpert MTB/XDR test to detect mutations that confer resistance to isoniazid.

Phenotypic drug susceptibility testing (pDST)

Phenotypic testing requires growth of *Mycobacterium tuberculosis* in the presence of drugs at a specific concentration that will inhibit the growth of susceptible bacteria or have no impact on growth of resistant bacteria.

Presumptive tuberculosis

Presumptive tuberculosis refers to a patient who presents with symptoms or signs suggestive of tuberculosis (WHO Definitions and Reporting 2020).

Promoter region

A promoter region is a sequence of DNA where the transcriptional machinery binds before transcribing the DNA into RNA that may then be translated into an amino acid sequence.

Reflex test

The term reflex test refers to a diagnostic approach in which an initial test meets predetermined criteria (e.g. outside of the normal range), and a second test is performed automatically, usually without a request from the health care worker. For example, a urinalysis may be followed by a culture (reflex test) if in the urine, the presence of nitrites is detected or the number of white blood cells is increased suggesting an infection. In the context of tuberculosis, culture may be used as a reflex test in a person living with HIV who has a Xpert MTB/RIF Ultra-negative result.

Resistance-determining region

A region of the *Mycobacterium tuberculosis* genome where mutations commonly cause resistance to a specific drug.

rrs

Gene target included in the Xpert MTB/XDR test to detect mutations that confer resistance to second-line injectable drugs, amikacin, kanamycin, and capreomycin.

Sanger sequencing

Technique for DNA sequencing based upon the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication, also known as 'the chain termination method'.

Targeted gene sequencing

The process for detecting predetermined mutations in DNA or genomic regions.

Whole genome sequencing (WGS)

The process of determining the complete genome sequence for a given organism (tuberculosis bacteria) at one time through next-generation sequencing methods. This method can determine the order of most nucleotides in a given genome and detect any variations relative to a reference genome using bioinformatics analyses.

Adapted from National Human Genome Research Institute 2022.

Appendix 2. Detailed search strategy

Ovid MEDLINE(R) and In-Process, In-Data-Review & Other Non-Indexed Citations < 1946 to present

1 Extensively Drug-Resistant Tuberculosis/ or Tuberculosis, Multidrug-Resistant/ or Tuberculosis, Pulmonary/ or Mycobacterium Tuberculosis/

2 ((tuberculosis adj3 (lung or pulmonary)) or (tuberculosis adj3 respiratory)).mp.

3 (tuberculosis adj3 (drug resistan* or multidrug resistan* or mdr or xdr)).mp.

4 (((isoniazid adj3 resistance) or isoniazid) adj3 resistant).mp.

5 ((Ethionamide adj3 resistance) or (ethionamide adj3 resistant)).mp

6 ((Amikacin adj3 resistance) or (amikacin adj3 resistant)).mp.

7 ((Fluoroquinolone adj3 resistance) or (Fluoroquinolone adj3 resistant)).mp.

8 (Second-line injectable drug adj3 resistance).mp.

9 (Second-line injectable drug adj3 resistant).mp.

10 ((SLID adj3 resistance) or (SLID adj3 resistant)).mp.

11 (MDR-TB or XDR-TB).tw.

12 ((isoniazid or fluoroquinolone or "second-line injectable drug" or SLID) adj3 (monoresist* or mono-resist*)).tw.

13 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12

14 (cartridge adj3 test*).mp.

15 cartridge*.ab. or cartridge*.ti.

16 (Molbio or Truenat or Cepheid or Xpert* or Bioneer or Hain).mp.

17 Genexpert*.mp.

18 exp Point-of-Care Systems/

19 (drug susceptibility test* or drug resistance test* or (rapid adj3 (detect* or test* or diagnos*)) or (poc or poct or "point of care")).mp.

20 14 or 15 or 16 or 17 or 18 or 19

21 13 and 20

22 Limit 21 to yrs “2015-Current”

Embase OVID

1 drug resistant tuberculosis/ or extensively drug resistant tuberculosis/ or multidrug resistant tuberculosis/ or lung tuberculosis/ or Mycobacterium Tuberculosis/

2 ((tuberculosis adj3 (lung or pulmonary)) or (tuberculosis adj3 respiratory)).mp.

3 (tuberculosis adj3 (drug resistan* or multidrug resistan* or mdr or xdr)).mp.

4 (((isoniazid adj3 resistance) or isoniazid) adj3 resistant).mp.

5 ((Ethionamide adj3 resistance) or (ethionamide adj3 resistant)).mp.

6 ((Amikacin adj3 resistance) or (amikacin adj3 resistant)).mp.

7 ((Fluoroquinolone adj3 resistance) or (Fluoroquinolone adj3 resistant)).mp.

8 (Second-line injectable drug adj3 resistance).mp.

9 (Second-line injectable drug adj3 resistant).mp.

10 ((SLID adj3 resistance) or (SLID adj3 resistant)).mp.

11 (MDR-TB or XDR-TB).tw.

12 ((isoniazid or fluoroquinolone or "second-line injectable drug" or SLID) adj3 (monoresist* or mono-resist*)).tw.

13 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12

14 (cartridge adj3 test*).mp.

15 cartridge*.ab. or cartridge*.ti.

16 (Molbio or Truenat or Cepheid or Xpert* or Bioneer or Hain).mp.

17 Genexpert*.mp.

18 exp Point-of-Care Systems/

19 (drug susceptibility test* or drug resistance test* or (rapid adj3 (detect* or test* or diagnos*)) or (poc or poct or "point of care")).mp.

20 14 or 15 or 16 or 17 or 18 or 19

21 13 and 20

22 Limit 21 to yrs “2015-Current”

CPCI-S, SCI-EXPANDED, Biosis (Web of Science)

#4 (#1) AND #2 and 2021 or 2020 or 2019 or 2018 or 2017 or 2016 or 2015 (Publication Years)

#3 (#1) AND #2

#2 (cartridge test*) or (Molbio or Truenat or Cepheid or Xpert* or Bioneer or Hain) or Genexpert* or Point-of-Care System* (Topic)

#1 (tuberculosis AND (drug resistan* or multidrug resistan* or mdr or xdr)) (Topic) or tuberculosis AND (isoniazid resist* or Ethionamide resist* or Amikacin resist* or Fluoroquinolone resist* or Second-line injectable drug resist*) (Topic)

Scopus

(TITLE-ABS-KEY ((cartridge AND test*) OR (molbio OR truenat OR cepheid OR xpert* OR bioneer OR hain) OR genexpert* OR point-of-care AND system*)) AND ((TITLE-ABS-KEY (tuberculosis AND (drug AND resistan* OR multidrug AND resistan* OR mdr OR xdr))) OR (TITLE-ABS-KEY (tuberculosis AND ((isoniazid AND resist*) OR (ethionamide AND resist*) OR (amikacin AND resist*) OR (fluoroquinolone AND resist*) OR (second-line AND injectable AND drug AND resist*))))) AND (LIMIT-TO (PUBYEAR , 2021) OR LIMIT-TO (PUBYEAR , 2020) OR LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR, 2015))

Database: LILACS

Search on: (tuberculosis AND (drug resistan$ or multidrug resistan$ or mdr or xdr)) [Words] and (cartridge test$) or (Molbio or Truenat or Cepheid or Xpert$ or Bioneer or Hain) or Genexpert$ or Point-of-Care System$ [Words] and 2015 OR 2016 OR 2017 OR 2018 OR 2019 OR 2020 OR 2021 [Country, year publication]

Clinicaltrials.gov, WHO ICTRP, ISRCTN

Xpert, Genexpert and Tuberculosis, Multidrug-Resistant ; Multi-Drug Resistant Tuberculosis; MDR Tuberculosis; MDR-TB; Multidrug-Resistant TB

ProQuest Dissertations & Theses A&I

ab(tuberculosis) AND ab(Xpert or genexpert or cartridge) limit to 2015-01-01 - 2021-09-16

Appendix 3. Data extraction form

Study	
Name of data extractor	1 – SP 2 – KRS 3 – other, specify GT, MdV, GD
First author	
Corresponding author and email	
Was author contacted?	1 – yes 2 – no If yes, dates(s)
Title of paper	
Year (of publication)	
Year (study start date)	
Language	1 – English 2 – other If other, specify:
Was the study conducted without industry sponsorship?	1 – yes 2 – no 9 – unknown/not reported

(Continued)

If industry sponsorship was present, select one item from the list	Answers ordered from least to most industry involvement 1 – donation of test for use in study 2 – test at a special preferred price 3 – receipt of educational support, grants, or speaking fees 4 – financial relationship – author is employee/consultant/stock-holder 5 – involvement in design, analysis, or manuscript production
Study addresses question A (detection of isoniazid only), B (detection of second-line only), (detection of both isoniazid and second-line) C	1 – A 2 – B 3 – C Circle as many options as required
What was the aim of this study in authors' own words?	
Country of laboratory where test was run	
World Bank Classification of laboratory country	1 – low 2 – middle 3 – high 8 – other
Laboratory setting; describe as written in the paper	1 – primary care laboratory 2 – intermediate-level laboratory 3 – central-level laboratory 8 – other, specify 9 – unknown/not reported
Study design	1 – cross-sectional 2 – cohort 3 – single gate diagnostic study 8 – other, specify 9 – unknown/not reported
Participant selection	1 – consecutive 2 – random 3 – convenience 8 – other, specify 9 – unknown/not reported

(Continued)

Comments about study design	
Number after screening by exclusion and inclusion criteria	9 – unknown/not reported
Number included in analysis (# screened – # exclusions)	9 – unknown/not reported
Did the study include specimens and/or cultured isolates for testing?	1 – specimens 2 – isolates 3 – both 9 – unknown/not reported
Characteristics of participants	
Age	mean SD median IQR range 9 – unknown/not reported
Gender	male female total # females/total (%) 9 – unknown/not reported
HIV status	positive negative unknown total # HIV positive/total (%) 9 – unknown/not reported
Previous tuberculosis treatment	yes no unknown total # previous tuberculosis/total (%) = 9 – unknown/not reported
Type of participants/specimens tested	1 – presumptive tuberculosis 2 – irrespective of rifampicin resistance 3 – with known (detected) rifampicin resistance

(Continued)

	8 – other, specify:
	9 – unknown/not reported

Reference standards
1 – pDST
2 – gDST
3 – composite
The composite reference standard is pDST and gDST, where at least one component test is positive.

Isoniazid	1 – pDST (specify type and critical concentrations)
	2 – sequencing of the *katG, inhA promoter,* and *fabG1* gene
	3 – both 1 and 2 in all specimens (specify culture information in 1)
	9 -unknown/not reported
	1a – MGIT, LJ, other
	1b – isoniazid critical concentration
	MGIT – 0.1 WHO concentration
	LJ – 0.2 WHO concentration
Fluoroquinolones	1 – pDST (specify type and critical concentrations)
	2 – sequencing of the *gyrA* and *gyrB* gene
	3 – both 1 and 2 in all specimens (specify culture info in 1)
	9 – unknown/not reported
	1a – MGIT, LJ, other
	1b – drugs used for this class and critical concentration
	Levofloxacin
	MGIT – 1.0 WHO concentration
	LJ – 2.0 WHO concentration
	Moxifloxacin (critical concentration)
	MGIT – 0.25 WHO concentration
	LJ – 1.0 WHO concentration
	Moxifloxacin (clinical breakpoint)
	7H10 – 2.0 WHO concentration
	MGIT – 1.0 WHO concentration
Ethionamide	1 – pDST (specify type and critical concentrations)
	2 – sequencing of the *inhA promoter* gene
	3 – both 1 and 2 in all specimens (specify culture information in 1)

(Continued)

	9 – unknown/not reported 1a – MGIT, LJ, other 1b – ethionamide critical concentration MGIT – 5.0 WHO concentration LJ – 40.0 WHO concentration
Amikacin	1 – pDST (specify type and critical concentrations) 2 – sequencing of the *rrs* gene 3 – both 1 and 2 in all specimens (specify culture info in 1) 9 – unknown/not reported 1a – MGIT, LJ, other 1b – amikacin critical concentration MGIT – 1.0 WHO concentration LJ – 30.0 WHO concentration
Test information	
Was microscopy used?	1 – yes 2 – no 9 – unknown/not reported
Smear status of specimens (if applicable)	positive negative unknown total
Specimen information	
Type of specimen (may include expectorated sputum) if test performed directly on a specimen	1 – all expectorated 2 – all induced 3 – both types 8 – other 9 – unknown/not reported describe
Were results for Xpert MTB/XDR and culture obtained using the same specimen?	1 – yes 2 – no 3 – not applicable 9 – unknown/not reported

(Continued)

Pretreatment processing procedure if performed for Xpert MTB/XDR specimen	1 – none 2 – NALC-NaOH 3 – NaOH (Petroff) 8 – other 9 – unknown/not reported
For Xpert MTB/XDR specimen, what was the condition of the specimen when tested?	1 – fresh 2 – frozen 3 – both 9 – unknown/not reported
If fresh, specify:	1 – tested after storage at room temperature or refrigerated within 48 hours of collection 2 – tested after storage at room temperature or refrigerated > 48 hours after collection 9 – unknown/not reported
If frozen, specify:	1 – tested after frozen < 1 year of storage 2 – tested frozen ≥ 1 year storage 9 – unknown/not reported
Proportion contaminated cultures, if provided:	= # of contaminated cultures total # cultures performed 9 – unknown/not reported
Proportion inconclusive sequencing results, if provided (does not apply to discrepant analysis)	= # of inconclusive sequencing total # sequencing performed 9 – unknown/not reported
Were patient-important outcomes evaluated?	1 – yes 2 – no 9 – unknown/not reported
Time to diagnosis and Time to report	Isoniazid Fluoroquinolone Ethionamide Amikacin 9 – unknown (45 days (27–122 days) for liquid culture)
Time to treatment initiation	Isoniazid

(Continued)

	Fluoroquinolone
	Ethionamide
	Amikacin
	9 – unknown

Tables

Tuberculosis detection

Tuberculosis detection, all		Culture		
		Yes	No	Total
Xpert MTB/XDR Result	Positive			
	Negative			
	Total			

Isoniazid resistance detection, direct testing, in people irrespective of rifampicin resistance

Isoniazid, all		pDST		
		Yes	No	Total
Xpert MTB/XDR Result	Positive			
	Negative			
	Total			

Isoniazid, smear positive		pDST		
		Yes	No	Total
Xpert MTB/XDR Result	Positive			
	Negative			
	Total			

Isoniazid, smear negative		pDST		
		Yes	No	Total
Xpert MTB/XDR Result	Positive			
	Negative			
	Total			

Add tables as needed.

Abbreviations: **gDST:** genotypic drug susceptibility testing; **IQR:** interquartile range; **LJ:** Löwenstein Jensen; **MGIT:** Mycobacteria Growth Indicator Tube; **pDST**: phenotypic drug susceptibility testing; **SD**: standard deviation; **WHO:** World Health Organization.

Appendix 4. QUADAS-2 tailored to the review

Domain 1: patient selection

Detection of pulmonary tuberculosis

Risk of bias: could the selection of patients have introduced bias?

Signalling question 1: was a consecutive or random sample of patients enrolled?

We answered yes if the study enrolled a consecutive or random sample of eligible participants; no if the study selected participants by convenience; and unclear if the study did not report the manner of participant selection or we could not determine this.

Signalling question 2: was a case-control design avoided?

We answered yes for all studies.

Signalling question 3: did the study avoid inappropriate exclusions?

We answered yes if the study included both smear-positive and smear-negative participants; no if the study included primarily or exclusively smear-positive or smear-negative participants; and unclear if we could not determine this. If, at the time of specimen collection, participants were receiving tuberculosis treatment, we answered no because treatment reduces the culturability of *M tuberculosis* quicker than it reduces the amount of MTB DNA. Treatment therefore confounds the relationship between Xpert MTB/XDR-positivity and culture-positivity (the reference standard), potentially leading to underestimation of specificity. We also judged high-risk of bias if we thought most participants were enrolled based on known rifampicin resistance.

Applicability: are there concerns that the included participants and setting do not match the review question?

We considered low concern if the included patients matched the review question; high concern if the included patients did not match the review question; and unclear concern if we could not determine. Our assessment included consideration of prior testing and the clinical setting. We answered low concern if participants were people with presumed pulmonary tuberculosis; high concern if participants received prior testing and were included based on a positive Xpert MTB/RIF or Xpert MTB/RIF Ultra result; and unclear concern if participants received prior testing but we could not tell if inclusion was based on a positive Xpert MTB/RIF or Xpert MTB/RIF Ultra result. We answered low concern if participants were evaluated as outpatients (with either expectorated or induced sputum) in local hospitals or primary care centres. We answered high concern if participants were evaluated exclusively as inpatients in tertiary care centres. We answered unclear concern if the clinical setting was not reported or there was insufficient information to make a decision. We also answered unclear concern if testing was performed at a central-level laboratory and the clinical setting was not reported or if, for example, it was difficult to determine whether the laboratory provided services mainly to very sick people or people with a broader clinical spectrum of illness. We also answered high concern if patients were on treatment or their treatment status was unclear, as such patients have already been diagnosed with tuberculosis.

Detection of drug resistance

Risk of bias: could the selection of participants have introduced bias?

Signalling question 1: was a consecutive or random sample of participants enrolled?

We answered the same as for detection of tuberculosis.

Signalling question 2: was a case-control design avoided?

We answered yes if the study enrolled people with tuberculosis with suspected or sufficiently high pretest probability (per World Health Organization guidelines) for resistance to isoniazid, second-line drugs, or both isoniazid and second-line drugs; no if the study enrolled people with tuberculosis with confirmed previously known resistance to the drug in question; and unclear for all other scenarios or if it was not clearly reported. We considered that accuracy studies may have a cross-sectional design even when the reference standard is performed before the index test if both cases and controls are sampled from a single source population.

Signalling question 3: did the study avoid inappropriate exclusions?

We answered yes for people who were previously treated for tuberculosis. We answered no if people who were previously treated were excluded. People previously tested for tuberculosis have a higher risk of having drug resistance and are likely to be the target population for initial use of Xpert MTB/XDR. If people with samples known to be heteroresistant (a mix of susceptible and resistant tuberculosis strains in the specimen) were excluded, which is particularly relevant for the fluoroquinolones, we answered answer no. We answered unclear if we could not determine this.

Applicability: are there concerns that the included participants and setting do not match the review question?

We answered low concern if the selected clinical specimens or isolates matched the review question, which reflects the way the test will be used in practice. We answered high concern if the selected specimens or isolates did not represent those for whom the test will be used in practice, such as in people who do not require investigation for resistance to the drugs in question. We answered unclear concern if we could not determine this.

Domain 2: index test

Detection of pulmonary tuberculosis

Risk of bias: could the conduct or interpretation of the index test have introduced bias?

Signalling question 1: were the index test results interpreted without knowledge of the results of the reference standard?

We answered yes for all studies since Xpert MTB/XDR results are automatically generated and the user is provided with printable test results, thus, avoiding subjective interpretation.

Signalling question 2: if a threshold was used, was it pre-specified?

We answered yes for all studies.

Applicability: are there concerns that the index test, its conduct, or its interpretation differ from the review question?

Variations in test technology, execution, or interpretation may affect estimates of the diagnostic accuracy of a test. We judged the study of low concern for applicability if the test was performed as recommended by the manufacturer. We judged the study of high concern if the test was applied differently than recommended by the manufacturer, for example, if the test was applied to summary sputa. We judged the study of unclear concern if we could not determine this.

Detection of drug resistance

Risk of bias: could the conduct or interpretation of the index test have introduced bias?

Signalling question 1: were the index test results interpreted without knowledge of the results of the reference standard?

We answered yes for all studies since Xpert MTB/XDR results are automatically generated and the user is provided with printable test results, thus, avoiding subjective interpretation.

Signalling question 2: if a threshold was used, was it pre-specified?

We answered yes for all studies.

Applicability: are there concerns that the index test, its conduct, or its interpretation differ from the review question?

We recorded the same judgements as for detection of pulmonary tuberculosis.

Domain 3: reference standard

Detection of pulmonary tuberculosis

Risk of bias: could the reference standard, its conduct, or its interpretation have introduced bias?

Signalling question 1: is the reference standard likely to correctly classify the target condition?

We answered yes for all studies because a microbiological reference standard for *M tuberculosis* is a criterion for inclusion in the review.

Signalling question 2: were the reference standard results interpreted without knowledge of the results of the index test?

We answered yes if the reference test provided an automated result (e.g. MGIT 960), blinding was explicitly stated, or it was clear that the reference standard was performed at a separate laboratory or performed by different people (or both). We answered no if the study stated that the reference standard result was interpreted with knowledge of the Xpert MTB/XDR test result. We answered unclear if we could not determine this.

Applicability: are there concerns that the target condition as defined by the reference standard does not match the question?

We answered high concern if a type of culture was not used as part of the reference standard, because studies that include only DNA-based tests do not directly measure live *M tuberculosis*. We answered low concern if culture was performed. We answered unclear concern if we could not determine this.

Detection of drug resistance

Risk of bias: could the reference standard, its conduct, or its interpretation have introduced bias?

We considered the reliability of the different reference standards for the different drugs (Heyckendorf 2018).

Signalling question 1.1: Is the reference standard likely to correctly classify the target condition, pDST?

Signalling question 1.2: Is the reference standard likely to correctly classify the target condition, gDST?

Signalling question 1.3: Is the reference standard likely to correctly classify the target condition, composite?

We answered these questions as follows.

Drug	pDST*	gDST using targeted sequencing	Composite (pDST* and gDST using targeted sequencing)	gDST using whole genome sequencing)	Composite (pDST* and gDST using whole genome sequencing)
Isoniazid	Yes	Unclear if few loci were investigated, and yes, if all relevant loci were analysed Loci required for yes: *katG*, *inhA* promoter, *oxyR-ahpC* intergenic region, and *fabG1*	Yes	Unclear if few loci were investigated, and yes, if all relevant loci were analysed Loci required for yes: *katG*, *inhA* promoter, *oxyR-ahpC* intergenic region, and *fabG1*	Yes
Fluoroquinolones	Yes, will depend on critical concentration used for moxifloxacin	Yes Loci required for yes: *gyrA* and *gyrB*	Yes	Yes Loci required for yes: *gyrA* and *gyrB*	Yes
Ethionamide	No, there is considerable overlap in the MICs of *M tuberculosis* isolates with and without resistance-causing variants. This means there is	Unclear if few loci were investigated, and yes, if all relevant loci were analysed Loci required for yes: *ethA, ethR,* and *inhA* promoter	Unclear	Unclear if few loci were investigated, and yes, if all relevant loci were analysed Loci required for yes: *ethA, ethR,* and *inhA* promoter No if only the *inhA* promoter was analysed	Unclear

(Continued)

	considerable overlap in the distribution of MICs for resistant and wild-type isolates	No if only the *inhA* promoter was analysed			
Amikacin	Yes	Yes, if all relevant loci were analysed Loci required for yes: *rrs* and *eis* promoter	Yes	Yes, if all relevant loci were analysed Loci required for yes: *rrs* and *eis* promoter	Yes

Abbreviations: **gDST**: genotypic drug susceptibility testing; **MIC**: minimum inhibitory concentration; **pDST**: phenotypic drug susceptibility testing.
*We used the currently recommended World Health Organization critical concentrations as a benchmark for judging risk of bias (Appendix 11). For *M tuberculosis*, the antimicrobial susceptibility testing critical concentration is defined as the lowest concentration of an anti-tuberculosis agent in vitro that will inhibit the growth of 99% of phenotypically wild type strains of *Mtuberculosis* complex (WHO Critical Concentrations 2018; WHO Critical Concentrations 2021).

We added the following signalling questions.

Signalling question 2.1: Were the reference standard results interpreted without knowledge of the results of the index tests, pDST?

Signalling question 2.2: Were the reference standard results interpreted without knowledge of the results of the index tests, gDST?

Signalling question 2.3: Were the reference standard results interpreted without knowledge of the results of the index tests, composite?

For pDST, we answered yes if the reference test provided an automated result (e.g. if liquid culture was used as in MGIT 960 DST), blinding was explicitly stated, or it was clear that the reference test was performed at a separate laboratory, or performed by different people, or both. Of note, pDST on solid media is not automated. We answered no if the study stated that the reference standard result was interpreted with knowledge of the Xpert MTB/XDR test result. We answered unclear if we could not determine this. For gDST, we answered yes for all studies since the results for the reference standard are automated.

We added the following signalling question.

Signalling question 3: Were the index test and reference standard performed using the same material (clinical specimen or sediment, or cultured isolate)?

Phenotypic DST (pDST) and genotypic DST (gDST) for reference standard testing can be performed on an isolate that has undergone (potentially multiple rounds) of culture in drug-free media. This may lead to the depletion of resistant strains present in the original specimen (which would have been used for the Xpert MTB/XDR testing if direct testing was performed) and cause discrepant results. We think this is an important question as it addresses heteroresistance, which often explains discordance between genotypic and phenotypic results.

For direct testing of a clinical specimen by Xpert MTB/XDR: we answered yes if the reference test was performed directly on the same clinical specimen; no if the reference standard was performed on a culture isolate; and unclear if we could not determine this. For indirect testing of a culture isolate by Xpert MTB/XDR: we answered yes if the reference test was performed on the same culture isolate (e.g. indirect sequencing); no if the reference standard was performed on a different culture isolate, or specimen; and unclear if we could not determine this.

Applicability: are there concerns that the target condition as defined by the reference standard does not match the question?

We judged applicability of low concern for all studies because specimens to be subsequently tested for drug resistance will have already been identified as *M tuberculosis* complex positive.

Domain 4: flow and timing

Detection of tuberculosis

Risk of bias: could the patient flow have introduced bias?

Signalling question 1: was there an appropriate interval between the index test and reference standard?

In most studies, we expected the reference standard to be performed at the same time as Xpert MTB/XDR. However, in some studies, the reference standard may have been performed on a different sample collected at an earlier time. This case applies to some cultured isolates, whose drug susceptibility profile might have been confirmed before Xpert MTB/XDR was available. We answered yes if Xpert MTB/XDR and the reference standard were performed at the same time or were separated by less than 14 days. We answered no if Xpert MTB/XDR and the reference standard were not performed at the same time and were separated by 14 days or more. As people suspected of second-line drug resistance are often receiving treatment for tuberculosis, it is possible that variation in the microbial population of specimens collected at different time points may occur. We answered unclear if we could not determine this.

Signalling question 2: did all patients receive the same reference standard?

We answered yes if the reference standard was applied to all participants or a random sample of participants, no if the reference standard was only applied to a selective group of participants, and unclear if it was not stated in the paper or if the authors failed to answer this question.

Signalling question 3: were all patients included in the analysis?

We determined the answer to this question by comparing the number of participants enrolled with the number of participants included in the 2×2 tables. We noted if the study authors reported the number of inconclusive test results. We answered yes if the number of participants enrolled was clearly stated and corresponded to the number presented in the analysis or if exclusions were adequately described. We answered no if there were participants missing or excluded from the analysis and there was no explanation given. We answered unclear if insufficient information was given to assess whether participants were excluded from the analysis.

Detection of drug resistance

We answered the same as for detection of pulmonary tuberculosis.

Judgements for risk of bias assessments for a given domain.

- If we answered all signalling questions for a domain yes, then we judged risk of bias as low.
- If we answered all or most signalling questions for a domain no, then we judged risk of bias as high.
- If we answered only one signalling question for a domain no, we discussed further the risk of bias judgement.
- If we answered all or most signalling questions for a domain unclear, then we judged risk of bias as unclear.
- If we answered only one signalling question for a domain unclear, we discussed further the risk of bias judgement for the domain.

Appendix 5. Xpert MTB/XDR inconclusive results and missed cases

We used the following approach to describe the different types of inconclusive results.

Xpert MTB/XDR NON-DETERMINATE: Among specimens initially tested, we determined the proportion of Xpert MTB/XDR NON-DETERMINATE results and, of these, the number of ERROR, INVALID, and NO RESULT results. We also determined the percentage of non-determinate results remaining following retesting.

Xpert MTB/XDR INDETERMINATE: Among specimens reporting Xpert MTB/XDR MTB DETECTED, we determined the proportion that were Xpert MTB/XDR INDETERMINATE (drug resistance is only evaluated when tuberculosis is detected). Among specimens with results reported as Xpert MTB/XDR INDETERMINATE, we further determined the percentage that were resistant or susceptible by the reference standard.

Xpert MTB/XDR MTB NOT DETECTED: Among specimens with pDST results available, we determined the percentage that were Xpert MTB/XDR MTB NOT DETECTED. Among specimens with results reported as Xpert MTB/XDR MTB NOT DETECTED, we further determined the percentage that were resistant or susceptible according to pDST.

Xpert MTB/XDR NON-DETERMINATE results

The summary proportion of Xpert MTB/XDR non-determinate results was estimated to be 2.90% (95% CI: 1.97% to 3.84%).

In Omar 2020, upon initial Xpert MTB/XDR testing, of 531 specimens tested, 15 resulted in non-determinate results. There were 10 Error results, one Invalid result, and four No Result results. Therefore, the proportion of non-determinate results upon initial testing was 2.8%. The 15 specimens were retested, and 14 gave valid results. Only one of the 15 retested specimens resulted in an Error following its repeat test. Therefore, the proportion of non-determinate results following retesting was 0.2% (1/531).

In Penn-Nicholson 2021, upon initial Xpert MTB/XDR testing, of 709 specimens tested, 21 resulted in non-determinate results. Therefore, the proportion of non-determinate results upon initial testing was 3.0% (21/709). The 21 specimens were retested, and 19 gave valid results. Therefore, the proportion of non-determinate results following retesting was 0.3% (2/709).

One study reported Xpert MTB/XDR non-determinate results by smear status (Penn-Nicholson 2021). In this study, the proportion of Xpert MTB/XDR non-determinate results was 4.2% (9/216) in smear-negative specimens and 2.4% (12/491) in smear-positive specimens.

The phenotypic status of non-determinate results was not discernable for either study.

Xpert MTB/XDR INDETERMINATE results

Isoniazid resistance

Of 530 specimens tested, 498 specimens had an Xpert MTB/XDR MTB DETECTED result. Of these 498 specimens, two (0.4%) had indeterminate results for detection of resistance. By the pDST reference standard, of these two specimens, two (100%) were resistant and zero (0%) were susceptible (Omar 2020).

Of 709 specimens tested, 657 had an Xpert MTB/XDR MTB DETECTED result. Of these 657 specimens, two (0.3%) had indeterminate results for detection of resistance. None were indeterminate upon retesting (Penn-Nicholson 2021).

Fluoroquinolone resistance

Of 530 specimens tested, 498 specimens had an Xpert MTB/XDR MTB DETECTED result. Of these 498 specimens, four (0.8%) had indeterminate results for detection of resistance. By the pDST reference standard, of these four specimens, zero (0%) were resistant and four (100%) were susceptible (Omar 2020).

Of 709 specimens tested, 657 had an Xpert MTB/XDR MTB DETECTED result. Of these 657 specimens, nine (1.4%) had indeterminate results for detection of resistance. None were indeterminate upon retesting (Penn-Nicholson 2021).

Ethionamide resistance

Of 530 specimens tested, 498 specimens had an Xpert MTB/XDR MTB DETECTED result. Of these 498 specimens, none (0%) had an indeterminate result for detection of resistance (Omar 2020).

Of 709 specimens tested, 657 had an Xpert MTB/XDR MTB Detected result. Of these 657 specimens, one (0.2%) had an indeterminate result for detection of resistance. This specimen was no longer indeterminate upon retesting (Penn-Nicholson 2021).

Amikacin resistance

Of 530 specimens tested, 498 specimens had an Xpert MTB/XDR MTB DETECTED result. Of these 498 specimens, eight (1.6%) had indeterminate results for detection of resistance. By the pDST reference standard, of these eight specimens, zero (0%) were resistant and eight (100%) were susceptible (Omar 2020).

Of 709 specimens tested, 657 had an Xpert MTB/XDR MTB DETECTED result. Of these 657 specimens, 23 (3.5%) had indeterminate results for detection of resistance. One was indeterminate upon retesting (Penn-Nicholson 2021).

In Penn-Nicholson 2021, among specimens with results reported as Xpert MTB/XDR INDETERMINATE, we could not determine the proportion that were resistant or susceptible by the pDST reference standard.

Xpert MTB/XDR MTB NOT DETECTED

One study reported information about when Xpert MTB/XDR did not detect tuberculosis to begin with (missed cases) (Omar 2020).

Table. Summary of Xpert MTB/XDR MTB NOT DETECTED results by drug and drug susceptibility status

Drug	**Total pDST results**	**No. (%) Xpert MTB/XDR MTB NOT DETECTED**	**№** (%) resistant	**№** (%) susceptible
Isoniazid	512	32 (6.3%)	2 (6.3%)	30 (93.8%)
Fluoroquinolones	453	32 (7.1%)	1 (3.1%)	31 (96.9%)
Ethionamide	260	30 (11.5%)	2 (6.7%)	28 (93.3%)
Amikacin	445	32 (7.2%)	0 (0.0%)	32 (100.0%)

Abbreviaitons: **№**: number; **pDST:** phenotypic drug susceptibility testing.

Appendix 6. Xpert MTB/XDR for detection of resistance to kanamycin and capreomycin

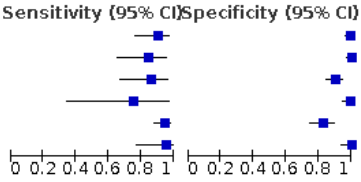
Figure 12

Figure 12. Forest plots of Xpert MTB/XDR sensitivity and specificity by direct testing for resistance to kanamycin and capreomycin by population and reference standard. Study in the forest plots refers to a study cohort within

a multicentre study. pDST = phenotypic drug resistance testing; TP = true positive; FP = false positive; FN = false negative; TN = true negative.

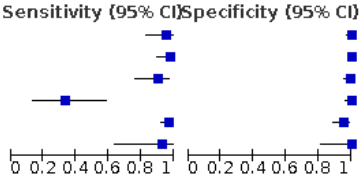
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	36	1	4	136	0.90 [0.76, 0.97]	0.99 [0.96, 1.00]		
Omar 2020 South Africa	22	0	4	140	0.85 [0.65, 0.96]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	24	14	4	135	0.86 [0.67, 0.96]	0.91 [0.85, 0.95]		
Penn-Nicholson 2021 India (New Delhi)	6	1	2	107	0.75 [0.35, 0.97]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 Moldova	111	19	7	93	0.94 [0.88, 0.98]	0.83 [0.75, 0.89]		
Penn-Nicholson 2021 South Africa	21	0	1	59	0.95 [0.77, 1.00]	1.00 [0.94, 1.00]		



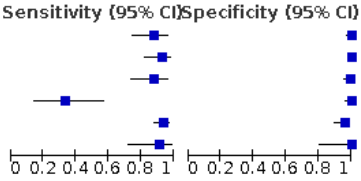
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	37	0	2	138	0.95 [0.83, 0.99]	1.00 [0.97, 1.00]		
Omar 2020 South Africa	51	0	1	234	0.98 [0.90, 1.00]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	36	1	4	120	0.90 [0.76, 0.97]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	6	0	12	91	0.33 [0.13, 0.59]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 Moldova	123	5	4	94	0.97 [0.92, 0.99]	0.95 [0.89, 0.98]		
Penn-Nicholson 2021 South Africa	12	0	1	18	0.92 [0.64, 1.00]	1.00 [0.81, 1.00]		



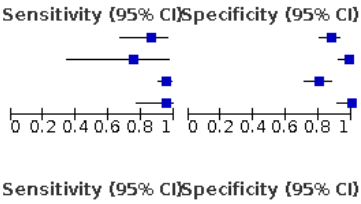
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, kanamycin, composite

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	37	0	5	141	0.88 [0.74, 0.96]	1.00 [0.97, 1.00]		
Omar 2020 South Africa	51	0	4	231	0.93 [0.82, 0.98]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	36	1	5	119	0.88 [0.74, 0.96]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	7	0	14	89	0.33 [0.15, 0.57]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 Moldova	126	4	9	89	0.93 [0.88, 0.97]	0.96 [0.89, 0.99]		
Penn-Nicholson 2021 South Africa	21	0	2	17	0.91 [0.72, 0.99]	1.00 [0.80, 1.00]		



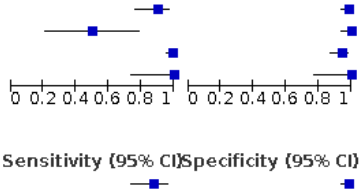
Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	24	14	4	102	0.86 [0.67, 0.96]	0.88 [0.81, 0.93]		
Penn-Nicholson 2021 India (New Delhi)	6	1	2	65	0.75 [0.35, 0.97]	0.98 [0.92, 1.00]		
Penn-Nicholson 2021 Moldova	109	19	5	79	0.96 [0.90, 0.99]	0.81 [0.71, 0.88]		
Penn-Nicholson 2021 South Africa	21	0	1	39	0.95 [0.77, 1.00]	1.00 [0.91, 1.00]		



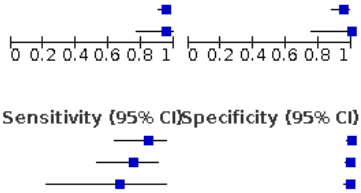
Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	36	1	4	91	0.90 [0.76, 0.97]	0.99 [0.94, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	6	0	6	54	0.50 [0.21, 0.79]	1.00 [0.93, 1.00]		
Penn-Nicholson 2021 Moldova	121	5	1	82	0.99 [0.96, 1.00]	0.94 [0.87, 0.98]		
Penn-Nicholson 2021 South Africa	12	0	0	14	1.00 [0.74, 1.00]	1.00 [0.77, 1.00]		



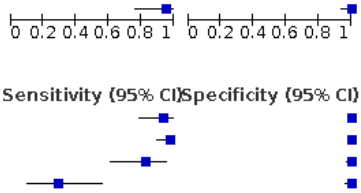
Xpert MTB/XDR, direct, with known rifampicin resistance, kanamycin, composite

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Penn-Nicholson 2021 India (Mumbai)	36	1	5	90	0.88 [0.74, 0.96]	0.99 [0.94, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	7	0	8	53	0.47 [0.21, 0.73]	1.00 [0.93, 1.00]		
Penn-Nicholson 2021 Moldova	124	4	6	77	0.95 [0.90, 0.98]	0.95 [0.88, 0.99]		
Penn-Nicholson 2021 South Africa	21	0	1	13	0.95 [0.77, 1.00]	1.00 [0.75, 1.00]		



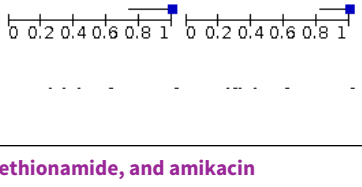
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, pDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 South Africa	21	0	4	142	0.84 [0.64, 0.95]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	18	1	6	153	0.75 [0.53, 0.90]	0.99 [0.96, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	4	1	2	109	0.67 [0.22, 0.96]	0.99 [0.95, 1.00]		
Penn-Nicholson 2021 Moldova	10	1	8	211	0.56 [0.31, 0.78]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	20	0	1	59	0.95 [0.76, 1.00]	1.00 [0.94, 1.00]		



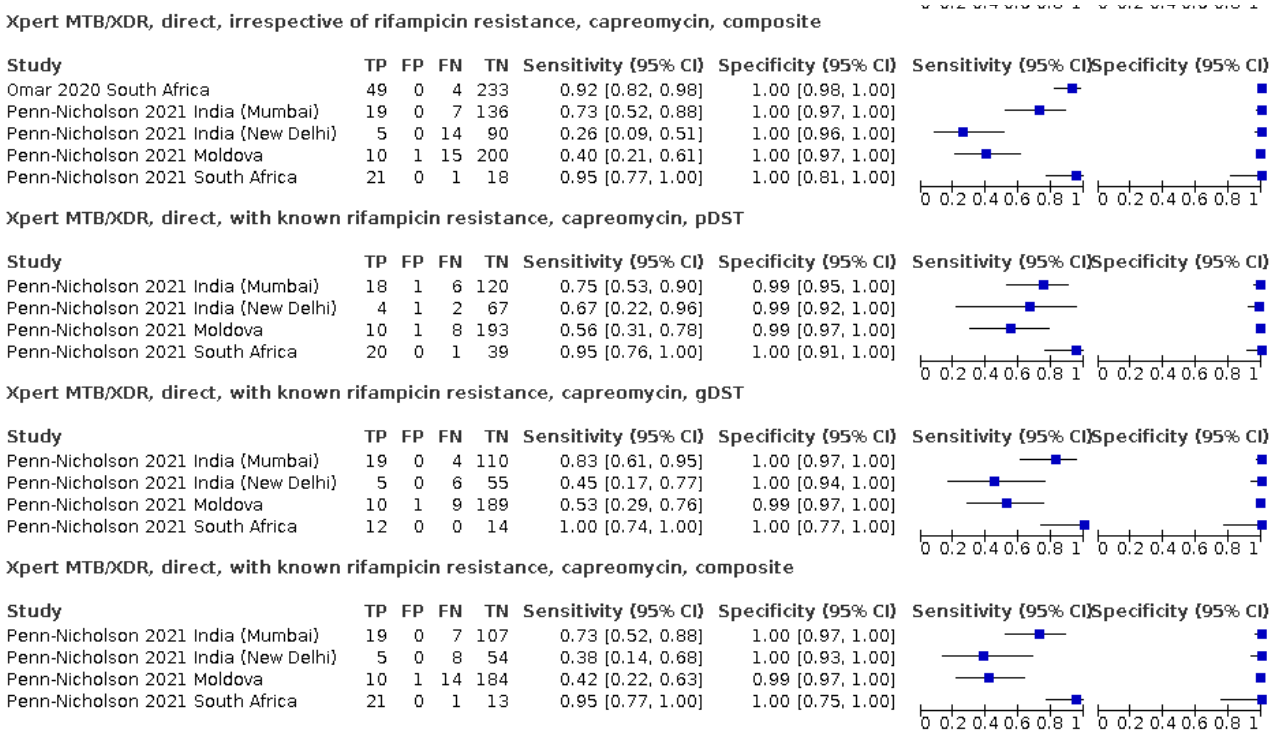
Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, gDST

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Omar 2020 China	29	0	2	146	0.94 [0.79, 0.99]	1.00 [0.98, 1.00]		
Omar 2020 South Africa	49	0	1	236	0.98 [0.89, 1.00]	1.00 [0.98, 1.00]		
Penn-Nicholson 2021 India (Mumbai)	19	0	4	139	0.83 [0.61, 0.95]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 India (New Delhi)	5	0	12	92	0.29 [0.10, 0.56]	1.00 [0.96, 1.00]		
Penn-Nicholson 2021 Moldova	10	1	10	205	0.50 [0.27, 0.73]	1.00 [0.97, 1.00]		
Penn-Nicholson 2021 South Africa	12	0	0	19	1.00 [0.74, 1.00]	1.00 [0.82, 1.00]		



Xpert MTB/XDR, direct, irrespective of rifampicin resistance, capreomycin, composite

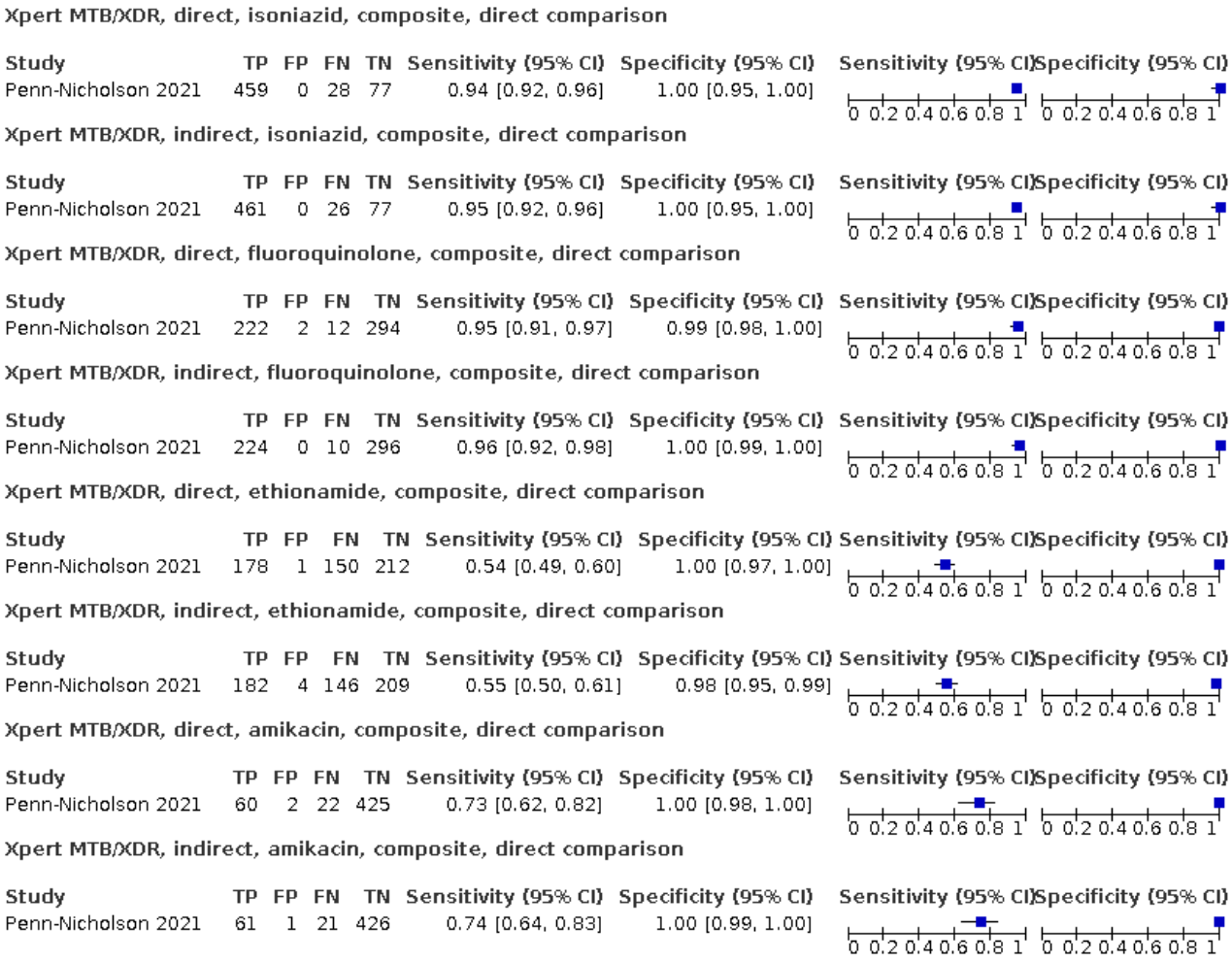
Figure 12. (Continued)



Appendix 7. Xpert MTB/XDR for detection of drug resistance, direct versus indirect testing

Figure 13

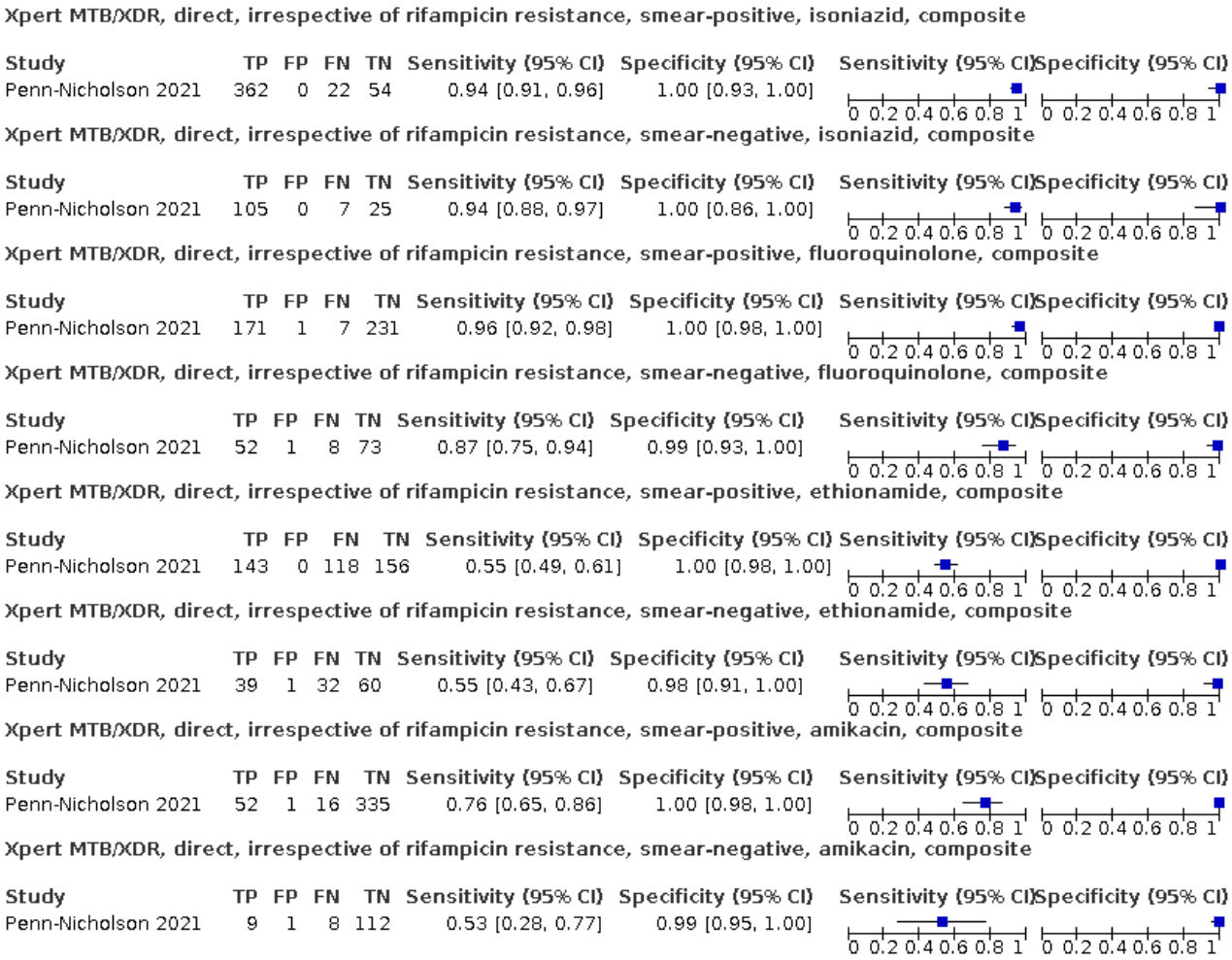
Figure 13. Forest plots of Xpert MTB/XDR sensitivity and specificity for resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin, testing on sputum (direct testing) versus testing on cultured isolates (indirect testing), composite reference standard. Data were reported for all study cohorts combined. TP = true positive; FP = false positive; FN = false negative; TN = true negative.



Appendix 8. Xpert MTB/XDR for detection of drug resistance by smear status

Figure 14

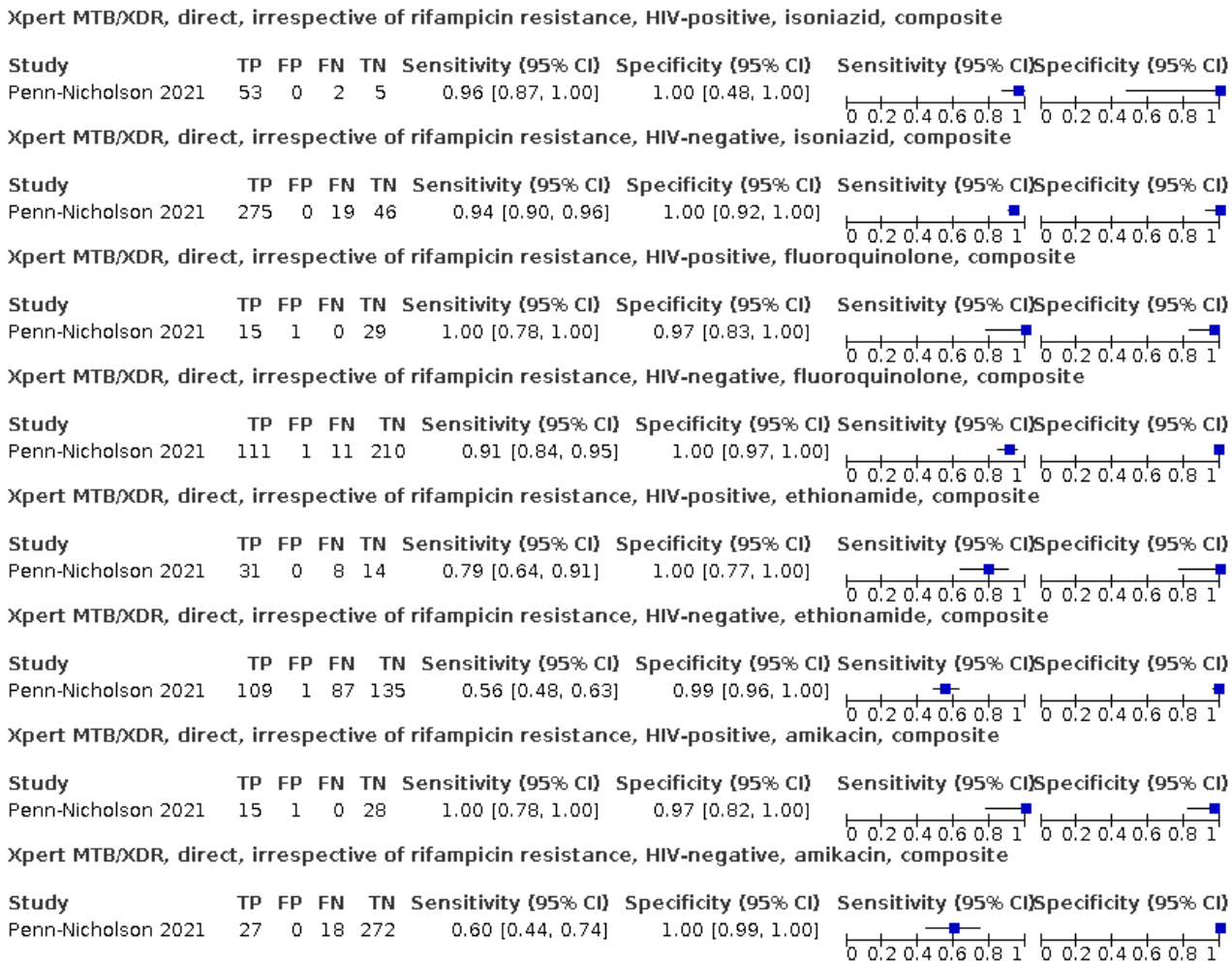
Figure 14. Forest plots of Xpert MTB/XDR sensitivity and specificity by direct testing for resistance to isoniazid, fluoroquinolone, ethionamide, and amikacin, by smear status, composite reference standard. Data were reported for all study cohorts combined. TP = true positive; FP = false positive; FN = false negative; TN = true negative.



Appendix 9. Xpert MTB/XDR for detection of drug resistance by HIV status

Figure 15

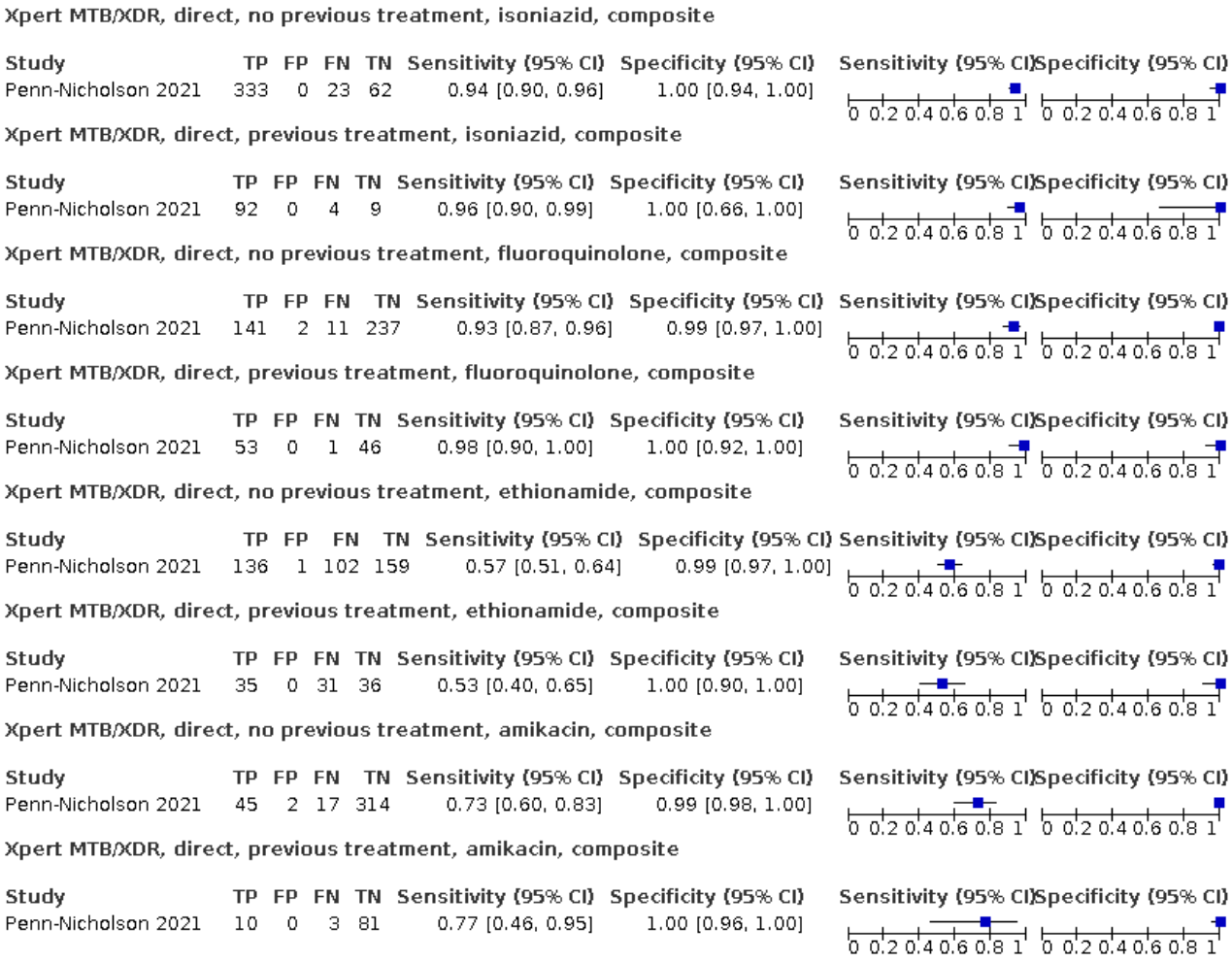
Figure 15. Forest plots of Xpert MTB/XDR sensitivity and specificity by direct testing for resistance to isoniazid, fluoroquinolone, ethionamide, and amikacin in HIV-positive and HIV-negative people, composite reference standard. Data were reported for all study cohorts combined. TP = true positive; FP = false positive; FN = false negative; TN = true negative.



Appendix 10. Xpert MTB/XDR for detection of drug resistance in in people with and without previous treatment for tuberculosis

Figure 16

Figure 16. Forest plots of Xpert MTB/XDR sensitivity and specificity by direct testing for resistance to isoniazid, fluoroquinolone, ethionamide, and amikacin in people with and without previous treatment for tuberculosis, composite reference standard. Data were reported for all study cohorts combined. TP = true positive; FP = false positive; FN = false negative; TN = true negative.



Appendix 11. Critical concentrations and clinical breakpoints for medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant tuberculosis

Drug groups	Drug	LJ	7H10	7H11	MGIT
First-line drugs	Isoniazid	0.2	0.2	0.2	0.1
Fluoroquinolones	Levofloxacin (CC)	2.0	1.0	—	1.0
	Moxifloxacin (CC)	1.0	0.5	0.5	0.25
	Moxifloxacin (CB)	—	2.0	—	1.0
	Gatifloxacin (CC)	0.5	—	—	0.25

(Continued)

Second-line injectable agents	Amikacin	30.0	2.0	—	1.0
	Capreomycin	40.0	4.0	—	2.5
	Kanamycin	30.0	4.0	—	2.5
Other second-line agents	Ethionamide	40.0	5.0	10	5.0

Abbreviations: **LJ:** Löwenstein–Jensen medium; **MGIT:** Mycobacteria Growth Indicator Tube.

Table adapted from WHO Critical Concentrations 2018 and WHO Critical Concentrations 2021.

All concentrations are in mg/L and apply to the proportion method with 1% as the critical proportion. Unless otherwise stated, they are critical concentrations (CCs), as opposed to clinical breakpoints (CBs). The clinical breakpoint is used to guide individual clinical decisions in patient treatment.

MGIT is proposed as the reference method for performing DST for second-line tuberculosis agents.

HISTORY

Protocol first published: Issue 6, 2021

CONTRIBUTIONS OF AUTHORS

SP, GRD, MDV, MC, KRS, and GT drafted the review.

MC and KRS wrote the statistical analysis section.

All review authors (SP, GRD, MC, MDV, SGS, RW, KRS, and GT) read and approved the final review draft.

DECLARATIONS OF INTEREST

SP received funding from USAID, administered by the World Health Organization (WHO) Global Tuberculosis Programme, Switzerland.

KRS received funding from USAID, administered by the WHO Global Tuberculosis Programme, Switzerland. In addition, she has received financial support from Cochrane Infectious Diseases (UK), McGill University (Canada), Baylor College of Medicine (USA), Maastricht University (the Netherlands), and the WHO Global Tuberculosis Programme (Switzerland) for the preparation of related systematic reviews and educational materials; consultancy fees from FIND, Switzerland (for the preparation of systematic reviews and GRADE tables); consultancy fees from Stellenbosch University, South Africa (for guidance on evidence syntheses), and honoraria, and travel support to attend WHO guideline meetings.

GRD received funding from USAID, administered by the WHO Global Tuberculosis Programme, Switzerland.

MC has no known conflicts of interest.

MDV is employed by the Foundation for Innovative New Diagnostics (FIND). FIND has conducted studies and published on Xpert MTB/RIF as part of a collaborative project between FIND, a Swiss non-profit, Cepheid, a US company, and academic partners. The product arising through this partnership was developed under a contract that obligated FIND to pay for development costs and trial costs and Cepheid to make the test available at specified preferential pricing to the public sector in low- and middle-income countries. In addition, FIND conducted studies for the Xpert MTB/RIF Ultra assay, which have also been published.

SGS was employed by the Foundation for Innovative New Diagnostics (FIND) while conducting the review. FIND has conducted studies and published on Xpert MTB/XDR and Xpert MTB/RIF as part of a collaborative project between FIND, a Swiss non-profit, Cepheid, a US company, and academic partners. Regarding Xpert MTB/RIF, the product developed through this partnership was developed under a contract that obligated FIND to pay for development costs and trial costs and Cepheid to make the test available at specified preferential pricing to the public sector in low- and middle-income countries. In addition, FIND conducted studies for the Xpert MTB/RIF Ultra assay, which have also been published.

RW has no known conflicts of interest.

GT received funding from USAID, administered by the WHO Global Tuberculosis Programme, Switzerland. In addition, he has received in-kind research consumable donations provided to employer by Cepheid to work on Xpert MTB/RIF and Xpert MTB/RIF Ultra (not Xpert MTB/

XDR) for diagnostic accuracy evaluations for tuberculosis detection. He is the group Principal Investigator for this work. Cepheid has also loaned instruments to conduct these studies. These studies are on different products to those potentially considered for inclusion in this Cochrane Review.

SOURCES OF SUPPORT

Internal sources

- Liverpool School of Tropical Medicine, UK

External sources

- Foreign, Commonwealth and Development Office (FCDO), UK

  Project number 300342-104
- World Health Organization Global Tuberculosis Programme, Switzerland

  Registration number 2020/1048818-0; purchase order 202582841

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Clinical pathway

- Scenario D. Xpert MTB/XDR used for detection of drug resistance in people being treated for pulmonary tuberculosis. We did not identify studies that assessed this role.

Objectives

- A secondary objective was to compare the diagnostic accuracy of Xpert MTB/XDR by direct testing (whereby Xpert MTB/XDR is tested directly on a sputum specimen) versus indirect testing (whereby Xpert MTB/XDR is run on an *M tuberculosis* isolate grown from culture). Our plan was to perform these analyses for those studies that made direct comparisons between test evaluations with the same participants by adding a covariate for the type of testing to the model (Takwoingi 2013). However, we only identified one study that compared Xpert MTB/XDR accuracy by direct and indirect testing. Instead, we narratively described these analyses and presented results in forest plots.

Methods

- Types of studies. We identified one report at a conference and included this report in the review.

- Conflicts of interest. We had planned to assess conflicts of interest using the Tool for Addressing Conflicts of Interest in Trials (TACIT) (Lundh 2020). However, this tool was not available while we performed the review. We extracted information about industry sponsorship and performed sensitivity analyses by repeating the meta-analyses and excluding the study sponsored by the manufacturer.

Statistical analyses

- Regarding fluoroquinolone resistance, we had planned to take the following approach. If multiple fluoroquinolones were tested by pDST and at least one was resistant, the patient would be classified as resistant. If no resistant results occurred and a least one pDST susceptible result was present, that patient would be classified as susceptible. However, none of the included studies tested more than one fluoroquinolone by pDST.

- Due to little observed variability in specificity and in the volume of analyses, we chose to present only forest plots, as such plots were more informative than corresponding summary receiver operator characteristics (SROC) plots.

- We did not perform a meta-analysis for Xpert MTB/XDR for pulmonary tuberculosis detection as heterogeneity, in terms of both characteristics of included participants and observed specificity values, would have rendered the summary sensitivity and specificity estimates uninterpretable and potentially misleading.

Inconclusive results

- We performed meta-analyses to estimate the summary proportion of non-determinate and indeterminate results using the metaprop command in Stata (Version 14) (Stata).

- We wrote in the protocol that we would extract data on discrepant analysis, where in each study, gene sequencing was applied only to resolve discordant
Xpert MTB/XDR-pDST results. However, the study cohorts evaluated Xpert MTB/XDR using both pDST and gDST as reference standards and we did not characterize discordant results further.

Investigations of heterogeneity

We had planned to explore the possible influence of the pre-specified categorical covariates, listed below, by adding these covariates to the meta-analysis models. However, data were insufficient to perform these analyses. Had we performed these analyses, we would have assessed the significance of the difference in test accuracy according to each covariate by performing a likelihood ratio test comparing models with and without covariate terms.

For detection of pulmonary tuberculosis, we had planned to investigate the following potential sources of heterogeneity.

- Smear status, smear positive or negative (we described narratively).
- HIV status, positive or negative.
- Previous tuberculosis treatment, previous treatment or no previous treatment. We changed 'History of tuberculosis treatment' (in the protocol) to 'previous tuberculosis treatment' (in the review).
- Treatment status, no treatment or currently receiving treatment.
- Treatment response status, culture conversion, yes or no.

For detection of drug resistance, we investigated the following potential sources of heterogeneity.

- Type of reference standard.
- Smear status, positive or negative (we described narratively).
- HIV status, positive or negative (we described narratively).
- Previous tuberculosis treatment, previous treatment or no previous treatment (we described narratively).

In addition, we had planned to investigate specific drugs (e.g. ofloxacin or moxifloxacin) used in the pDST reference standard for determining fluoroquinolone resistance; however data were not available to do this.

We had also planned to investigate 'Was the WHO-recommended critical drug concentration used for the pDST reference standard (WHO Critical Concentrations 2018; WHO Critical Concentrations 2021), yes or no? However, the included studies used the currently recommended concentration for each drug.

Sensitivity analyses

- For Xpert MTB/XDR for detection of drug resistance against the pDST reference standard, we had planned to perform sensitivity analyses for studies meeting the QUADAS-2 criteria listed below. However, there were only two studies in the review and the sensitivity analyses are less meaningful with few studies.

1. Was a consecutive or random sample of participants/specimens enrolled?
2. Were the reference standard results interpreted without knowledge of the results of the index test results?
3. Was the test applied in the manner recommended by the manufacturer (index test domain, low concern about applicability)?

Questions numbered 2 and 3 were satisfied by all studies.

- For Xpert MTB/XDR for detection of resistance to isoniazid and fluoroquinolones in people irrespective of rifampicin resistance, we performed sensitivity analyses by repeating the meta-analyses and excluding the study (reporting on two study cohorts) sponsored by the manufacturer. For detection of resistance to ethionamide and amikacin in people with known rifampicin resistance, we did not perform sensitivity analyses because the main analyses included only one study (reporting on four study cohorts), which was not sponsored by the manufacturer.

INDEX TERMS

Medical Subject Headings (MeSH)

Amikacin [pharmacology] [therapeutic use]; *Antibiotics, Antitubercular [pharmacology] [therapeutic use]; Drug Resistance, Bacterial [genetics]; Ethionamide [pharmacology] [therapeutic use]; Fluoroquinolones [pharmacology] [therapeutic use]; Isoniazid [pharmacology] [therapeutic use]; Microbial Sensitivity Tests; *Mycobacterium tuberculosis [genetics]; Rifampin [pharmacology] [therapeutic use]; Sensitivity and Specificity; *Tuberculosis, Lymph Node [diagnosis]; *Tuberculosis, Multidrug-Resistant [diagnosis] [drug therapy]; *Tuberculosis, Pulmonary [diagnosis] [drug therapy]

MeSH check words

Adult; Humans

Chapter 5

Melting the *eis*: Non-detection of kanamycin resistance markers by routine diagnostic tests and identification of new *eis* promoter variants

Ley, S.D*., **Pillay, S***., Streicher, E.M., van der Heijden, Y.F., Sirgel, F., Derendinger, B., de Kock, M., Gagneux, S., Warren, R.M., Theron, G. and de Vos, M., 2021. Melting the *eis*: Non-detection of Kanamycin Resistance Markers by Routine Diagnostic Tests and Identification of New *eis* Promoter Variants. *Antimicrobial agents and chemotherapy*, *65*(7), pp. e02502-20.
doi: 10.1128/AAC.02502-20

****Contributed equally***

Published status: published [*note Supplementary Material is in Appendix V*]

Key findings:

We found that AMK alone cannot be used as a surrogate marker for KAN resistance as low-level drug resistance can be missed leading to ineffective treatment regimen. In instances where cases were missed by MTBDR*sl* but detected by sequencing, the mutations identified did not form part of the *eis* promoter region targeted by MTBDR*sl* and hence were associated with minimal confidence to predict resistance. Most common resistance markers identified as circulating in the Western Cape were -12 C > T and -10 G > A.

Candidate's role:

Assisted in clinical data collection, performing and running of all tests molecular and phenotypic tests for study, data interpretation, data analysis and preparation of manuscript.

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MECHANISMS OF RESISTANCE

Melting the *eis*: Nondetection of Kanamycin Resistance Markers by Routine Diagnostic Tests and Identification of New *eis* Promoter Variants

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[g]The Aurum Institute, Johannesburg, South Africa

Serej D. Ley and Samantha Pillay contributed equally to this work. Serej D. Ley wrote the manuscript.

ABSTRACT *Eis* promoter mutations can confer reduced *Mycobacterium tuberculosis* kanamycin susceptibility. GenoType MTBDR*sl,* a widely used assay evaluating this region, wrongly classified 17/410 isolates as *eis* promoter wild type. Six out of seventeen isolates harbored mutations known to confer kanamycin resistance, and the remainder harbored either novel *eis* promoter mutations (7/11) or disputed mutations (4/11). GenoType MTBDR*sl* can miss established and new variants that cause reduced susceptibility. These data highlight the importance of reflex phenotypic kanamycin testing.

KEYWORDS *Mycobacterium tuberculosis*, extensive drug resistance, second-line injectables

The drugs amikacin (AMK), kanamycin (KAN), and capreomycin (CAP) have been part of the recommended second-line antituberculosis treatment since the 1970s. The most common genetic resistance marker for these drugs is a single-nucleotide variant (SNV) at position 1401 of the rRNA 16S encoding gene, *rrs* (1, 2). An alternative mechanism conferring (low-level) resistance to KAN includes SNVs in the promoter region of *eis* (Rv2416c) (Fig. S1 in the supplemental material) (3). Amikacin is often used as a surrogate for KAN phenotypic drug-susceptibility testing (pDST) based on the assumption of complete cross-resistance. Similarly, if the strain was susceptible to AMK, KAN susceptibility was assumed, and low-level KAN resistance was potentially overlooked. Until 2017, *eis* promoter mutations were not routinely tested for in South Africa, leading to undetected resistance and less effective treatment.

This study investigated the presence, type, and detection of *eis* promoter mutations in clinical *Mycobacterium tuberculosis* isolates collected in South Africa using the line probe assay GenoType MTBDR*sl* VER 2.0 (MTBDR*sl*; Hain Lifescience, Germany), Sanger sequencing, and whole-genome sequencing (WGS).

Two unique sample sets were analyzed. Sample set 1 consisted of 951 *M. tuberculosis* isolates from Xpert MTB/RIF (Cepheid) rifampin (RIF)-resistant specimens from South Africa that were collected between June 2016 and June 2017 as part of routine diagnostics by the National Health Laboratory Services, Cape Town. These isolates

Citation Ley SD, Pillay S, Streicher EM, van der Heijden YF, Sirgel F, Derendinger B, de Kock M, Gagneux S, Warren RM, Theron G, de Vos M. 2021. Melting the *eis*: nondetection of kanamycin resistance markers by routine diagnostic tests and identification of new *eis* promoter variants. Antimicrob Agents Chemother 65:e02502-20. https://doi.org/10.1128/AAC.02502-20.

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were analyzed using the GenoType MTBDR*plus* assay (detecting resistance against RIF and/or isoniazid [INH]) and MTBDR*sl* (4). To determine the number of *eis* promoter mutations missed by MTBDR*sl*, isolates that were phenotypically susceptible to AMK, wild type (WT) for *eis* promoter and *rrs* by MTBDR*sl*, and available in the Stellenbosch University biobank ($n = 398$) were Sanger sequenced (i.e., the region covering 222 bp upstream of the transcriptional start site of the *eis* gene, subsequently referred to as "*eis* promoter region"; Fig. S1). Sample set 2 consisted of a convenience sample of 2,863 whole-genome sequences of clinical *M. tuberculosis* isolates derived from sputum samples collected between 1993 and 2018 and sequenced as part of different research projects (5–9). These sequences were screened *in silico* for *eis* promoter mutations (genome positions 2715332 to 2715582 of *M. tuberculosis* H37Rv; GenBank accession no. AL123456). Of those isolates with *eis* promoter mutations, representatives for each (combination of) mutation(s) were selected for further analyses with targeted Sanger sequencing, MTBDR*sl*, and pDST. An overview of the study workflow for both sample sets is given in Fig. 1.

For isolates of sample set 1, PCR amplification—and subsequent Sanger sequencing—was conducted on thermal lysates, whereas purified DNA was used for sample set 2. Briefly, the PCR mixture contained the following final concentrations: 1× HotStartTaq Plus master mix (Qiagen, San Diego, CA, USA), 500 nM each primer (forward, 5′-CCATGGGACCGGTACTTGCT-3′; reverse, 5′-ACTTCACCAGGCACCGTCAA-3′), and 1× SYTO 9 green fluorescent nucleic acid stain (Thermo Fisher Scientific). As a template, 1 μl of thermal lysate (sample set 1) or purified DNA (sample set 2) was added to the reaction mixture. Amplification of the *eis* promoter region of the selected isolates was carried out using a CFX96TM real-time system C1000 Touch thermal cycler (Bio-Rad) running the following thermocycling protocol: initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 1 min, annealing at 62°C for 1 min, and elongation at 72°C for 1 min, followed by a final elongation at 72°C for 10 min. Successful amplification was confirmed by a high-resolution melt from 80°C to 95°C with an increment of 0.5°C, each increment temperature held for 5 s. Successfully amplified PCR products were sent to the Central DNA Sequencing Facilities of Stellenbosch University for targeted Sanger sequencing using the forward PCR primer. The MTBDR*sl* assay was conducted according to the manufacturer's protocol using the same DNA used for WGS. The assay defines specific banding patterns (i.e., presence or absence of WT and MUT bands) for the following most common *eis* promoter mutations: −37 G > T, −14 T > C, −12 T > C, −10 G > A, and −2 A > C. In this study, these mutations were therefore defined as "detectable by MTBDR*sl*." However, only the mutation −14 T > C is explicitly detected by a MUT probe (4). Other known *eis* promoter mutations (Fig. S1) may also cause one of the WT bands to fail but appear not to have been validated by the manufacturer. In this study, these mutations were therefore defined as "not included in MTBDR*sl*." Phenotypic DST was performed on all isolates using solid Löwenstein-Jensen medium according to the 1% proportion method at clinical breakpoints of 0.2 μg/ml for INH, 40.0 μg/ml for RIF, 30 μg/ml for AMK, and 2 μg/ml for ofloxacin (10, 11). MICs for KAN were subsequently determined for isolates with an *eis* promoter mutation missed by the MTBDR*sl* (sample set 1) and for representatives of each additional (combination of) *eis* promoter mutation(s) (sample set 2). These MICs were done using 2-fold serial dilutions ranging from 10.0 μg/ml to 1.25 μg/ml using the Bactec MGIT 960 system with the TB eXiST module of the EpiCentre software (12). Susceptibility to KAN was determined using the 1% proportion method based on a clinical breakpoint of 2.5 μg/ml. For WGS, each isolate was recultured from culture stocks, and DNA was extracted as previously described (13). Whole-genome sequencing libraries were prepared according to the manufacturer's protocol (Illumina, Inc, San Diego, CA), and sequenced on an Illumina HiSeq or Illumina NextGen Seq platform. The resulting sequencing reads were mapped to the *M. tuberculosis* H37*Rv* reference strain (GenBank accession no. AL123456). Variant calling and annotation were conducted using a within-house pipeline as previously described (6). The genotypic

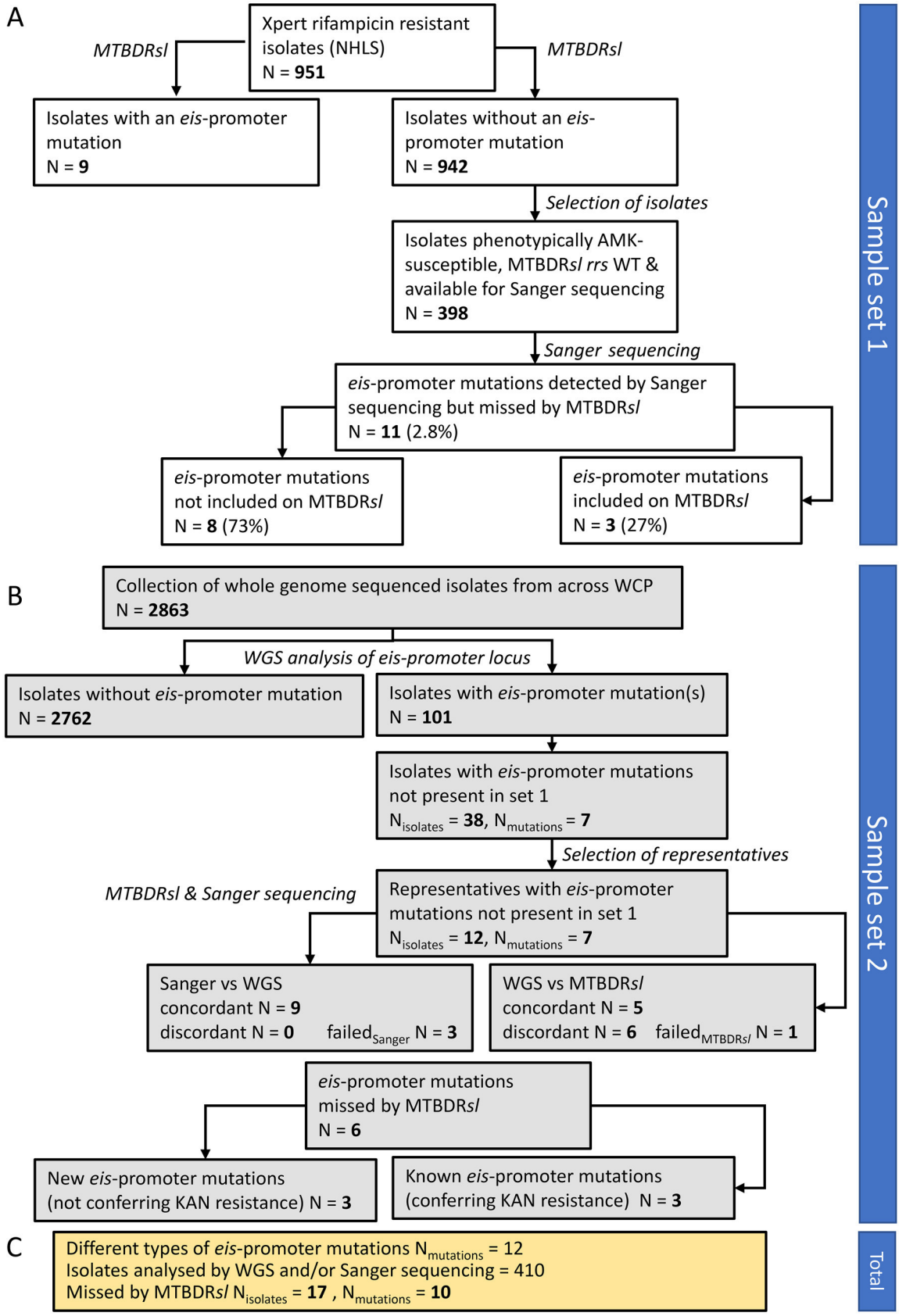

FIG 1 Workflow diagram. (A) Workflow and number of isolates included in each step for sample set 1. (B) Workflow and number of isolates included in each step for sample set 2. (C) Total number of *eis* promoter mutations detected and missed by routine MTBDR*sl* across both sample sets. NHLS, National Health Laboratory Services; WCP, Western Cape Province; WGS, whole-genome sequencing.

drug resistance profile of each isolate was determined using markers defined by Miotto et al. and Coll et al. (14, 15). Raw sequencing reads of the isolates listed in Tables 1 and 2 have been deposited at the European Nucleotide Archive (ENA accession no. PRJEB41458). Additional details for all methods are described in Supplemental File 1.

In sample set 1, *eis* promoter mutations were detected in 9/951 (0.95%) isolates by MTBDR*sl*. These isolates were phenotypically AMK susceptible with no *rrs* 1401 mutation (Table 3). From the 951 isolates, 398 were phenotypically AMK susceptible, *eis* promoter, and *rrs* WT, based on the MTBDR*sl*, and available in the biobank (Fig. 2). Sanger sequencing revealed that 11/398 (2.8%) isolates classified as *eis* promoter and *rrs* WT by routine diagnostics harbored at least one *eis* promoter mutation (Table 4). Three of those 11 carried the known KAN resistance markers 12 C > T and −10 G > A and should have been detected by the MTBDR*sl*. As Sanger sequencing revealed no heteroresistance for these isolates, it is unlikely that MTBDR*sl* missed this mutation because of the detection limit. Two of the three were phenotypically resistant to KAN (Table 4). The failure to detect these mutations therefore falsely classified the isolates as KAN susceptible, impacting the patient's treatment options. The third isolate was phenotypically susceptible to KAN despite carrying an *eis* promoter mutation, −12 C > T. Previous studies also reported variable KAN pDST results for this mutation, including KAN susceptibility (2, 16–19). The *eis* promoter mutations of the remaining eight isolates could potentially have been detected through failing WT bands but were missed by the MTBDR*sl*. These mutations are either considered not to confer KAN resistance ($n = 4$; *eis* promoter mutation, −10 G > C) or undescribed ($n = 4$; *eis* promoter mutations, −50 T > C and −100 C > T) (Table 4). The latter are unlikely to affect the transcription of the *eis* gene, as they are located upstream of the usual promoter area. Since none of these mutations elevated the KAN MIC, patient treatment should not have been affected despite undetected mutations.

The screening of 2,863 WGS of clinical *M. tuberculosis* isolates (sample set 2) identified 101 isolates from 69 patients that carried at least one mutation in the *eis* promoter region (Tables S1 and S2). Seven mutations (−6 G > A, −8 C > A, −14 C > T, −15 G > A, −32 C > T, −37 G > T, −104 G > A) were not present in sample set 1. The mutations −6 G > A, −32 C > T, and −104 G > A were previously undescribed. More in-depth analyses of 12 representative isolates revealed that 6 (50%) were wrongly classified as *eis* promoter WT by the MTBDR*sl*, 4 with *eis* promoter mutations not included in the MTBDR*sl* and 2 isolates with mutations detectable by MTBDR*sl* (−37 G > T; −10 G > A and −15 C > G) (Tables 1 and 2). The reasons for the failure of detecting these mutations remain unclear. However, the assay failed to detect the −10 G > A mutation when in combination with −15 C > G in all four isolates with that *eis* promoter combination (Table S2), even when the majority of the WGS reads belonged to the *M. tuberculosis* subpopulation with the −10 G > A mutation (i.e., 63% of reads versus 36%; Tables 1 and 2). It is therefore unlikely that the mutant subpopulation was missed due to the detection limit of the assay. As all other isolates with different combinations of *eis* promoter mutations were correctly identified as mutant, the presence of more than one SNV in the same isolate does not generally seem to affect the assay's performance. For one isolate with three *eis* promoter mutations (−12 C > T, −14 C > T, and −37 G > T), MTBDR*sl* correctly identified all mutations, but the result would not have been properly interpretable without the additional information of WGS and Sanger sequencing.

The phenotypic and genotypic results were partially discrepant (Tables 1 and 2): three of six isolates misclassified as WT carried an *eis* promoter mutation known to confer low-level KAN resistance (−8 C > A, −37 G > T, and −10 G > A) and were thereby falsely classified as KAN susceptible. At the time these isolates were collected, the routine diagnostic algorithm did not yet include MTBDR*sl* but only pDST. All three isolates were phenotypically AMK resistant, which, following the national treatment guidelines, would have led to the exclusion of KAN from the treatment regimen for those patients. An isolate with *eis* promoter mutation −32 C > T was phenotypically KAN susceptible, yet intermediate growth (<1%) was observed at all drug concentrations measured (1.25, 2.5, 5.0, and 10.0 μg/ml). The latter is usually an indication of heteroresistance with an underlying resistant *M. tuberculosis*

TABLE 1 Genotypic drug susceptibility testing results of selected representative isolates[b]

Patient	Isolate no.	Collection yr	WGS *eis* promoter mutation (% of reads)	WGS *rrs* 1401 mutation (% of reads)	Sanger sequencing *eis* promoter	MTBDR*sl* result *eis* promoter	MTBDR*sl eis* result interpretation	MTBDR*sl* result *rrs*	MTBDR*sl rrs* result interpretation	WGS vs MTBDR*sl eis* promoter	Lineage
P3	WGS-3	2009	−6 G > A (17)	WT	−6 G > K	All WT bands present, no MUT band	WT	WT1 and WT2 present, MUT1 present	Heteroresistance, *rrs* 1401 A > G and WT	Discrepant (not detectable by *sl*)	2.2
P14	WGS-22	2007	−32 C > T (91)	WT	−32 C > Y	All WT bands present, no MUT band	WT	WT1 and WT2 present, no MUT band	WT	Discrepant (not detectable by *sl*)	4.1.1.3
	WGS-22	2007	NA	NA	No *eis* promoter mutation detected (additional Sanger sequencing of *rrs* locus found *rrs* 1401A > G)	All WT bands present, no MUT band	WT	WT1 missing, MUT1 present	*rrs* 1401 A > G	NA	NA
P17	WGS-26	2008	−10 G > A (63) and −15 C > G (36)	WT	−10 G > R and −15 C > S	All WT bands present, no MUT band	WT	WT1 and WT2 present, no MUT band	WT	Discrepant (detectable by *sl*)	2.2
P19	WGS-30	2009	−14 C > T (17)	*rrs* 1401 A > G (21)	Failed	Failed	NA	Failed	NA	NA	2.2
P27	WGS-48	2014	−14 C > T (67)	WT	−14 C > T	WT2 missing, MUT1 present	*eis* promoter mutation −14	WT1 and WT2 present, no MUT band	WT	Concordant	4.1.1.3
P29	WGS-50	2010	−37 G > T (22)	*rrs* 1401 A > G (35)	−37 G > K	All WT bands present, no MUT band	WT	WT1 and WT2 present, MUT1 present	Heteroresistance, *rrs* 1401 A > G and WT	Discrepant (detectable by *sl*)	2.2
P31	WGS-54	2010	−12 C > T (18) and −14 C > T (9)[a] and −37 G > T (60)	WT	−12 C > Y and −14 C > Y[a] and −37 G > K	WT3 present; WT1 and WT2 weakly present, MUT present	Heteroresistance, *eis* promoter mutations −12 or −10, −14, −37 and WT mixed (not interpretable without additional information)	WT1 and WT2 present, no MUT band	WT	Concordant	4.9
P34	WGS-58	2012	−14 C > T (11) and −37 G > T (11)	*rrs* 1401 A > G (53)	−14 C > Y[a] and −37 G > K[a]	All WT bands present and MUT band present	Heteroresistance, *eis* promoter mutation −14 and WT	WT1 and WT2 present, MUT1 present	Heteroresistance, *rrs* 1401 A > G and WT	Concordant	2.2
P40	WGS-71	2012	−14 C > T (45)	*rrs* 1401 A > G (8)	−14 C > Y	All WT bands present and MUT band present	Heteroresistance, *eis* promoter mutation −14 and WT	WT1 and WT2 present, MUT1 present	Heteroresistance, *rrs* 1401 A > G and WT	Concordant	2.2
P40	WGS-72	2012	−14 C > T (39) and −10 G > A (12)	*rrs* 1401 A > G (41)	Failed	All WT bands present and MUT band present	Heteroresistance, *eis* promoter mutation −14 and WT	WT1 (weak) and WT2 present, MUT1 present	Heteroresistance, *rrs* 1401 A > G and WT	Concordant	2.2
P47	WGS-79	2015	−8 C > A (93)	WT	Failed	All WT bands present and MUT band present	WT	WT1 and WT2 present, no MUT band	WT	Discrepant (not detectable by *sl*)	2.2
P65	WGS-97	2015	−104 G > A (62)	WT	−104 G > R	All WT bands present, no MUT band	WT	WT1 and WT2 present, no MUT band	WT	Discrepant (not detectable by *sl*)	4.9
Summary				7/12 WT, 5/12 *rrs* 1401 A > G and WT	9/12 confirmed WGS, 3/12 failed	6/12 WT, 1/12 *eis* promoter −14 MUT, 3/12 *eis* promoter −14 MUT and WT, 1/12 combination of *eis* promoter mutations, 1/12 NA		5/12 WT, 5/12 *rrs* 1401 and WT, 1/12 1× WT, 1× *rrs* 1401 (P14), 1/12 NA		5/12 concordant, 4/12 discrepant (not detectable by *sl*), 2/12 discrepant (detectable by *sl*), 1/12 no result	8/12 lineage 2.2, 2/12 lineage 4.9, 2/12 lineage 4.1.1.3

[a]Difficult to distinguish from background noise.

[b]Changes in *rrs* or *eis* promoter are indicated as nucleotide changes using the IUPAC nucleotide code. WGS, whole-genome sequencing; *sl*, MTBDR*sl* assay; WT, wild type; MUT, mutant; NA, not applicable.

TABLE 2 Phenotypic drug susceptibility testing results of selected representative isolates[b]

Patient	Isolate No.	Collection yr	Amikacin pDST result (routine diagnostics)	gDST vs pDST (routine diagnostics) of AMK resistance	Amikacin pDST result (repeat by SU)	Kanamycin pDST result (SU)	Kanamycin MIC (μg/ml)
P3	WGS-3	2009	S	Discrepant	ND	Failed to regrow	NA
P14	WGS-22	2007	R	Discrepant	R	S	1.25 and intermediate growth at all measured concentrations
	WGS-22	2007	R[a]	Concordant[a]	ND	R	>10
P17	WGS-26	2008	R	Discrepant	S	R	>10
P19	WGS-30	2009	R	Concordant	R	R	>10
P27	WGS-48	2014	S	Concordant	ND	Failed to regrow	NA
P29	WGS-50	2010	R	Concordant	ND	R	>10
P31	WGS-54	2010	R	Discrepant	ND	R	10
P34	WGS-58	2012	R	Concordant	R	R	>10
P40	WGS-71	2012	R	Concordant	ND	R	>10
P40	WGS-72	2012	S	Concordant	ND	R	>10
P47	WGS-79	2015	R	Discrepant	ND	R	>10
P65	WGS-97	2015	S	Concordant	S	S	2.5
Summary			8/12 R, 4/12 S	7/12 concordant, 5/12 discrepant	7/12 ND, 4/12 confirmed DST at diagnosis, 1/12 discrepant to diagnosis	8/12 R, 2/12 S, 2/12 failed	7/12 > 10, 1/12 10, 1/12 2.5, 1/12 1.25 and intermediate growth, 2/12 failed

[a]Result from the original diagnostic isolate.

[b]Changes in *rrs* or *eis* promoter are indicated as nucleotide changes using the IUPAC nucleotide code. SU, Stellenbosch University; WGS, whole-genome sequencing; gDST, genotypic drug susceptibility testing; pDST, phenotypic drug susceptibility testing; AMK, amikacin; R, resistant; S, susceptible; ND, not done; NA, not applicable.

TABLE 3 *eis* promoter mutations detected in sample set 1 by the MTBDR*sl* assay as part of routine diagnostics[a]

Patient	Isolate	MTBDR*sl* result *eis* promoter (banding pattern)	*eis* promoter mutation	AMK pDST result
Pa-1	NHLS-1	WT2 and MUT1 missing	−10 G > A or −12 C > T	S
Pa-2	NHLS-2	WT2 and MUT1 missing	−10 G > A or −12 C > T	S
Pa-3	NHLS-3	WT2 and MUT1 missing	−10 G > A or −12 C > T	S
Pa-4	NHLS-4	WT2 and MUT1 missing	−10 G > A or −12 C > T	S
Pa-5	NHLS-5	WT1-3 and MUT1 present	WT and −14 C > T mixed	S
Pa-6	NHLS-6	WT1-3 and MUT1 present	WT and −14 C > T mixed	S
Pa-7	NHLS-7	WT1-3 and MUT1 present	WT and −14 C > T mixed	S
Pa-8	NHLS-8	WT1-3 and MUT1 present	WT and −14 C > T mixed	S
Pa-9	NHLS-9	WT1-3 and MUT1 present	WT and −14 C > T mixed	S

[a]WT, wild type; pDST, phenotypic drug susceptibility testing; AMK, amikacin; S, susceptible; MUT, mutation.

subpopulation. However, the *eis* promoter mutant subpopulation was found to be the dominant subpopulation by both WGS (*eis* promoter mutation, −32 C > T in 91% of reads) and Sanger sequencing, indicating that the −32 C > T mutation may not be the reason for the intermediate growth under KAN pressure. For this isolate, additional pDST under KAN pressure was conducted, and subsequent MTBDR*sl* and Sanger sequencing revealed the *rrs* 1401 mutation but not the −32 C > T *eis* promoter mutation as being present in this subpopulation. Phenotypic DST for this isolate showed high KAN resistance (MIC > 10 μg/ml). This subpopulation had been present in a concentration below the detection limit of the pDST (1%) in the original culture but is clinically relevant, as treatment with KAN could have failed due to high-level KAN resistance (20).

In addition to the *eis* promoter mutations, the presence of the *rrs* 1401 mutation was investigated (Tables 1 and 2). Phenotypic DST revealed AMK resistance in 8/12 isolates at diagnosis, but for only 4/8, the genotypic marker *rrs* 1401 was detected by MTBDR*sl* and/or WGS. For two isolates with no *rrs* 1401 mutation, pDST was repeated, confirming

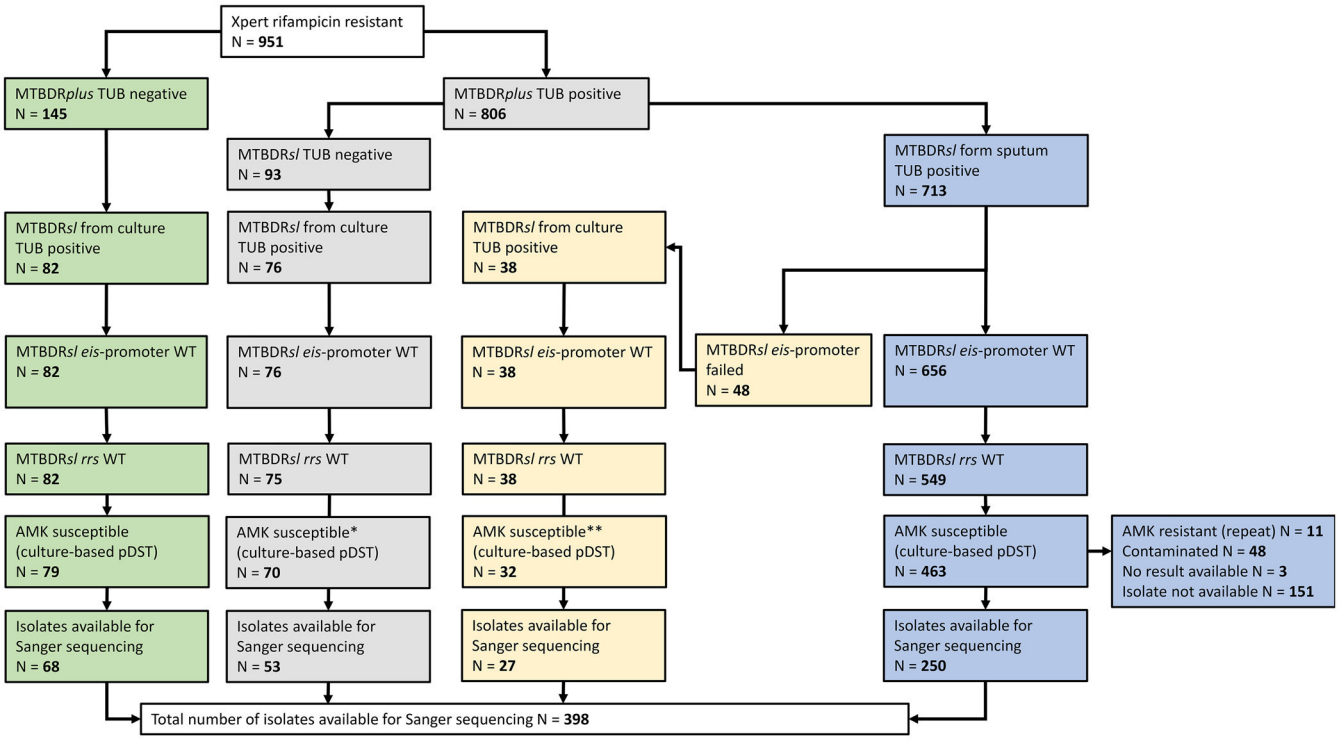

FIG 2 Flowchart describing the sample selection for sample set 1. WT, wild type; TUB, tuberculosis control band of the assay; AMK, amikacin; pDST, phenotypic drug susceptibility testing. *, the remaining 5 cultures were contaminated and pDST could therefore not be performed; **, the remaining 6 cultures were contaminated, and pDST could therefore not be performed.

TABLE 4 *eis* promoter mutations and kanamycin MICs of isolates diagnosed as *eis* promoter wild type by the MTBDR*sl* assay[b]

Patient	Isolate	*eis* promoter mutation	Detectable by MTBDR*sl*[a]	AMK pDST result	KAN pDST result	KAN MIC (ug/ml)
Pa-10	NHLS-10	−10 G > C	No	S	S	2.5
Pa-11	NHLS-11	−12 C > T	Yes	S	S	2.5
Pa-12	NHLS-12	−100 C > T	No	S	S	2.5
Pa-13	NHLS-13	−10 G > C	No	S	S	2.5
Pa-14	NHLS-14	−10 G > C	No	S	S	2.5
Pa-15	NHLS-15	−50 T > C	No	S	S	2.5
Pa-16	NHLS-16	−100 C > T	No	S	S	2.5
Pa-17	NHLS-17	−12 C > T	Yes	S	R	5
Pa-18	NHLS-18	−10 G > C	No	S	Failed regrowth	Failed regrowth
Pa-19	NHLS-19	−10 G > A	Yes	S	R	10
Pa-20	NHLS-20	−100 C > Y	No	S	Failed regrowth	Failed regrowth

[a]"Detectable by MTBDR*sl*" refers to those mutations for which the MTBDR*sl* provides specific banding patterns (see text).
[b]MICs are reported as the lowest concentration tested at which no growth was observed; however, the MIC can be lower than the reported number. S, susceptible; R, resistant; AMK, amikacin; KAN, kanamycin.

the phenotypic resistance for one isolate, whereas the other was phenotypically susceptible, matching the genotypic results. Genotypic and phenotypic results correlated for 3/4 isolates that were typed AMK susceptible at diagnosis, but for 1, MTBDR*sl* detected heteroresistance (i.e., WT and *rrs* 1401 present). WGS, however, did not detect the *rrs* 1401 mutation, suggesting a false-positive MTBDR*sl* result (Tables 1 and 2).

This study used comprehensive data sets that nevertheless bare limitations (for a more comprehensive discussion of the limitations, see Supplemental File 1). Not all isolates of set 1 were available for Sanger sequencing; the proportion of missed *eis* promoter mutations could therefore be higher. Despite analyzing data collected over 25 years, no conclusions about the prevalence of *eis* promoter mutations across that period can be drawn, as sample set 2 was a convenience sample from several studies. All WGS isolates were screened for *eis* promoter mutations, but only representatives were further analyzed. However, in combination, our data provide insights on the type and frequency of *eis* promoter mutations present in South Africa and reflect the complexity of antibiotic resistance in *M. tuberculosis*. Our results indicate the most reliable option for comprehensive individual DST to be a combination of genotypic methods, including (targeted) WGS and the phenotypic analysis of consecutively collected isolates of a patient. This reduces the limitations of current diagnostic algorithms and allows adaptation to newly emerging resistance markers (5, 21) but remains an unaffordable option for low- and middle-income countries where most tuberculosis (TB) cases occur. With more and less expensive WGS-based tools becoming available, targeted use of this strategy for severe cases could nevertheless be implemented (22).

The prevalence of *eis* promoter mutations detected in routine surveillance data and the proportion of missed low-level KAN resistance were low in this setting but nevertheless represent a potential cause of treatment failure. WHO released new tuberculosis treatment guidelines in 2019, no longer recommending the use of KAN (23). However, some *eis* promoter mutations (e.g., −14 C > T) also cause low-level resistance to AMK, which remains part of the WHO-recommended treatment guidelines. More importantly, though, many countries may not be able to timely implement the new treatment recommendations and will continue using AMK or KAN (23, 24). It therefore remains important to continue monitoring the prevalence of *eis* promoter mutations in circulating *M. tuberculosis* to preserve as many treatment options as possible.

Ethics. This study was designed and carried out in accordance with relevant guidelines and regulations. It was reviewed and approved by the Health Research Ethics Committee of Stellenbosch University (HREC) and the Western Cape Province Department of Health.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.
SUPPLEMENTAL FILE 1, DOCX file, 0.06 MB.
SUPPLEMENTAL FILE 2, XLSX file, 0.01 MB.
SUPPLEMENTAL FILE 3, XLSX file, 0.01 MB.

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Hain Lifesciences donated consumables used to generate some of the data analyzed; however, Hain Lifesciences had no say in the results or interpretation.

We declare no conflict of interest.

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Chapter 6

Discussion

Chapter 2:

GenoType MTBDR*plus* and GenoType MTBDR*sl* both v2 are widely-deployed WHO-endorsed tests for the rapid detection of first- and second-line TB drug resistance. As noted by the WHO and other policy making bodies, performance data of the latest versions of these important assays is scarce, particularly on sputum specimens with low *Mycobacterium tuberculosis* concentrations (smear-negative).

We showed that while both LPA tests were accurate as a rule-in test on smear-negative specimens for detection of resistance, the failure (non-actionable result) rates remained high. Thus, resistant cases were predominantly missed not because of a false-susceptible result (low sensitivity) but rather because these reflex DSTs were unable to detect TB DNA in the first place; precluding drug resistance reporting.

We also showed that Xpert, has a higher performance when detecting RR than MTBDR*plus*. This challenges the current standard-of-care in many settings, where MTBDR*plus* is used to "confirm" Xpert RR.

In addition, where RR is often assumed to also be INH-resistant (and therefore MDR resistant), we showed that 1 in 4 Xpert RIF-resistant patients had INH-susceptible-TB per MTBDR*plus*. This challenges the notion of using RR as a proxy for automatically assuming MDR and suggests that INH is still likely useful in many patients with RR-TB (Dean et al., 2020).

Our study provided significant clinical findings to improve algorithms used to diagnose and confirm resistance to first- and second-line drugs in people diagnosed with TB, and gives evidence that, in a real-world programmatic context, the standard-of-care is improved. Furthermore, we highlighted key gaps (e.g., the frequent inability to generate a resistance call in paucibacillary specimens) that should be targeted for improvement and prioritisation by funders and test developers.

Chapter 3

LPAs remain standard-of-care in many settings but have limitations, most so poor performance on paucibacillary smear-negative specimens where LPAs often fail (failure rates ~40%). This results in wasteful attempts at testing therefore laboratories opt out of direct LPA testing of smear-negatives and rely on culture, which is unacceptably slow.

We showed that rates of drug-resistance in smear-negative specimens is similar to that in smear-positives. Laboratories that do not directly test smear-negatives will miss opportunities to rapidly diagnose resistance. Selectively testing smear-negative specimens that fall below certain C_{Tmin} thresholds will reduce non-actionable result rates of LPAs but still allow high levels of LPA-detectable resistance to be detected (compared to when LPAs are done universally). An Xpert semi-quantitation category ≥medium would permit LPA testing to be expanded to almost half of smear-negatives.

Overall, our findings provide a framework that laboratories can apply in their own settings to make LPA testing on smear-negatives more efficient. Furthermore, this concept of using upstream molecular test information to better select patients for downstream reflex DSTs is relevant to many future tests and should be applied as programmes implement new DSTs. Our findings have implications for diagnostic laboratories and designers of laboratory algorithms, including policy makers, and can directly benefit the diagnostic care-cascade.

Chapter 4

Xpert XDR assay became one of the newly WHO-endorsed low-complexity assays approved for use of first- and second-line drug testing (World Health Organization, 2021b). Although this assay received conditional recommendations by WHO (World Health Organization, 2020d), the uptake in

South Africa has been slow and only a few studies have been published globally (Bainomugisa et al., 2020, Penn-Nicholson et al., 2021, Chakravorty et al., 2017).

We performed a systematic review and meta-analysis using the current published literature available to assess the diagnostic accuracy of Xpert XDR assay in patients with pulmonary TB and drug resistance to INH, FQ, ETH and AMK.

Our findings from the review illustrated that the assay cannot detect TB as a follow-on test if the initial test was found as Ultra trace-positive and this was due to the limit of detection of the assay.

The assay accurately detected resistance to FQ and INH. With high performance of this assay treatment initiation for all-oral 9-12 standardized shorter regimen with FQ can be established sooner, and INH resistance, which is often undiagnosed in RIF susceptible patients, can be used to optimise drug regimens. Suboptimal performance for the detection of ETH resistance was observed and this was due to the assays ability to target the *inhA* gene only. Hence, resistance detection may be missed in some cases.

The findings in this study had profound implications as it aided in informing policy making decisions. Furthermore, this assay can be used to guide treatment decisions and allow for rapid initiation of effective therapy. Routine diagnostics laboratories can save on time and costs involved with the collection and processing of secondary sputa and the additional consequences of a patient remaining infectious and contributing to transmission.

Chapter 5

With rapidly emerging drug resistance, it is essential to understand the mechanisms related to drug resistance. At the time of the study KAN formed part of the DR-TB regimen (World Health Organization, 2016c), however, DST for this individual drug was not performed routinely as it was

assumed AMK had complete cross resistance to KAN, hence AMK pDST could be conducted alone. This meant that low-level KAN resistance may have been missed in some patients hence potential exposure to ineffective regimens which may in turn lead to treatment failure. Therefore, with the introduction of MTBDR*sl* into the routine diagnostic algorithm which included *eis* promoter region used for the detection of low-level KAN resistance we aimed to determine the number of missed low-level KAN resistant cases.

In this paper we found 17/410 cases were missed for the detection of KAN resistance by MTBDR*sl*. We have also shown that of the isolates that were sequenced, a small proportion did not confer resistance. However, it important to acknowledge the limitations of MTBDR*sl* as this assay only targets the "hot spot" area of the *eis* promotor region and not the entire genome. Therefore, it is essential for both genotypic and phenotypic drug testing to be performed in parallel on isolates in order to capture drug resistance effectively.

Furthermore, we identified the most common resistant markers identified circulating in the Western Cape were -12 C > T and -10 G > A. Although the information generated in this study had little significance in our settings it is still crucial as it contributes to the global surveillance of drug resistance. With the use of new and repurposed drugs these findings are still warranted in regions where new all oral treatment regimens is not taken-up.

Along with the published articles included in this thesis, I additionally co-authored a number of research articles that dealt with a various way of improving the detection of second-line drug resistant tuberculosis through frontline diagnostic assays. These ancillary studies were closely linked to the primary focus of my PhD. Furthermore, I was involved in a study which used CE remnants to perform second-line drug testing with the use of only one sputum specimen. Additionally, I participated in a

survey study, which showed how corrected ramp rates improved the detection rates of first-and second-line drug resistance.

Chapter 7

Conclusion and Future Work

In conclusion, the studies involved in this thesis showed potential ways in which current diagnostic assays including LPAs can be optimized for use programmatically, TB diagnosis could be improved, and drug regimens could be guided thereby evading empirical treatment and improving treatment outcomes. We have acknowledged the role non-actionable results play in diagnostic tests and identified profound strategies to reduce unnecessary LPA testing and expand the framework of smear negative testing if not taken up already. We performed a systematic review and meta-analysis on the latest second-line diagnostic tool implemented by WHO in 2021 (Xpert MTB/XDR) which provides rapid diagnosis and treatment initiation, thereby reducing most of the shortcomings associated with LPAs. These new and novel findings have the potential to pave the pathway to new diagnostic algorithms and enclose the diagnostic gap that exists in primary healthcare which is the entry point to strong diagnostic capacity.

Future Work

Future work should include:

1. More studies investigating the performance of line probe assay, specifically for MTBDR*sl,* using paucibacillary clinical specimens, treatment outcomes, hetero-resistance, people living with HIV and mutational patterns circulating in different geographic settings for drug surveillance.
2. Follow-up validation studies using programmatic Ultra generated data which consists of Xpert C_{Tmin} values, semi-quantitation category and smear-microscopy are needed in different settings which could help guide additional reflex testing with Xpert XDR assay.
3. Additional studies with Xpert XDR cartridges using varying populations including children, HIV positive and negative patients, pulmonary samples, and smear-negative specimens in different geographical settings.

4. Further research needs to be performed looking at the effect of new diagnostic algorithms which include a combination of Xpert Ultra and Xpert XDR on treatment outcomes using routine programmatic data since.

5. Cost-effective studies on current diagnostics and new diagnostics in low- and middle-income countries.

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Chapter 8

Additional academic outputs and supplementary paper for Chapter-specific papers

Appendix I

Frequent Suboptimal Thermocycler Ramp Rate Usage Negatively Impacts GenoType MTBDR*sl* VER 2.0 Performance for Second-Line Drug-Resistant Tuberculosis

Derendinger, B., de Vos, M., **Pillay, S.**, Venter, R., Metcalfe, J., Ghebrekristos, Y., Minnies, S., Dolby, T., Beylis, N., Warren R.M., Theron, G., in *Journal of Molecular Diagnostics*, 2022
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Key findings:

We showed in smear-negative specimens, missing TUB-band and indeterminate results were faint or missing banding patterns which contributed to the high number (40%) of non-actionable results. With further investigation, we showed that by implementing the manufacturer recommended ramp rate of 2.2ºC/s as compared to vs. 4.0°C/s, indeterminate results, detection of drug resistance and incorrect scoring of banding patterns were reduced, however, there was no difference in TUB-band detection in clinical specimens. Overall, the number of valid results improved by 21%, indicating that repeat testing on a cultured isolate will no longer be needed.

Candidate's role:

Assisted in a proportion of clinical data collection, performing, and running of molecular tests MTBDR*sl* for study; data interpretation and reviewing of manuscript.

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Frequent Suboptimal Thermocycler Ramp Rate Usage Negatively Impacts GenoType MTBDR*sl* VER 2.0 Performance for Second-Line Drug-Resistant Tuberculosis Diagnosis

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Strengthening second-line drug-resistant tuberculosis (TB) detection is a priority. GenoType MTBDR*plus* VER 2.0 performance is reduced with non-recommended ramp rate usage (temperature change speed between PCR cycles); however, ramp rate's effect on GenoType MTBDR*sl* VER 2.0 (MTBDR*sl*) performance, is unknown. Fifty-two Xpert MTB/RIF Ultra-positive rifampicin-resistant smear-negative sputa and a *Mycobacterium tuberculosis* dilution series were tested at a manufacturer-recommended (2.2°C/second) or suboptimal (4.0°C/second) ramp rate. *M. tuberculosis*—complex-DNA positivity, indeterminates, fluoroquinolone- and second-line injectable-resistance accuracy, banding differences, and, separately, inter-reader variability were assessed. Five (39%) of 13 re-surveyed laboratories did not use the manufacturer-recommended ramp rate. On sputum, 2.2°C/second improved indeterminates versus 4.0°C/second (0 of 52 versus 7 of 51; $P = 0.006$), incorrect drug-class diagnostic calls (0 of 104 versus 6 of 102; $P = 0.013$), and incorrect banding calls (0 of 1300 versus 54 of 1275; $P < 0.001$). Similarly, 2.2°C/second improved valid results [(52 of 52 versus 41 of 51; +21% ($P = 0.001$)] and banding call inter-reader variability [34 of 1300 (3%) versus 52 of 1300 (4%); $P = 0.030$]. At the suboptimal ramp rate, false-resistance and false-susceptible calls resulted from wild-type band absence rather than mutant band appearance, resulting in misclassification of moxifloxacin resistance level from high-to-low. Suboptimal ramp rate contributes to poor MTBDR*sl* performance. Laboratories must ensure that the manufacturer-recommended ramp rate is used. *(J Mol Diagn 2022, 24: 494—502; https://doi.org/10.1016/j.jmoldx.2022.01.003)*

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In 2019, approximately 10 million individuals fell ill with tuberculosis (TB) and approximately 1.3 million individuals died.[1] Drug-resistant TB is a global health problem. Approximately 465,000 individuals having multidrug-resistant TB (MDR-TB), ≥6% of whom have additional resistance to fluoroquinolones (FQs) and second-line injectables (SLIDs) (WHO Global Tuberculosis Report 2020). Worldwide in 2019, only 52% of patients with MDR-TB were tested for resistance to both these drug classes, and only 58% of those who start treatment successfully complete it (WHO Global Tuberculosis Report 2020). Phenotypic culture-based drug susceptibility testing is slow and costly, and patients need to wait up to 6 months before being placed on effective treatment, if at all.[2] FQs are becoming incorporated into first-line drug regimens, which will require drastic scale-up of drug susceptibility testing. The World Health Organization (WHO) also recommends moxifloxacin for isoniazid-monoresistant TB in the newly endorsed shortened rifapentine regimen.[3]

GenoType MTBDR*sl* VER 2.0 (MTBDR*sl*) (Hain Lifescience, Nehren, Germany) is one of two commercially available rapid molecular WHO-endorsed assays for the detection of *Mycobacterium tuberculosis* complex and resistance to FQs and SLIDs.[4,5] According to the WHO, MTBDR*sl* should be performed directly on sputum irrespective of smear microscopy status to reduce the delay associated with culture for indirect testing.[4]

However, performance data for direct use on sputum are heterogeneous. In a systematic review and meta-analysis, smear-negative sensitivity estimates were imprecise: 80% [95% CI, 28−99], 80% (95% CI, 28−99), and 50% (95% CI, 1−99) for FQs, SLIDs, and extensively drug-resistant TB (XDR-TB) (using the then contemporaneous definition), respectively.[6] This affected the certainty of evidence of the WHO recommendation and undermined uptake of MTBDR*sl*.

MTBDR*sl* requires thermocycling for DNA amplification. The manufacturer recommends a ramp rate of ≤2.2°C/second, which is the speed of temperature change between PCR cycles. It was previously shown that performance of GenoType MTBDR*plus* VER 2.0 (MTBDR*plus*) (Hain Lifescience), which is an assay for first-line resistance, is reduced when suboptimal thermocycler ramp rates are used, mainly on smear-negative specimens.[7] These findings are incorporated into laboratory external quality assessment programs and the WHO TB laboratory training material (*https://openwho.org/courses/multi-drug-resistant-tb*, last accessed July 6, 2021).

If MTBDR*sl* is also vulnerable to this phenomenon, this would result in some of the thousands of individuals who receive this assay each day having drug resistance diagnoses missed, thereby resulting in resistance to the drugs critical to protect new regimens (eg, FQ to limit bedaquiline resistance acquisition in the oral second-line regimen) remaining delayed or undiagnosed.[8,9] More broadly, this issue of ramp rate is increasingly pertinent as manufacturers are designing instruments with faster thermocycling (and hence faster ramp rates) to decrease time-to-result. Furthermore, many thermocyclers, especially those at entry level (ie, with fewer customizable settings compared with more advanced models that are typically more expensive), do not have a customizable ramp rate.

It is hypothesized that the heterogeneous and suboptimal sensitivities reported for MTBDR*sl* on smear-negative specimens were partly attributable to suboptimal ramp rate, and the goal was to generate empirical evidence of this theory. The current study assessed whether laboratories that reported use of suboptimal ramp rates during the authors' previous MTBDR*plus* evaluation[7] had switched to the manufacturer-recommended ramp rate and what the observed effect had been.

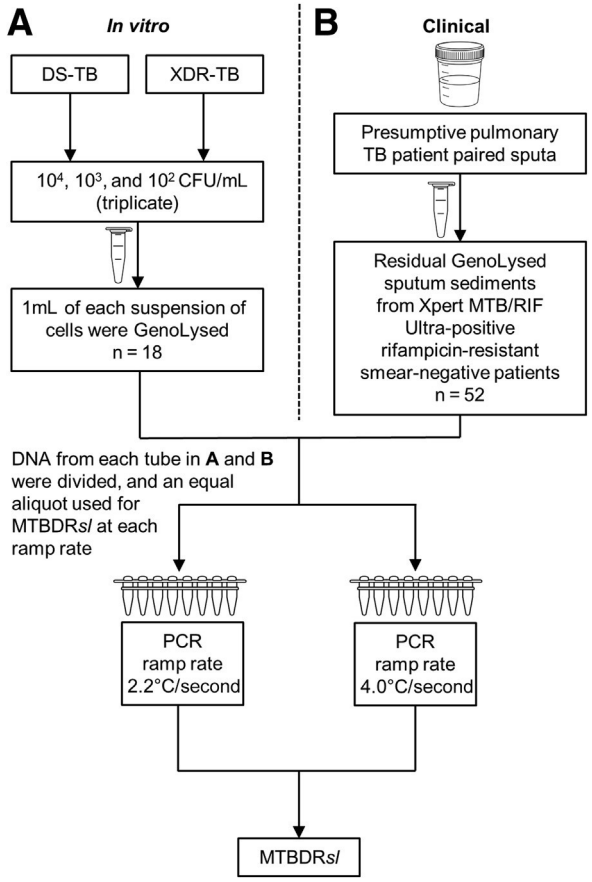

Figure 1 Study flow diagram for an *in vitro* [a dilution series of cells (10^4, 10^3, and 10^2 colony-forming units per milliliter [CFU/mL])] experiment (**A**) and clinical experiment (sputa) (**B**) to assess the impact of thermocycler ramp rate on GenoType MTBDR*sl* VER 2.0 (MTBDR*sl*). DNA extracted from the dilution series and clinical specimens was split and MTBDR*sl* compared head-to-head at the manufacturer-recommended ramp rate of 2.2°C/second or 4.0°C/second. DS-TB, drug-susceptible tuberculosis; TB, tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

Table 1 MTBDR*sl* Performance on a Dilution Series of Drug-Susceptible-TB and XDR-TB Strains (10^4, 10^3, and 10^2 CFU/mL) at Ramp Rates of 2.2°C/second (Manufacturer-Recommended) or 4.0°C/second (3 Replicates in Triplicate for Each Ramp Rate; 18 Total MTBDR*sl* Results)

		TUB-band—positive			
Ramp rate (°C/second)	TUB-band—positive	Indeterminate for any gene locus	Incorrect banding call	Incorrect drug class diagnostic call	Valid result
2.2	16/18* (89)	2/16† (13)	22/400‡ (6)	2/32§ (6)	14/16† (88)
4.0	17/18* (94), $P = 0.547$	3/17† (18), $P = 0.680$	33/425¶ (8), $P = 0.193$	2/34‖ (6), $P = 0.950$	14/17† (82), $P = 0.680$

Data are expressed as *n/N* (%). Accuracy for *M. tuberculosis*—complex-DNA (TUB-band) and then further analysis of indeterminate rates, incorrect banding calls, and incorrect drug class diagnostic calls were done. No significant differences were seen between ramp rates using dilution series. *P* values are for within-column comparisons between different ramp rates. CFU, colony-forming units; Incorrect banding call, the presence or absence of a band deviating from the true banding call; Incorrect drug class diagnostic call, the presence or absence of banding patterns resulting in deviation of the true susceptibility to a drug class; Indeterminate, one or more gene locus control is absent; MTBDR*sl*, GenoType MTBDR*sl* VER 2.0; TB, tuberculosis; TUB-band—positive, positive for *Mycobacterium tuberculosis*—complex-DNA; Valid result, TUB-band—positive, determinate for all gene locus controls, thus having diagnostic calls for both drug classes; XDR, extensively drug resistant.

*Two strains × 3 replicates × 3 dilutions.

†TUB-positive strips.

‡Sixteen TUB-band—positive strips × 25 bands per strip.

§Sixteen TUB-band—positive strips × 2 drug class diagnostic calls.

¶Seventeen TUB-band—positive strips × 25 bands per strip.

‖Seventeen TUB-band—positive strips × 2 drug class diagnostic calls.

Materials and Methods

Ethics Statement

This study was approved by the Health Research Ethics Committee of Stellenbosch University (N16/04/045) and Western Cape Research Ethics Committee (WC_2016RP18_637). All methods were in accordance with relevant guidelines and regulations. Permission was granted to access anonymized residual specimens collected as part of routine diagnostic practices, and thus patient informed consent was waived.

Experimental Design

Ramp rate assessment was performed in both an *in vitro* dilution series and clinical sputa (Figure 1). DNA extracted from dilution series and clinical specimens were split and compared head-to-head at the manufacturer-recommended ramp rate of 2.2°C/second or the most common suboptimal ramp rate of 4.0°C/second identified previously in a survey.[7] MTBDR*sl* was performed on all amplified DNA per manufacturer's instructions for use (Hain Lifescience) [kit lot #39B (expiry date September 2, 2019); strip lot #ABB0117A161 (expiry date September 18, 2019)]. All experiments for this study were performed before the kits' expiration dates. Strips were interpreted by using the WHO-endorsed Global Laboratory Initiative line probe assay interpretation guide (GLI, *http://www.stoptb.org/wg/gli/assets/documents/LPA_test_web_ready.pdf*; WHO, *https://openwho.org/courses/multi-drug-resistant-tb/items/49CT8rhOFxxXzbJYsIIZlK*, last accessed October 19, 2021) and the authors agree with the recommendations in these guidelines. For sputa, programmatic MTBDR*sl* results (performed at the recommended ramp rate) were compared. All equipment is annually calibrated and serviced.

MTBDR*sl* Calls and Result Definitions

Conjugate Control Band

The conjugate control (CC)-band must be present for a strip to be valid as it indicates that hybridization occurred.

Amplification Control Band

The amplification control (AC)-band is present when the assay is performed correctly. Per the manual (GenoType MTBDR*sl* Instructions for Use IFU-317A-04; Hain Lifescience), there are rare cases in which the AC-band disappears due to competition during the amplification reaction. In this scenario, an absent AC-band in combination with *M. tuberculosis*—complex-DNA (TUB-band) and locus control bands is still a valid result.

Locus Control Bands (*gyrA*, *gyrB*, *rrs*, and *eis*)

The locus control bands (*gyrA*, *gyrB*, *rrs*, and *eis*) need to be present for a call from that locus to not be indeterminate.

Positive for *M. tuberculosis*—Complex-DNA

The TUB-band indicates the presence of *M. tuberculosis*—complex-DNA.

Strip Banding Call

For a band to be classified as present, it must be equal or darker than the AC-band. Overall, there are 27 possible strip bands on MTBDR*sl*. When only the CC- and AC-bands are present, this represents a valid TUB-negative result.

Table 2 MTBDR*sl* Performance on Smear-Negative Sputa at Ramp Rates of 2.2°C/second (Manufacturer-Recommended) or 4.0°C/second (52 Isolates)

		TUB-band—positive			
Ramp rate (°C/second)	TUB-band—positive	Indeterminate for any gene locus	Incorrect banding call	Incorrect drug class diagnostic call	Valid result
2.2	52/52* (100)	0/52† (0)	0/1300‡ (0)	0/104§ (0)	52/52† (100)
4.0	51/52* (98), $P = 0.315$	7/51† (14), **$P = 0.006$**	54/1275¶ (4), **$P < 0.001$**	6/102‖ (6), **$P = 0.013$**	41/51† (80), **$P = 0.001$**

Data are expressed as *n/N* (%). Accuracy for *Mycobacterium tuberculosis*—complex-DNA, and then further analysis of indeterminate rates, incorrect banding calls, and incorrect drug class diagnostic calls were done. The number of valid results [52 of 52 (100%) versus 41 of 51 (80%)] improved by 21% (95% CI, 8—34; $P < 0.001$). *P* values are for within-column comparisons between different ramp rates. Significant *P* values are marked in bold. Incorrect banding call, the presence or absence of a band deviating from the true banding call; Incorrect drug class diagnostic call, the presence or absence of banding patterns resulting in deviation of the true susceptibility to a drug class; Indeterminate, one or more gene locus control is absent; MTBDR*sl*, GenoType MTBDR*sl* VER 2.0; TB, tuberculosis; TUB-band—positive, positive for *Mycobacterium tuberculosis*—complex-DNA; Valid result, TUB-band—positive, determinate for all gene locus controls, thus having diagnostic calls for both drug classes.

*Total number of clinical specimens.

†TUB-positive strips.

‡Fifty-two TUB-band—positive strips × 25 bands per strip.

§Fifty-two TUB-band—positive strips × 2 drug class diagnostic calls.

¶Fifty-one TUB-band—positive strips × 25 bands per strip.

‖Fifty-one TUB-band—positive strips × 2 drug class diagnostic calls.

Drug Class Diagnostic Call

Band presence or absence in a gene region determines whether the result is classified as susceptible or resistance to a drug class (two drug class diagnostic calls possible for MTBDR*sl*: FQs or SLIDs).

(In)determinate for a Gene Region and/or Drug Class

For a specific gene region and/or drug class to be determinate, locus control band(s) must be present. A strip was called indeterminate for a drug class if at least one gene locus control was absent.

Valid Result

TUB-band—positive strip determinate for all gene locus controls and thus has diagnostic calls for both drug classes (eg, TUB-band—positive, FQ-resistant, SLID-susceptible).

Additional Amikacin Resistance (*rrs* C1402T and *eis* C-14T)

These are new guidelines released by the WHO indicating resistance to amikacin. *rrs* C1402T translates to *rrs* WT1 band not binding and *eis* C-14T translates to the *eis* MUT1 band binding.[10] The MTBDR*sl* will need to be updated.

Impact of Thermocycler Ramp Rate on MTBDR*sl* Performance on a Dilution Series

A phenotypically and genotypically resistant clinical XDR strain (*gyrA* D94N, *gyrB* wild type, *rrs* A1401G, and *eis* wild type) and a drug-susceptible strain (H37Rv, ATCC 25618) were grown to mid-exponential phase in Middlebrook 7H9 media (Becton Dickinson, Franklin Lakes, NJ) supplemented with Middlebrook Oleic Albumin Dextrose Catalase (Becton Dickinson) and adjusted to a McFarland 1.0 standard [approximately 10^8 colony-forming units per milliliter (CFU/mL)] (GLI Mycobacteriology Laboratory Manual, *http://www.stoptb.org/wg/gli/assets/documents/gli_mycobacteriology_lab_manual_web.pdf*, last accessed July 23, 2021). Serial dilutions in phosphate buffer supplemented with 0.025% Tween 80 (Merck, Sandton, South Africa) were inoculated onto Middlebrook 7H10 solid media (Becton Dickinson) and incubated for 21 days at 37°C for CFU calculations. These experiments were performed in biological triplicate. One milliliter of the 10^4, 10^3, and 10^2 CFU/mL suspensions were GenoLysed (Hain Lifescience) and MTBDR*sl* performed per the manufacturer's instructions (Hain Lifescience). The two lower dilutions approximate to smear-negative disease (<10,000 CFU/mL),[11] expected to be most affected by a suboptimal ramp rate. DNA was amplified with the CFX96 thermocycler (Bio-Rad Laboratories, Sandton, South Africa) at ramp rates of 2.2°C/second and 4.0°C/second. Two experienced readers recorded bands in a blinded manner. Accuracy analyses for TUB-band positivity, indeterminate rates, incorrect banding calls, and incorrect drug class diagnostic calls were done.

Impact of Thermocycler Ramp Rate on MTBDR*sl* Performance on Clinical Specimens

GenoLysed samples ($n = 52$) remaining after programmatic line probe assay test results were collected from a TB laboratory in Cape Town, South Africa. These samples were, per the national algorithm, derived from the paired sputum specimen of a presumptive pulmonary TB patient who received Xpert MTB/RIF Ultra (Ultra) (on separate sputum), MGIT 960 culture, and Auramine O microscopy (on the same sputum before being GenoLysed). All sputa were smear-negative and Ultra-positive rifampicin-resistant.

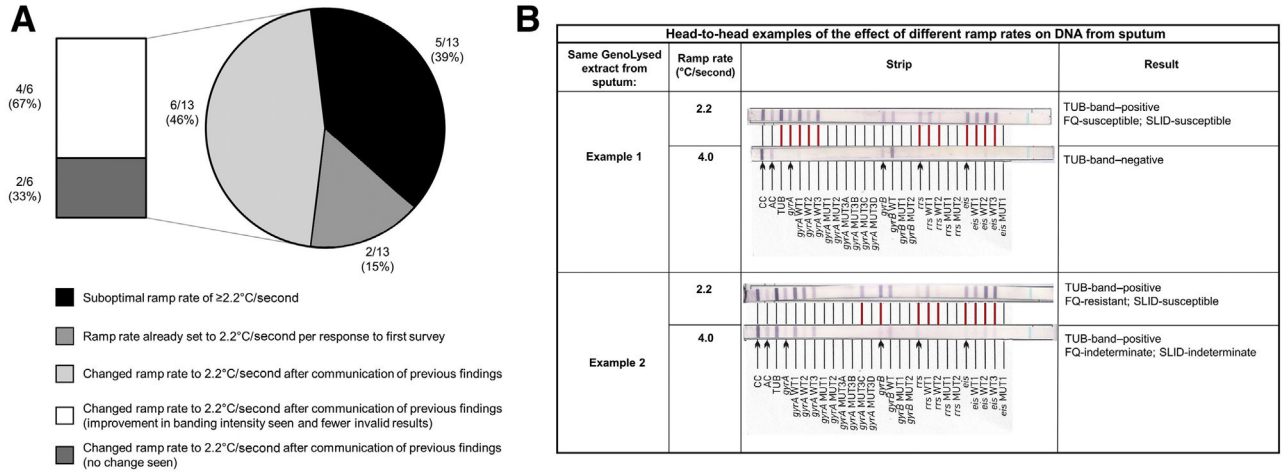

Figure 2 **A:** Follow-up survey results summarizing thermocycler ramp rates for GenoType MTBDR*sl* VER 2.0. Two (15%) of 13 initially surveyed laboratories already had their ramp rate set to 2.2°C/second, and five (39%) of 13 were still using a suboptimal ramp rate of ≥2.2°C/second upon resurveying. Six (46%) of 13 laboratories had, since the first survey on GenoType MTBDR*plus* VER 2.0, changed the GenoType MTBDR*sl* VER 2.0 ramp rate to the recommended ramp rate. Of these, four (67%) of six reported an improvement in banding intensity and fewer invalid results. **B:** An illustrative example of differences in banding patterns (and consequences for patient diagnoses) caused using suboptimal ramp rate. In example 1, at the suboptimal ramp rate (4.0°C/second), no tuberculosis or drug susceptibility information would be generated. In example 2, at the suboptimal ramp rate (4.0°C/second), again no drug susceptibility information would be generated, but, in this case, it would lead to a missed diagnosis of fluoroquinolone (FQ) resistance. Different banding patterns between strips are shown with a **red line**. SLID, second-line injectables; TUB-band—positive, positive for *Mycobacterium tuberculosis*—complex-DNA.

Smear-positive specimens were not included as it was previously shown that ramp rate had no effect on MTBDR*plus* performance on smear-positive specimens.[7] Residual GenoLysed samples were stored at −20°C.

Samples were categorized by using programmatic line probe assay results as: 17 MDR-TB, 24 pre-XDR, and 11 XDR-TB. For the experiment, DNA was amplified by using a CFX96 thermocycler (Bio-Rad, Hercules, CA) at 2.2°C/second (manufacturer-recommended) and 4.0°C/second. MTBDR*sl* was performed per the manufacturer's instructions (Hain Lifescience), and two experienced readers recorded bands in a blinded fashion. Accuracy analyses for TUB-band positivity, indeterminate rates, incorrect banding calls, and incorrect drug class diagnostic calls were done.

Calculation of Laboratory Savings from an Improvement in MTBDR*sl* Performance on Smear-Negative Specimens Stemming from Ramp Rate

Calculations were performed on how much the routine laboratory, from which GenoLysed remnants were received, would save if the proportional increase, which was found in valid results when the optimal versus the suboptimal ramp rate was used, was applied. This cost savings calculation was based on the average number of MTBDR*sl* tests performed indirectly on cultured isolates per month (which would now be reduced due to direct testing on smear-negative specimens having improved performance) and the cost of each test (including consumables, labor, and overheads; the sum is pre-calculated and supplied by the laboratory provider).

Inter-reader Agreement

An additional three experienced readers, independent of the aforementioned two readers, read all strips from the dilution series and clinical specimens at either ramp rate independently from one another and blinded to each other's calls as well as any other information regarding the specimens or strains used. Banding calls were assessed between readers, as well as resultant differences in final drug class diagnostic calls. Excluding the CC-bands and AC-bands, and including the TUB-band, gene locus control-bands, and gene-specific wild type- and mutant-bands, there are 25 possible bands per MTBDR*sl* strip. There are hence 450 possible bands total for the 18 samples in the dilution series and 1300 possible bands for the 52 clinical isolates. Each strip results in two drug class diagnostic calls, and there are hence 36 possible drug class diagnostic calls in total for 18 samples in the dilution series and 104 possible drug class diagnostic calls in total for the 52 clinical isolates.

Follow-Up Survey of TB Diagnostic and Research Laboratories

Prior respondents to the initial MTBDR*plus*-focused survey[7] were re-surveyed ($n = 29$) to gather information on the current MTBDR*sl* conditions. Other laboratories newly known to us as performing MTBDR*sl* on smear-negative specimens ($n = 11$) were also surveyed for the first time, and initial nonresponders were re-contacted at least twice. Survey questions included whether ramp rate changed and impact on nonvalid results (Supplemental Appendix S1[7]). Permission to use data in an anonymized manner was

Table 3 Comparison of Banding and Drug Class Diagnostic Calls Done on a Dilution Series of DS-TB and XDR-TB Strains and Clinical Specimens Interpreted by Three Experienced Readers

	DS-TB strain		XDR-TB strain		Clinical specimens	
Ramp rate (°C/second)	Different banding call between readers	Different drug class diagnostic call between readers	Different banding call between readers	Different drug class diagnostic call between readers	Different banding call between readers	Different drug class diagnostic call between readers
2.2	0/225* (0)	0/18† (0)	1/225* (0.4)	0/18† (0)	34/1300‡ (3)	5/104§ (5)
4.0	1/225* (0.4), $P = 0.317$	1/18† (6), $P = 0.311$	3/225* (1), $P = 0.313$	0/18† (0), $P > 0.999$	52/1300‡ (4), **$P = 0.030$**	8/104§ (8), $P = 0.390$

Data are expressed as *n/N* (%). Differences in banding calls or drug class diagnostic calls did not differ between the three readers at either ramp rate for the dilution series of cells, neither did the drug class diagnostic calls in the clinical specimens; however, significant difference between readers for banding calls on the clinical sputa occurred. *P* values are for within-column comparisons between different ramp rates. Significant *P* values are marked in bold. banding call, the presence or absence of a band deviating from the true banding call; diagnostic call, the presence or absence of banding patterns resulting in deviation of the true susceptibility to a drug class; DS-TB, drug-susceptible tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

*One strain × 3 replicates × 3 dilutions × 25 bands per strip.

†One strain × 3 replicates × 3 dilutions × 2 drug class diagnostic calls.

‡Fifty-two clinical specimens × 25 bands per strip.

§Fifty-two clinical specimens × 2 drug class diagnostic calls.

received from the Faculty of Medicine and Health Sciences Human Research Ethics Committee of Stellenbosch University (N16/04/045).

Statistical Analyses

Data were analyzed using Stata version 15 (StataCorp, College Station, TX) and GraphPad Prism version 8.0.1 (GraphPad Software, La Jolla, CA) using two-sided *t*-tests with $\alpha = 0.05$. McNemar's test was used to calculate differences for paired data (ie, the same DNA tested at both ramp rates). The two-sample proportion test was used for comparisons between proportions.

Results

MTBDR*sl* on the Dilution Series at Different Ramp Rates

Overall, there were no differences between ramp rates of 2.2°C/second and 4.0°C/second for TUB-band detection [16 of 18 (89%) versus 17 of 18 (94%); $P = 0.547$], indeterminate results [2 of 16 (13%) versus 3 of 17 (18%); $P = 0.680$], incorrect banding calls [22 of 400 (6%) versus 33 of 425 (8%); $P = 0.193$)], or incorrect drug resistance calls [2 of 32 (6%) versus 2 of 34 (6%); $P = 0.950$] (Table 1). Therefore, valid results did not differ significantly [14 of 16 (88%) versus 14 of 17 (82%); $P = 0.680$].

MTBDR*sl* on Clinical Sputa at Different Ramp Rates

No TUB-band detection differences were seen at 2.2°C/second versus 4.0°C/second [52 of 52 (100%) versus 51 of 52 (98%); $P = 0.315$; one MDR-TB patient was TUB-negative only at 4.0°C/second]. However, indeterminate rates improved at 2.2°C/second [0 of 52 (0%) versus 7 of 51 (14%); $P = 0.006$], as did the proportion of bands that appeared incorrectly [0 of 1300 (0%) versus 55 of 1275 (4%); $P < 0.001$)] and drug-resistance calls [0 of 104 (0%) versus 6 of 102 (6%); $P = 0.013$] (Table 2). The proportion of patients with a valid result was therefore 52 (100%) of 52 versus 41 (80%) of 51. In other words, the patients who successfully received testing for FQs and SLIDs thus improved 21% (95% CI, 8–34; $P < 0.001$).

Programmatic Ultra semi-quantitative data were available for 41 (79%) of 52 sputa. When bacterial load in sputa that gave a valid result at 2.2°C/second was compared versus sputa that gave a valid result at 4.0°C/second, there were no differences [median (interquartile range) minimum cycle threshold (C_{Tmin}), 18.7 (17.7–19.9) versus 18.8 (18.0–19.9); $P = 0.899$]. It was expected that 2.2°C/second would result in an improved limit of detection in MTBDR*sl* (better ability to detect higher C_{Tmin} and therefore fewer bacilli); however, no differences were detected.

Head-to-head examples of the effect of different ramp rates on DNA from sputum are provided in Figure 2B.

Banding patterns from both the dilution series and clinical sputa are listed in Supplemental Tables S1 and S2. For the dilution series (Supplemental Table S1), irrespective of ramp rate, MTBDR*sl* did not classify the XDR-TB strain correctly at 10^2 CFU/mL across all replicates (Table 1). Overall, for dilution series (both strains, all dilutions), the overall effect was missed resistance due to a TUB-negative, indeterminate, or a missing gene-specific band, or false-resistance due to an erroneously absent wild-type band. For clinical sputa (Supplemental Table S2) at the suboptimal ramp rate, there was worse detection of the TB and locus control bands and, when TB was detected and the locus control bands present, gene-specific bands that should have been present were absent. In the dilution series, one replicate (XDR-TB, 10 to 2 dilution) missed amikacin resistance at the suboptimal ramp rate. In clinical specimens, two samples (RR2-31 and RR2-38) with high-level moxifloxacin

Table 4 Laboratories That Indicated Their Ramp Rate Had Not yet Changed to the Manufacturer-Recommended Ramp Rate of ≤2.2°C/second Since the Last Survey, the Reason Why, and Total Number of Line Probe Assays Performed per Month

Country	Reason given	No. of line probe assays performed per month by this respondent laboratory
Kenya	Do not know	240
South Africa	Ramp rate change was not necessary as MTBDR*plus* assays are performed on cultured isolates only and no MTBDR*sl* assays are performed, as well as any changes to a standard operating procedure requires a validation process	40
Belarus	Ramp rate change in a standard operation procedure is not permitted without a prior approval process	155
Denmark	Ramp rate was not changed due to the run time of the original amplification protocol being faster	25
Spain	The thermocycler did not permit a ramp rate change	12

These laboratories perform either GenoType MTBDR*plus* VER 2.0 (MTBDR*plus*), GenoType MTBDR*sl* VER 2.0 (MTBDR*sl*), or both on smear-negative specimens, but data on the subtotals for each assay were not collected.

resistance were incorrectly classified at the suboptimal ramp rate as low-level resistant (RR2-38) or susceptible (RR2-31). At the suboptimal ramp rate of 4.0°C/second, 55 gene locus bands were erroneous. The breakdown is as follows: *gyrA*, 14 of 55 (25%); *gyrB*, 5 of 55 (9%); *rrs*, 28 of 55 (51%); and *eis*, 8 of 55 (15%).

Laboratory Savings

If the improvement in FQ and SLID testing due to optimal ramp rate usage is applied, there would be a 21% decrease in the number of tests required to be performed indirectly (which would require culture and a second MTBDR*sl*). At a local reference laboratory, approximately 320 MTBDR*sl* assays, initially attempted on smear-negative sputa, are performed per month and are subsequently repeated on culture isolates. Hence, in a scenario in which this laboratory was using an incorrect ramp rate and changed to the correct rate, they would perform approximately 67 fewer indirect MTBDR*sl* assays per month. At a total per test cost of US$60 (6% per annum inflation),[12] this translates to a savings of US$48,240 per year (only factoring in pure laboratory costs).

Inter-reader Agreement

In the dilution series, diagnostic calls did not differ between the three readers at either ramp rate. All readers incorrectly classified the XDR-TB strain (as either TUB-band−negative or indeterminate) at all 10^2 CFU/mL replicates and the drug-susceptible−TB strain (as indeterminate) at one of the three replicates at 10^2 CFU/mL (Table 3). The proportion of disagreement between readers (banding calls) did not differ at suboptimal versus optimal ramp rates [for the drug-susceptible (1 of 225 versus 0 of 225; $P = 0.317$) or the XDR (3 of 225 versus 1 of 225; $P = 0.313$)] strain.

In clinical sputa, however, although the disagreement in drug class diagnostic calls did not differ between readers at the optimal versus suboptimal ramp rate [5 of 104 (5%) versus 8 of 104 (8%); $P = 0.390$], banding calls did differ [34 of 1300 (3%) versus 52 of 1300 (4%); $P = 0.030$].

Additional Survey

Twenty-nine follow-up surveys were sent to the original respondents and 11 to new laboratories. Thirteen total responses were received (45%), including four from new respondents (Figure 2A). Two (15%) of 13 respondents already had their ramp rate at 2.2°C/second (per their response to the first survey), and six (46%) of 13 had subsequently changed their ramp rate to 2.2°C/second after the previous findings were communicated.[7] Concerningly, five (39%) of 13 had not changed, for which varied reasons were offered (Table 4). Of the laboratories who changed to 2.2°C/second, four (67%) of six reported that this resulted in an improvement in banding intensity and fewer nonvalid results for MTBDR*plus* and MTBDR*sl*.

Discussion

The current study evaluated for the first-time the impact of thermocycler ramp rates on the most widely used molecular test for second-line drug-resistant TB (MTBDR*sl*). This study shows: i) in sputa, valid results improved by 21% when using the optimal ramp rate, which results in significant laboratory cost savings and would decrease diagnostic delay; ii) banding call and drug susceptibility call reader disagreement worsened at the suboptimal ramp rate; and iii) several laboratory respondents had not corrected their line probe assay ramp rate but, those that had, reported fewer nonvalid results from MTBDR*sl* on smear-negative specimens.

In a previous study, the authors found that a suboptimal thermocycler ramp rate negatively affects the diagnostic accuracy of potentially thousands of MTBDR*plus* assays, especially on smear-negative sputa,[7] and ramp rate

monitoring was incorporated into laboratory quality control and training documentation (WHO Drug-resistant tuberculosis: how to interpret rapid molecular test results, *https://openwho.org/courses/multi-drug-resistant-tb*, last accessed July 6, 2021). The current study shows that a 21% increase in MTBDR*sl* diagnoses (valid results) in smear-negative specimens is possible through ramp rate correction. This is not a niche problem; diagnostic laboratories that still do not perform MTBDR*sl* correctly were identified. This correction, which this study has now provided MTBDR*sl*-specific empirical evidence, could reduce drug-resistant TB diagnostic care cascade gaps: a recent study found that only 65% of MDR-TB cases were evaluated for FQ resistance.[13]

Critically, ramp rate correction will reduce repeat MTBDR*sl* testing on isolates. Most directly, this will translate into substantial laboratory cost savings in high-burden countries, especially when TB services are fragile due to the COVID-19 pandemic, not to mention the myriad of other individual and population benefits that can stem from improved drug susceptibility testing[14]; these include reduced time to treatment, transmission, and mortality.

Most laboratories in the follow-up survey had corrected the ramp rate; however, a significant amount, including those responsible for routine diagnostic testing on smear-negative specimens, still used a suboptimal ramp rate. It should be emphasized that: i) laboratories must ensure that they are using the optimal ramp rate; ii) thermocycler ramp rate monitoring should be added to laboratory external quality assurance programs and accreditation processes for MTBDR*sl*; and iii) the manufacturer should make the recommended ramp rate more prominent in assay documentation. It is worth evaluating further why incorrect ramp rates continued to be used. This may be due to quality assurance lapses, a deliberate choice (eg, to potentially speed up turn-around-time) without an awareness of downsides, or a design limitation of available thermocyclers.

When a band was present at the optimal ramp rate (2.2°C/second) and not the suboptimal ramp rate (4.0°C/second), FQ and/or SLID diagnoses were missed completely due to gene locus control bands not binding. False drug class diagnostic calls for FQs and/or SLIDs (false resistance) due to the inability of a band to bind were also seen. No false resistance was observed due to the binding of mutant probes when the suboptimal ramp rate was used. However, false resistance calls due to an erroneous absence of wild-type bands occurred. It was noted that more than one-half of the incorrect bands in sputa occurred in one gene locus (*rrs*), which may be due to secondary structures that interfere with PCR and detection.

A more prominent performance difference was seen between ramp rates in clinical sputa than in spiked solution. Bacilli in mucus sputa matrices behave differently from bacilli spiked in *in vitro* experiments, and these findings illustrate potential downsides to investigating the effect of PCR parameters on molecular assays when *in vitro* or mock specimens are used.

The current evaluation has strengths and limitations. A wider ramp rate range or different thermocycler models were not assessed due to limited sputa and cost. The utility of additional testing when a useful (ie, valid) result failed to be generated was also not evaluated. The most frequently reported incorrect ramp rate from the previous survey was used.[7] DNA from samples was not directly quantified; however, when comparing Ultra semi-quantitative (C_{Tmin}) data between valid results across ramp rates, no differences occurred. When there is an indeterminate result for a gene locus, regardless of whether that indeterminate result is caused by optimal ramp rate, it may influence the reliability of other diagnostic calls from loci with valid control bands. However, this requires a large diagnostic accuracy study to investigate, and the current work was not designed to do so.

The survey results would have also been subjected to selection, response, and reporting biases. The authors suggest that a formal survey be done by the manufacturer and/or the appropriate regulatory and oversight agency (the study survey was done independently). Savings stemming from quicker diagnosis, treatment initiation, and long-term reductions in transmission and mortality due to improved performance were not evaluated; there is already a saving in laboratory costs alone, with no downside.

In conclusion, this study found that a still incorrectly configured and innocuous technical setting (ramp rate) has a real-world negative impact on patients' diagnoses for second-line drug resistance using MTBDR*sl*. Patients with smear-negative specimens, for whom early diagnosis is important to curtail transmission of drug resistance, are especially vulnerable. All stakeholders must ensure that the optimal thermocycler ramp rate for MTBDR*sl* is used, and the impact of this source of technical variation should be investigated for other molecular diagnostics.

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Author Contributions

B.D., M.d.V., G.T., and R.W. conceived the experiments; T.D., N.B., and S.P. provided specimens and data from the National Health Laboratory Service; B.D. conducted the experiments and analyzed the data; S.P, Y.G., R.V., and S.M. assisted with analysis of results; J.M. provided critical input. All authors reviewed the manuscript.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2022.01.003*.

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Supplement

MTBDR*sl* thermocycler ramp rate survey sent to laboratory respondents who completed our first survey on MTBDR*plus*[1]

This new survey was also sent to laboratories known to be doing line probe assays on smear-negative specimens but not originally contacted.

1. Name
2. Name of your organisation
3. Email address
4. What country is your laboratory based in?
5. Since completing the previous survey, did your laboratory change the thermocycler ramp rate to 2.2°C/s when doing MTBDR*plus* and/or MTBDR*sl*?
    - Yes
    - No
6. When did your laboratory change the ramp rate? (mm/yyyy format)
7. If the answer was no to question 5, why did your laboratory NOT change the ramp rate to 2.2°C/s? (Please complete question 8 and 9 then skip to the end of the survey and click submit)
    - Thermocycler did not permit ramp rate change
    - Do not know
    - Changes to test SOP are not permitted without a prior approval process
    - Thermocycler ramp rate was already set to 2.2°C/s
    - Other:_____
8. Did your laboratory change any other MTBDR*plus* and/or MTBDR*sl* test parameters?
    - Yes
    - No
9. If yes to question 8, please elaborate:
10. Did the correction of ramp rate result in an improvement in banding intensity when doing MTBDR*plus* and/or MTBDR*sl*?
    - Yes
    - No
11. If yes to question 10, please elaborate (e.g. are there specific bands that have improved the most (including control bands):

12. Did the correction of ramp rate result in fewer non-actionable (TUB band-negative or indeterminate for any drug locus control band) MTBDR*plus* results?
    - Yes
    - No
    - Do not know
    - Other:_____
13. Did the correction of ramp rate result in fewer non-actionable (TUB band-negative or indeterminate for any drug locus control band) MTBDR*sl* results?
    - Yes
    - No
    - Do not know
    - Other:_____
14. In the 3 months BEFORE your laboratory corrected the ramp rate, is there data available on how many MTBDR*plus* tests were done directly on smear-negative sputum?
    - Yes
    - No
        i. If no to question 14, why?
            - Not recorded
            - Too difficult to retrieve data
            - Other:_____

        ii. If yes to question 14, how many MTBDR*plus* tests were done directly on smear-negative sputum?

        iii. How many of these, done directly on smear-negative sputum, were TUB-band negative (TB-negative)?

        iv. How many of these, done directly on smear-negative sputum, were TUB-band positive, but indeterminate for any gene locus (*rpoB*, *katG* or *inhA*)?
15. In the 3 months BEFORE your laboratory corrected the ramp rate, is there data available on how many MTBDR*sl* tests were done directly on smear-negative sputum?
    - Yes
    - No
        i. If no to question 15, why?
            - Not recorded
            - Too difficult to retrieve data
            - Other:_____

ii. If yes to question 15, how many MTBDR*sl* tests were done directly on smear-negative sputum?

iii. How many of these, done directly on smear-negative sputum, were TUB-band negative (TB-negative)?

iv. How many of these, done directly on smear-negative sputum, were TUB-band positive, but indeterminate for any gene locus (*gyrA*, *gyrB*, *rrs* or *eis*)?

16. In the 3 months AFTER your laboratory corrected the ramp rate, is there data available on how many MTBDR*plus* tests were done directly on smear-negative sputum?

- Yes
- No

i. If no to question 16, why?

- Not recorded
- Too difficult to retrieve data
- Other:_____

ii. If yes to question 16, how many MTBDR*plus* tests when done directly on smear-negative sputum?

iii. How many of these, done directly on smear-negative sputum, were TUB-band negative (TB-negative)?

iv. How many of these, done directly on smear-negative sputum, were TUB-band positive, but indeterminate for any gene locus (*rpoB*, *katG* or *inhA*)?

17. In the 3 months AFTER your laboratory corrected the ramp rate, is there data available on how many MTBDR*sl* tests were done directly on smear-negative sputum?

- Yes
- No

i. If no to question 17, why?

- Not recorded
- Too difficult to retrieve data
- Other:_____

ii. If yes to question 17, how many MTBDR*sl* tests were done directly on smear-negative sputum?

iii. How many of these, done directly on smear-negative sputum, were TUB-band negative (TB-negative)?

iv. How many of these, done directly on smear-negative sputum, were TUB-band positive, but indeterminate for any gene locus (*gyrA*, *gyrB*, *rrs* or *eis*)?

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Appendix II

Mycobacterial genomic DNA from used Xpert MTB/RIF cartridges can be utilised for accurate second-line genotypic drug susceptibility testing and spoligotyping

Venter, R., Derendinger, B., De Vos, M., **Pillay, S**., Dolby, T., Simpson, J., Kitchin, N., Ruiters, A., Van Helden, P.D., Warren, R.M. and Theron, G., 2017. Mycobacterial genomic DNA from used Xpert MTB/RIF cartridges can be utilised for accurate second-line genotypic drug susceptibility testing and spoligotyping. *Scientific reports*, *7*(1), pp.1-9.
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Publication status: published

Key findings:

MTB DNA from the PCR-mix in Xpert cartridges can successfully be used for second-line DST and spoligotyping is also possible using the Xpert extract.

Candidate's role:

Assisted in collection of data and reviewing of manuscript.

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Mycobacterial genomic DNA from used Xpert MTB/RIF cartridges can be utilised for accurate second-line genotypic drug susceptibility testing and spoligotyping

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Xpert MTB/RIF (Xpert) is a widely-used test for tuberculosis (TB) and rifampicin-resistance. Second-line drug susceptibility testing (DST), which is recommended by policymakers, typically requires additional specimen collection that delays effective treatment initiation. We examined whether cartridge extract (CE) from used Xpert TB-positive cartridges was, without downstream DNA extraction or purification, suitable for both genotypic DST (MTBDR*plus*, MTBDR*sl*), which may permit patients to rapidly receive a XDR-TB diagnosis from a single specimen, and spoligotyping, which could facilitate routine genotyping. To determine the limit-of-detection and diagnostic accuracy, CEs from dilution series of drug-susceptible and -resistant bacilli were tested (MTBDR*plus*, MTBDR*sl*). Xpert TB-positive patient sputa CEs (n = 85) were tested (56 Xpert-rifampicin-susceptible, MTBDR*plus* and MTBDR*sl*; 29 Xpert-rifampicin-resistant, MTBDR*sl*). Spoligotyping was done on CEs from dilution series and patient sputa (n = 10). MTBDR*plus* had high non-valid result rates. MTBDR*sl* on CEs from dilutions $\geq 10^3$CFU/ml ($C_T \leq 24$, >"low" Xpert semiquantitation category) was accurate, had low indeterminate rates and, on CE from sputa, highly concordant with MTBDR*sl* isolate results. CE spoligotyping results from dilutions $\geq 10^3$CFU/ml and sputa were correct. MTBDR*sl* and spoligotyping on CE are thus highly feasible. These findings reduce the need for additional specimen collection and culture, for which capacity is limited in high-burden countries, and have implications for diagnostic laboratories and TB molecular epidemiology.

Of the 10.4 million individuals with active tuberculosis (TB) in 2015, 580 000 were rifampicin (RIF) resistant or multidrug-resistant (MDR), defined as resistance to isoniazid (INH) and RIF[1]. Only ~20% of MDR-TB cases were diagnosed and started on treatment, and only half started on treatment were cured[1]. Extensively drug-resistant (XDR)-TB, which is MDR with resistance to a fluoroquinolone (FQ) and a second-line injectable drug (SLID) comprises 10% of MDR-TB cases, yet is even more underdiagnosed than MDR-TB, very costly to treat, and represents an emerging public health emergency[2–6].

Xpert MTB/RIF (Xpert) (Cepheid, United States) is a Food and Drug Administration and World Health Organization (WHO)-endorsed nucleic acid amplification test (NAAT) that rapidly detects *Mycobacterium tuberculosis* complex-DNA and RIF-resistance directly from sputa[7–9]. Over 25 million Xpert MTB/RIF cartridges have been consumed and over 30 000 test modules are installed worldwide[10]. The WHO and several national programmes recommend that if Xpert detects resistance, an additional sputum is collected for further drug

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susceptibility testing (DST) using line probe assays (LPAs), such as MTBDR*plus* (RIF and INH) and MTBDR*sl* (FQs and SLIDs), or phenotypic testing[1,9,11,12].

Patients, however, often do not rapidly return to the clinic to give another sputum or receive DST results. For example, a study in South Africa found that, even after MTBDR*plus* roll-out, time-to-treatment since initial diagnosis was unacceptably long (~55 days), and that this was partly due to challenges with patient loss-to-follow-up[13]. Furthermore, many patients do not produce sufficient sputum of adequate quality, especially in settings with high rates of HIV[14–17].

MTBDR*plus* and MTBDR*sl* have suboptimal sensitivity on specimens, and culture is often required prior to DNA extraction and further genotypic testing. Not only can this cause diagnostic delay, but many high burden countries lack the necessary biosafety and laboratory infrastructure for mycobacterial culture and DNA extraction[18–21]. Furthermore, culture can result in the loss of potentially clinically-meaningful resistance[22]. There is hence a need to reduce delays in the diagnosis of drug-resistant TB and use rapid methods that minimise reliance on culture through the direct testing of specimens[23].

Poor adherence to diagnostic algorithms using MTBDR*plus* and MTBDR*sl* has been reported[5,24,25]. For example, in South Africa, 34% of Xpert RIF-resistant patients failed to receive MTBDR*plus* and, of those confirmed to have MDR-TB, 28% did not receive second-line DST with MTBDR*sl* – despite both LPAs being mandated by the national programme[21]. Novel approaches to reduce this gap in the TB care cascade, which is worsened by the requirement for extra patient visits and additional specimen collection, is a major research priority[26,27]. If TB-testing and first- and second-line DST were possible on the first available specimen, fewer patients would potentially be lost and patients could be diagnosed earlier. This could result in earlier effective treatment initiation, fewer patient- and health systems-costs, and better long-term clinical outcomes.

We therefore conducted a proof-of-concept evaluation on whether *M. tuberculosis*-complex genomic DNA in the PCR-reaction mix from used Xpert cartridges (cartridge extract; CE) - that would otherwise be discarded - was detectable in an accurate manner using MTBDR*plus* and MTBDR*sl*. The feasibility of genotyping on CE by spoligotyping was also tested as this would potentially be useful for research laboratories and programmes seeking to implement routine strain surveillance. We explored the feasibility of Sanger sequencing on CE, as this may be useful for additional genotypic DST. Critically, we evaluated CE for all tests without additional downstream DNA extraction or purification, as not only would extraction require equipment not readily available in routine diagnostic laboratories in high burden settings, but it would complicate laboratory workflows and reduce the attractiveness of our approach. If the CE approach was feasible, it would mean that many laboratories would already have instrumentation available for mycobacterial genomic DNA extraction in the form of GeneXpert[10] and not need to procure new equipment.

Material and Methods

Ethics statement. Methods and protocols were carried out in accordance with relevant guidelines and regulations. The study was approved by the Health Research Ethics Committee of Stellenbosch University (N09/11/296) and the City of Cape Town (#10570). Permission was granted to use anonymised residual specimens collected as part of routine diagnostic practice and thus informed consent was waived.

Xpert MTB/RIF on dilution series of drug-susceptible- and drug-resistant bacilli. A triplicate ten-fold dilution series was made using phenotypically-confirmed drug-susceptible (DS)-TB, MDR-TB and XDR-TB clinical isolates (0–10^6 CFU/ml) in phosphate buffer (33 mM Na_2HPO_4, 33 mM KH_2PO_4; pH 6.8) with 0.025% Tween80 (Sigma-Aldrich, United States). Colony counts were done by plating on 7H11 Middlebrook agar (BD Biosciences, United States). Dilutions containing bacilli (1 ml aliquots) were tested by Xpert (54 in total: six dilutions ranging from 10^1–10^6 CFU/ml in triplicate for three strains and hence 18 dilutions each for the DS, MDR, and XDR strains) as well as 0 CFU/ml controls in triplicate, according to the manufacturer's instructions[9]. Used cartridges were stored at 4 °C prior to CE extraction within 24 h and freezing of the CE at −20 °C.

Xpert MTB/RIF on clinical specimens. Used Xpert-*TB*-positive cartridges done on sputa from people with symptoms suggestive of TB tested as part of the South African national TB diagnostic algorithm were collected between February 2016 and November 2016 from the National Health Laboratory Services (NHLS), a South African National Accreditation System-accredited, quality-assured diagnostics laboratory in Cape Town, South Africa[11]. Cartridges were stored at 4 °C prior to CE extraction within 5 days. Fifty-six Xpert TB-positive, RIF-susceptible cartridges and 29 Xpert-TB-positve RIF-resistant cartridges were collected. When the NHLS did a MGIT 960 liquid culture on sputum from RIF-resistant patients, we collected the isolate [20/29 (69%) had available isolates]. Isolates were not available from Xpert TB-positive, RIF-susceptible specimens as culture is not routinely done in these patients[11,28].

Recovery of mycobacterial genomic DNA from used Xpert MTB/RIF cartridges. The transparent diamond-shaped reaction chamber on the back of the cartridge was punctured with a sterile fixed-needle insulin syringe (1 ml; 29 G) (Fig. 1) in a biosafety level 2 cabinet. The full CE volume, typically ~15 µl, was withdrawn and stored in sterile, safe-lock micro-centrifuge tubes at −20 °C prior to analysis. Each cartridge and the surrounding surface was wiped down thoroughly with 1% sodium hypochlorite and 70% EtOH before and after extraction and UV sterilization was done after each batch of extraction. Used needles were discarded in a sharps container containing 1% sodium hypochlorite. Before and after each cartridge extraction session, hood surface area was decontaminated with sodium hypochlorite and EtOH and UV sterilised. No DNA extraction or purification steps were done on CE.

Line probe assays on cartridge extract. MTBDR*plus* and MTBDR*sl* (both version 2.0) were done according to the manufacturer's instructions[29,30] except for Xpert TB-positive, RIF-susceptible clinical specimens CE (n = 56), 7.5 µl CE was used as imput volume into MTBDR*plus* and MTBDR*sl*. For the Xpert TB-positive,

Figure 1. Cartridge extract extraction procedure. (**a**) The arrow indicates the diamond-shaped reaction chamber where the PCR amplification takes place and contains cartridge extract with mycobacterial genomic DNA. The needle is placed at the top of the diamond and the film is slowly and carefully pierced. (**b**) The needle is then slowly inserted deeper into the pocket and cartridge extract mix drawn out without piercing the other side.

RIF-resistant clinical specimen CEs (n = 29) and the dilution series, 5 µl (the recommended imput volume) CE was used in order to have enough CE remaining for Sanger sequencing. MTBDR*plus* and MTBDR*sl* results were reported as susceptible or resistant (RIF and INH for MTBDR*plus*; FQ and SLID for MTBDR*sl*), indeterminate [*M. tuberculosis* complex DNA-positive (reported by the test as TUB-positive) but no gene loci control bands] or TUB-band negative. LPA strips were read by two independent, experienced readers blinded to each other's calls and Xpert results (and, for dilution series, the strain used).

Spoligotyping on cartridge extract. Spoligotyping was done as described[31,32] on 2 µl CE from the MDR-TB dilution series. A set of Xpert TB-positive, RIF-susceptible cartridges (n = 10) done on specimens and separate from those used for genotypic DST on CE were collected with paired culture isolates from an ongoing research study. To determine whether the correct spoligotype was obtained from CE, crude DNA extracted through heat inactivation from the corresponding culture isolates was spoligotyped. SITVIT was used to identify strain families[33].

Targeted Sanger sequencing on cartridge extract. For dilution series, PCR clean-up and Sanger sequencing on 5 µl CE was done by the Stellenbosch University Central Analytical Facility using primers overlapping with LPA-binding sites (Supplementary Table 1). The *gyrA* and *rrs* regions in the DS-TB and XDR-TB strains were sequenced.

Data availability. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Results

Patient characteristics. A summary of the patient demographic and clinical data is in Table 1. For Xpert TB-positive, RIF-susceptible patients the median age (IQR) was 40 (31–49) years and for RIF-resistant specimens was 35 (23–42) years. 37/55 (67%) of RIF-susceptible patients and 12/20 (60%) of RIF-resistant patients were male.

Feasibility and diagnostic accuracy of MTBDR*plus* and MTBDR*sl* on dilution series Xpert TB-positive cartridge extract. Xpert detected *M. tuberculosis*-complex DNA in all dilutions $\geq 10^2$ CFU/ml and correctly identified RIF-susceptibility and -resistance (Fig. 2). MTBDR*plus* showed poor overall sensitivity for *M. tuberculosis*-complex DNA [22% (12/54) TUB-band-positive] in CE from Xpert TB-positive cartridges. MTBDR*plus* had high rates of non-actionable (TUB-band negative or TUB-band positive but indeterminate) and false RIF-heteroresistant results (Figs 2 and 3).

In contrast, MTBDR*sl* on CE had high sensitivity and specificity [87% (47/54) and 100% (9/9) respectively] for *M. tuberculosis*-complex DNA and a limit of detection of 10^3 CFU/ml. Susceptibility and resistance to FQs and SLIDs were correctly detected for all strains $\geq 10^3$ CFU/ml, corresponding to $C_T \leq 24$ (the higher C_T range of the Xpert "low" semiquantitation category) in all but one sample (one replicate of the MDR-TB strain was indeterminate for FQs; Fig. 3). Once non-actionable results were excluded, overall sensitivities and specificities of 87% (13/15) and 96% (25/26) for FQ-resistance and 94% (15/16) and 97% (30/31) for SLID-resistance, respectively were obtained. When the threshold of $\geq 10^3$ CFU/ml ($C_T \leq 24$) was applied, the sensitivity and specificity were both 100% (12/12 and 23/23, respectively) for FQs and for SLIDs (12/12 and 24/24, respectively).

Patient Characteristics	Xpert TB-positive	
	Xpert rifampicin-susceptible (n = 56)	Xpert rifampicin - resistant (n = 29)
Age, median (IQR)	40 (30–49)	35 (23–42; p = 0.086)
Male gender (%)	37/55 (67)*	12/20 (60)*
Smear-positivity (%)	37/50 (74)*	6/16 (38)*
Culture-positivity (%)	Not done	19/21 (90)*
TTP, median (IQR)	N/A	10 (8–20)
Xpert C_T, median IQR	17.9 (16.3–22.1)	20.5 (16.9–24.8)

Table 1. Patient demographic and clinical data. *Missing data: Gender (n = 1 for RIF-susceptible, n = 9 for RIF-resistant); Smear status (n = 6 for Xpert RIF susceptible, n = 13 for Xpert RIF-resistant); Culture results (n = 8 for RIF-resistant results). Abbreviations: Xpert - Xpert MTB/RIF; IQR - interquartile range; TTP - time-to-positivity; C_T - cycle threshold values.

Diagnostic accuracy of MTBDR*plus* and MTBDR*sl* on clinical specimen Xpert TB-positve cartridge extract. *Xpert MTB/RIF rifampicin-susceptible specimens.* As with the dilution series, MTBDR*plus* had high rates of indeterminate and false-resistance results on clinical specimen CE (Table 2). However, most MTBDR*sl* results from Xpert TB-positive, RIF-susceptible clinical specimen CE were valid (TUB-positive, not indeterminate, and no false-susceptible or -resistant results): 53/56 (95%) for FQ (two TUB-band negative, one indeterminate) and 51/56 (91%) for SLID (two TUB-band negative, three indeterminate). The few CEs that yielded indeterminate MTBDR*sl* results corresponded to "low" or "very low" Xpert semiquantitation levels ($C_T > 24$). The median (IQR) C_T of indeterminate (26.3, 24.4–26.7) vs. determinate (17.62, 15.6–20.6) MTBDR*sl* results differed ($p < 0.001$), indicating that indeterminate results are likely a function of low DNA concentrations in CE. There was not enough CE volume remaining or a matching clinical isolate for confirmatory testing from the three MTBDR*sl*-detected SLIDs resistant patients.

Xpert MTB/RIF rifampicin-resistant specimens. MTBDR*sl* on Xpert TB-positive, RIF-resistant CE had 24/29 (83%) valid results. For FQs, 14/24 (58%) were susceptible and 10/24 (42%) were resistant. For SLIDs, 15/24 (63%) were susceptible and 9/24 (37%) resistant. The five non-valid results were TUB-band-negative [2/29 (7%)] or indeterminate for both FQs and SLIDs [3/29 (10%); Table 2]. All CEs corresponding to the higher C_T ranges of the Xpert "low" semiquantitation category ($C_T \leq 24$) had valid results, whereas those that had indeterminate or TUB band-negative results corresponded to the lower semiquantitation levels ($C_T > 25.0$). The median (IQR) C_T of indeterminate (29.1, 26.5-31.1) vs. determinate (20.5, 16.−23.2) results differed significantly ($p < 0.001$).

MTBDRplus and MTBDRsl performance on Xpert MTB/RIF cartridge extract by smear status. MTBDR*plus* had high non-valid result rates irrespective of smear status. However, MTBDR*sl* on CE from smear-negative sputums had significantly higher rates of non-actionable results [5/23 (22%) vs. 1/43 (2%) for FQ, p = 0.01; 6/23 (23%) vs. 2/43 (5%) for SLIDs, p = 0.01] compared to smear-positive patients (Supplementary Table 2).

Concordance of MTBDRsl results on cartridge extract and culture isolates. Of the 29 Xpert TB-positive, RIF-resistant patients, 20 (69%) matched culture isolates were collected while the remaining nine had negative or contaminated cultures. The CEs and isolates showed 18/20 (90%) matching MTBDR*sl* FQ results and 17/20 (84%) matching SLID results. There were 2/20 (10%) discordant TUB-band MTBDR*sl* results on culture isolates (one TUB-positive and FQ and SLID sensitive, one TUB-positive and FQ and SLID resistant) where both CE results were TUB-band negative. There was also 1/20 (5%) discordant SLID result (CE showed resistance but the isolate showed susceptibility). Importantly, all three discordant results corresponded to a "very low" semiquantitation ($C_T > 28.0$). All TUB-band, susceptibility and resistance calls were concordant at $C_T \leq 24$, indicating that the diagnostic accuracy of MTBDR*sl* on CE vs. isolates is likely comparable at this threshold.

Spoligotyping on cartridge extract. *Dilution series.* Spoligotyping resulted in a readable strain type for dilutions $\geq 10^3$ CFU/ml, corresponding to the same threshold seen for MTBDR*sl*.

Clinical specimens. Spoligotyping on specimen CE and crude DNA from matched culture isolates were highly concordant 10/10 (100%) at the threshold defined by the dilution series (Table 3). A variety of strain families were observed with Beijing as the predominant family type [6/10 (60%)] as well as 2/10 (20%) LAM and 2/10 (20%) T1 family type.

Targeted sequencing on extract from used Xpert MTB/RIF cartridges. *Dilution Series.* Targeted Sanger sequencing was done on dilution series CE. For the *rrs* PCR on CE, sequence shorter than the expected length was observed. PCR of *gyrA* from CE from dilutions 10^3–10^4 CFU/ml resulted in sequence expected length, however high background noise occurred and the sequence did not align to H37Rv [NC_000962]. *gyrA* on CE from dilutions 10^5–10^6 CFU/ml aligned to the reference genome, however, several SNPs known to be present in the resistance determining regions (identified by sequencing of the corresponding isolate) were not detected. Due to the relatively poor limit of detection and accuracy of Sanger sequencing on dilution series CE, we did not do sequencing on clinical specimen CEs.

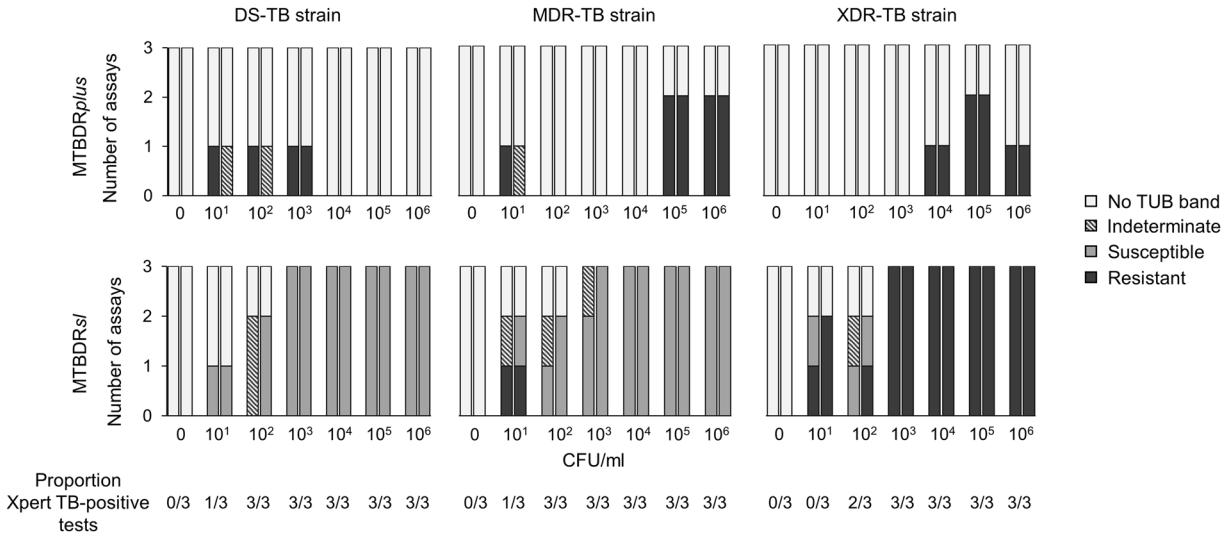

Figure 2. Results of MTBDR*plus* and MTBDR*sl* on Xpert CE from a dilution series of DS-, MDR- and XDR-TB strains. MTBDR*plus* (irrespective of concentration and strain) had high TUB-band negativity and indeterminate rates. However, MTBDR*sl* had high sensitivity and specificity and low indeterminate rates. For each dilution, left bars are for rifampicin (MTBDR*plus*, top row) or fluoroquinolones (MTBDR*sl*, bottom row) and right bars are for isoniazid (MTBDR*plus*) or second-line injectables (MTBDR*sl*). Data from LPA on DS-TB, MDR-TB and XDR-TB strains are shown. The experiment was done in triplicate. Abbreviations: CFU – colony forming; DS-TB – drug susceptible TB; MDR-TB – multidrug resistant TB; XDR-TB – extensively drug resistant TB; units; Xpert - Xpert MTB/RIF.

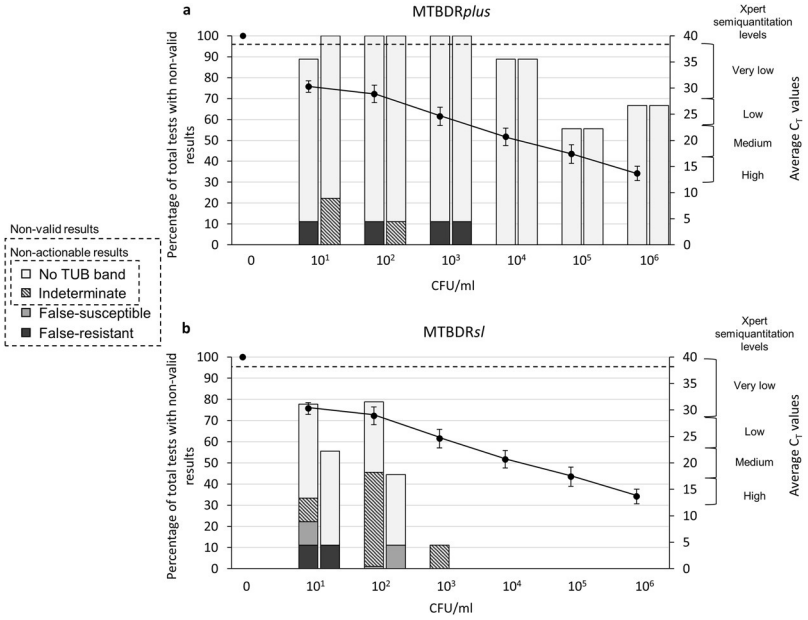

Figure 3. Xpert MTB/RIF quantitative information [average cycle threshold (C_T) values] (line graph, right y-axes) versus bacterial load (CFU/ml) in a triplicate dilution series for MTBDR*plus* (**a**) and MTBDR*sl* (**b**) done on CE. Left y-axes (bars) show the proportion of assays with non-valid results, disaggregated into non-actionable (TUB-band negative, indeterminate) and non-valid (false-susceptible, false-resistant). For each dilution, left bars are for rifampicin (MTBDR*plus*, top) or fluoroquinolones (MTBDR*sl*, bottom) and right bars are for isoniazid (MTBDR*plus*) or second-line injectables (MTBDR*sl*). Beyond 10^3 CFU/ml, there were no false resistance or susceptibility calls for MTBDR*sl*, which corresponds to $C_T \leq 24$. $C_T \geq 38$ (horizontal dashed line) correspond to a negative Xpert. Error bars show standard error (SE) of average C_T. Right y-axes show C_T corresponding to Xpert semiquantitation levels of very low ($C_T > 28$), low ($C_T = 22–28$), medium ($C_T = 16–22$) and high ($C_T < 16$). Pooled data from LPAs on DS-TB, MDR-TB and XDR-TB strains are shown. Abbreviations: CFU – colony forming; DS-TB – drug susceptible TB; MDR-TB – multidrug resistant TB; XDR-TB – extensively drug resistant TB; CFU – colony forming units; Xpert - Xpert MTB/RIF.

All Xpert TB-positive specimens						Xpert TB-positive specimens with $C_T \leq 24$					
Xpert rifampicin-susceptible				Xpert rifampicin-resistant		Xpert rifampicin-susceptible				Xpert rifampicin-resistant	
MTBDR*plus* (n = 56)		MTBDR*sl* (n = 56)		MTBDR*sl* (n = 29)*		MTBDR*plus* (n = 49)		MTBDR*sl* (n = 49)		MTBDR*sl* (n = 20)*	
TUB-band positive (%) 47/56 (84)		TUB-band positive (%) 47/54 (96)		TUB-band positive (%) 27/29 (93)		TUB-band positive (%) 45/49 (92)		TUB-band positive (%) 49/49 (100)		TUB-band positive (%) 20/20 (100)	
Rifampicin (%)		**Fluoroquinolones (%)**		**Fluoroquinolones (%)**		**Rifampicin (%)**		**Fluoroquinolones (%)**		**Fluoroquinolones (%)**	
Susceptible	0/47 (0)	Susceptible	53/54 (98)	Susceptible	14/27 (52)	Susceptible	0/56 (0)	Susceptible	49/49 (100)	Susceptible	11/20 (55)
Resistant	47/47 (100)	Resistant	0/54 (0)	Resistant	10/27 (37)	Resistant	45/49 (92)	Resistant	0/49 (0)	Resistant	9/20 (45)
Indeterminate	0/47 (0)	Indeterminate	1/54 (2)	Indeterminate	3/27 (11)	Indeterminate	0/49 (0)	Indeterminate	0/49 (0)	Indeterminate	0/20 (0)
Isoniazid (%)		**Second-line injectables %)**		**Second-line injectables (%)**		**Isoniazid (%)**		**Second-line injectables (%)**		**Second-line injectables (%)**	
Susceptible	11/47 (23)	Susceptible	48/54 (88)	Susceptible	15/27 (56)	Susceptible	11/49 (23)	Susceptible	46/49 (94)	Susceptible	14/20 (70)
Resistant	0/47 (0)	Resistant	3/54 (6)	Resistant	9/27 (33)	Resistant	0/49 (0)	Resistant	1/49 (2)	Resistant	6/20 (30)
Indeterminate	36/47 (77)	Indeterminate	3/54 (6)	Indeterminate	3/27 (11)	Indeterminate	34/49 (69)	Indeterminate	2/49 (4)	Indeterminate	0/20 (0)
TUB-band negative (%) 9/56 (16)		**TUB-band negative** (%) 2/56 (4)		**TUB-band negative** (%) 2/29 (7)		**TUB-band negative** (%) 4/49 (8)		**TUB-band negative** (%) 0/49 (0)		**TUB-band negative** (%) 0/20 (0)	

Table 2. Results of MTBDR*plus* and MTBDR*sl* drug susceptibility testing using cartridge extract on clinical specimens. MTBDR*plus* had high indeterminate results and rifampicin-resistance false-positive rates. MTBDR*sl* had low indeterminate rates for both DS-TB and DR-TB specimens and performance improved when MTBDR*sl* was done only on specimens with $C_T \leq 24$. *For the 29 Xpert RIF-resistant specimens we were able to retrieve 20 paired culture isolates used for MTBDRsl. 18/20 matched for FQs and 17/20 for SLIDs, the 2/20 done on crude DNA had LPA results whereas the LPA on CE was TUB-band negative. 1/20 did not match for the SLID resistance. Both the TUB-band negative and discordant SLIDs result corresponded to "very low" semi-quantitation level. When defined threshold of $C_T \leq 24$ was applied all LPAs on CE matched LPA from culture isolates.

Material used for spoligotyping	Xpert MTB/RIF semi-quantitation level	C_T	Spoligotyping pattern	Family
Negative Control			N/A	N/A
H37Rv				H37Rv
BCG				BOVIS1_BCG
Isolate (a)	-	-		T1
CE (a)	MEDIUM	20.85		T1
Isolate (b)	-	-		BEIJING
CE (b)	HIGH	12.28		BEIJING
Isolate (c)	-	-		BEIJING
CE (c)	HIGH	16.20		BEIJING
Isolate (d)	-	-		BEIJING
CE (d)	HIGH	15.00		BEIJING
Isolate (e)	-	-		BEIJING
CE (e)	LOW	24.40		BEIJING
Isolate (f)	-	-		BEIJING
CE (f)	HIGH	14.74		BEIJING
Isolate (g)	-	-		LAM9
CE (g)	LOW	20.86		LAM9
Isolate (h)	-	-		BEIJING
CE (h)	MEDIUM	21.35		BEIJING
Isolate (i)	-	-		LAM3
CE (i)	HIGH	16.28		LAM3
Isolate (j)	-	-		T1
CE (j)	MEDIUM	21.88		T1

Table 3. Spoligotyping results performed on CE done on sputum specimens and paired culture isolates at defined threshold ($C_T \leq 24$).

Discussion

Our key findings are: (1) MTBDR*sl* on CE enabled genotypic drug-susceptibility testing for FQs and SLIDs with high accuracy and low indeterminate rates when the Xpert semiquantitation category was at least "medium" or $C_T \leq 24$ (corresponding to $\geq 10^3$ CFU/ml), (2) spoligotyping was feasible and accurate on CE at the same threshold, (3) MTBDR*plus* was not feasible or accurate on CE and (4) neither was Sanger sequencing. These data have implications for the routine diagnosis of drug-resistant TB, researchers, and test developers.

Xpert is one of the most widely used tests for TB and drug-resistance[9,34] and although it is a significant advancement, time-to-treatment – especially for MDR- and XDR-TB - is still very long[35–38]. Our results show that accurate second-line drug testing using MTBDR*sl* is possible on CE from Xpert cartridges that would otherwise be discarded. This potentially allows for a rapid, single-specimen diagnosis of XDR-TB without additional specimen collection. Importantly, we defined a threshold at which this approach is feasible, meaning that MTBDR*sl* assays do not need to be wasted on CE unlikely to give a valid result. Using this threshold, we showed that on clinical specimen CEs, susceptibility and resistance calls were concordant with those from the isolate[19,39]. Furthermore, we showed that it is possible to do spoligotyping on CE at this threshold, which will inform strain surveillance and research studies on relapse and reinfection where specimens are limited. Collectively, these findings may reduce the need for culture.

Although our data suggest that the MTBDR*sl* will work on CE from cartridges with an Xpert semiquantitation category of at least "low", we suggest that, in laboratories where C_T cannot be readily calculated, a category of at least "medium" is used to guide use of this strategy unless the laboratory is comfortable with some semiquantitation low specimens not having a valid MTBDR*sl* result. Alternatively, if smear microscopy is available, smear-positivity may be used to guide use of CE, however, some smear-negative specimens in whom this approach would work (10^3-10^4 CFU/ml) would be unnecessarily excluded.

When considering the CE approach, it is important to identify a safe and sterile environment to avoid contamination. Although Xpert sample reagent as well as the sonication lysis step within the cartridge helps ensure *M. tuberculosis* is no longer culturable (and therefore poses minimal infectious risk[40]), steps to minimise the risk of *rpoB* amplicon cross-contamination should be implemented. These can include working in a dedicated cabinet or room and sterilising the work area with UV and disinfectant after CE is collected. Importantly, however, cross-contamination of other Xpert cartridges with *rpoB* amplicons appears unlikely. Although Xpert's automated pre-amplification wash step does not remove large pieces of debris-associated genomic DNA, it does efficiently remove high concentrations of contaminating *rpoB* amplicons from assays like MTBDR*plus*[41,42]. NAATs without such a wash step may be more vulnerable to CE *rpoB* amplicon cross-contamination.

Our study differed from a previous study which showed that sequencing, MTBDR*plus*, spoligotyping and MIRU-VNTR typing are feasible on the sputum mixed with Xpert sample reagent[43]. However, this sample reagent method has a number of disadvantages: 1) often no volume remains, 2) prolonged exposure to sample reagent degrades DNA and potentially introduces mutations[9,40], and 3) it still requires DNA extraction prior to PCR. Furthermore, DNA extraction adds cost and is not always feasible in laboratories in high burden countries; whereas the CE method yields directly usable material and does not need additional extraction or purification steps. An advantage, however, of using the sputum mixed with Xpert reagent buffer, is that it likely avoids high MTBDR*plus* error rates (TUB-band negative, indeterminate, false-positive) seen with CE. This could be due to the large amount of *rpoB* amplicons in Xpert TB-positive CE, which share binding sites with MTBDR*plus* probes and confound the assay resulting in non-valid results. Furthermore, the *rpoB* PCR that occurs as part of MTBDR*plus* may sequester reagents away from the multiplex *inhA* and *katG* amplification reactions. Testing for mutations conferring INH resistance using CE might hence be possible with the Genoscholar INH II line probe assay (which does not contain *rpoB* probes)[44]. Sequencing from CE thus primarily appears to be driven by *rpoB* amplicon interference (although a PCR clean-up was done prior to sequencing, this would have co-purified *rpoB* amplicons). Further investigation with primers optimised for minimal-input DNA may be warranted, however, it appears that, for sequencing, the best approach to avoid contaminating amplicons might be to PCR from the specimen-Xpert sample reagent mixture[43]. Given the rates of non-valid CE results below the defined threshold, we suggest that specimen-Xpert sample reagent mix be kept in the event that C_T falls >24.

The results presented here should be interpreted in context of their limitations. For the clinical specimens tested from the NHLS, matched culture isolates were not available for Xpert RIF-susceptible specimens, as per the national algorithm. However, the dilution series experiments showed very high concordance between MTBDR*sl* on CE vs. the isolates. The utility of CE depends on the downstream test used and MTBDR*sl* susceptible or non-valid results should be interpreted from CE the same as when they are done on patient specimens (i.e., further investigation, including culture, is recommended)[45]. Realistically, cartridges may need to be transported from remote locations and so the effect of storing cartridges for prolonged duration (>5 days) and at ambient temperature requires further systematic testing. Using bacilli in buffer can have limitations, which is why we also used patient clinical specimens, which are a better material to test than bacilli added to sputum (the former has bacilli within a sputum matrix, whereas in the latter bacilli are typically freely floating in bubbles).

In conclusion, CE contains template DNA for second-line DST using MTBDR*sl*, resulting in accurate results highly concordant with those from isolates, provided bacillary load in the specimen corresponds to at least a "medium" Xpert semiquantitation category of $C_T \leq 24$. This potentially facilitates XDR-TB detection within days from a single specimen. Spoligotyping is also feasible on CE and works consistently at this threshold. Our method provides an opportunity to potentially reduce the burden associated with addition specimen collection, such as patient treatment delay, pre-treatment loss-to-follow-up, and increased patient and provider costs. Furthermore, it shows that material that would otherwise be discarded still holds diagnostic utility.

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Author Contributions

G.T., R.W. and M.D.V. conceived the experiments. R.V., B.D., S.P., N.K. and A.R. conducted the experiments. J.S. and T.D. provided specimens and data from the NHLS. R.V. and B.D. analysed the data. All authors reviewed the manuscript.

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Mycobacterial genomic DNA from used Xpert MTB/RIF cartridges can be utilised for accurate second-line genotypic drug susceptibility testing and spoligotyping

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Supplementary

Table 1: Primer details for amplification of fragments of the *gyrA* and *rss* regions using cartridge extract from the dilution series

Target region	Primer name	Primer sequence (5' – 3')	Annealing temperature (°C)	Expected size (bp)
gyrA	gyrA_F	TGACATCGAGCAGGAGATGC	62	344
	gyrA_F	GGGCTTCGGTGTACCTCATC		
rrs290	rrs290_F	TGCTACAATGGCCGGTACAA	62	290
	rrs290_R	CTTCCGGTACGGCTACCTTG		

Table 2: Results of MTBDR*plus* and MTBDR*sl* drug susceptibility testing using cartridge extract on clinical specimens stratified to smear status. MTBDR*plus* had high indeterminate results rifampicin-resistance false-positive rates for both smear positive and smear negative specimens. MTBDR*sl* had low indeterminate rates for both RIF-susceptible and RIF–resistant specimens. Smear negative specimens had higher rates of non-actionable results for MTBDR*sl*.

Xpert positive rifampicin-susceptible and -resistant cartridges*								
Smear-positive specimens				**Smear-negative specimens**				
MTBDR*plus* (n=37)		**MTBDR*sl* (n=43)**		**MTBDR*plus* (n=13)**		**MTBDR*sl* (n=23)**		**p-values†**
TUB-band positive 32/37 (86)		**TUB-band positive 42/43 (98)**		**TUB-band positive 10/13 (77)**		**TUB-band positive 21/23 (91)**		
Rifampicin (%)		**Fluoroquinolones** (%)		**Rifampicin** (%)		**Fluoroquinolones** (%)		p=0.01
Susceptible	0/32 (0)	Susceptible	41/42 (98)	Susceptible	0/10 (0)	Susceptible	17/21 (81)	
Resistant	32/32 (100)	Resistant	1/42 (2)	Resistant	10/10 (100)	Resistant	1/21 (5)	
Indeterminate	0/32 (0)	Indeterminate	0/42 (0)	Indeterminate	0/10 (0)	Indeterminate	3/21 (14)	
Isoniazid (%)		**Second-line injectables** (%)		**Isoniazid** (%)		**Second-line injectables** (%)		p=0.01
Susceptible	10/32 (31)	Susceptible	39/42 (93)	Susceptible	0/10 (0)	Susceptible	16/21 (76)	
Resistant	0/37 (0)	Resistant	2/42 (5)	Resistant	0/10 (0)	Resistant	1/21 (5)	
Indeterminate	22/32 (69)	Indeterminate	.1/42 (2)	Indeterminate	10/10 (100)	Indeterminate	4/21 (19)	
TUB-band negative				**TUB band-negative**				p=0.015
5/37 (14)		1/43 (2)		3/13 (23)		2/23 (9)		

* Table shows results from both Xpert positive RIF-susceptible and RI-resistant specimens. RIF-susceptible samples had MTBDR*plus* and MTBDR*sl* done. RIF-resistant specimens only had MTBDR*sl*. 37/56 (66%) RIF-susceptible specimens were smear positive; 13/56 (23%) were smear negative and 6/56 (11%) had no smear results. 6/29 (20%) of RIF-resistant specimens were smear positive while 10/29 (35%) were smear negative and 13/29 (45%) had no smear result.

Appendix III

Chapter 2 Supplement

Non-actionable Results, Accuracy, and Effect of First- and Second-line Line Probe Assays for Diagnosing Drug-Resistant Tuberculosis, Including on Smear-Negative Specimens, in a High-Volume Laboratory

Supplement Table of Contents:

Methods

Additional pDST information

Solid media were incubated for two weeks. Colony forming units (CFUs) on controls without drugs were compared to the drug-containing tube and, if more numerous on drug media, designated resistant. H37Rv #25177 was the control strain. Ziehl Neelsen (ZN) smear microscopy and the TB Ag MPT64 Rapid antigen test (KAT Laboratory and Medical, South Africa) on a culture-positive isolate was used as the confirmatory *Mtb* complex test. pDST was performed programmatically using the 7H11 indirect proportion agar slants method with WHO-recommended critical concentrations of 0.2 µg/ml, 2.0µg/ml, and 2.0µg/ml for isoniazid, ofloxacin and amikacin, respectively [1, 2].

Additional information on the definition of "discrepant" results and Sanger sequencing

An LPA result was classified as discrepant to a comparator if the LPA, when repeated on the isolate if done initially directly, gave a differential final call to the comparator. For *rpoB*, the rifampicin-resistant determining region (RRDR) was sequenced whereas for isoniazid *katG* (315 gene region) and *inhA* promoter (-8, -15, -16 region) were sequenced (only MTBDR*plus*-susceptible, pDST-resistant discrepants underwent sequencing). The *gyrA* (85-97 region) quinolone resistance determining region (QRDR) and *rrs* (1401,1402 region) were sequenced [3-7]. No resistance was detected by MTBDR*sl* in *gyrB* and *eis* and these regions were not sequenced. Standard genomic DNA preparation kits (Illumina, San Diego, USA) were used per the manufacturer. Briefly, a 200µl isolate aliquot was heat inactivated at 100 °C for 30 min. PCR on a mixture containing 2µl crude template DNA, 5µl Q buffer, 2.5µl 10×buffer, 2µl 25mM $MgCl_2$, 4µl of deoxynucleoside triphosphates (dNTPs) (10 mM concentration of each), primer rpoB set (50pmol/µl) (5′-CGATCACACCGCAGACG-3′), primer katG set (50pmol/µl) (forward, 5′-CATGAACGACGTCGAAACAG-3′; and reverse, 5′-CTCTTCGTCAGCTCCCACTC-3′), primer inhA promoter set (50pmol/µl) (forward, 5′-AGAAAGGGATCCGTCATGGT-3′; and reverse, 5′-GTCACATTCGACGCCAAAC-3′), QRDR primer set (50pmol/µl) (forward, 5′-TGACATCGAGCAGGAGATGC-3′; and reverse, 5′-GGGCTTCGGTGTACCTCATC-3′), the rrs primer set (50pmol/µl) (forward, 5′-GTAATCGCAGATCAGCAAC-3′; and reverse, 5′-GTGATCCAGCCGCACCTT-3′), and 0.125µl HotStarTaq DNA polymerase (Qiagen, Germany) and made to 25µl with distilled water (dH_2O). Amplification was initiated by incubation at 95°C for 15 min, followed by 35 to 45 cycles of 94°C for 45s, 62°C for 45s, and 72°C for 45s. After the last cycle, the samples were incubated at 72°C for 10

min. Amplification products were sequenced using an ABI 3130XL analyzer, and chromatographs were analyzed using BioEdit Sequence Alignment Editor (v7.2.5) comparing them to the *Mtb* H37Rv reference genome (accession number:AL123456).

Additional information on the diagnostic algorithms in the "before" and "after" periods and analysis

During the before period, MTBDR*plus* was performed directly using only smear-positive specimens, while DST was performed on confirmed culture positive isolates using solid media for second-line drug testing. For smear-negative specimens, MTBDR*plus* was performed indirectly using the culture positive isolate with pDST following. In the after period, first- and second-line drug testing was performed directly using MTBDR*plus* and MTBDR*sl*, irrespective of smear status, followed by pDST on the culture isolate (**Figure 1**). Data compared across periods included sputum collection dates, test result dates, number of patients who failed to receive first- or second-line DST, treatment initiation times, and drug susceptibility results. In the "before period", clinicians sent multiple specimens per patient for DST and for every specimen a new barcode number was assigned by the laboratory. De-duplication was done by removing repeated initials and dates of birth; however, these fields had not always been captured by the programme. Data on failed second-line pDSTs (lost viability, contaminated) was not available in the "before period". In the "after period", specimens from the same individual received the same barcode; thereby enabling the identification of unique specimens and easy de-duplication. When analysing "after period", MTBDR*plus* rifampicin-resistant results were used to select patients for inclusion in analyses.

Results

TB Detection

MTBDR*plus*: Of the Xpert rifampicin-resistant specimens, 90% (849/951) were culture-positive. For direct testing, sensitivity was 88% (751/849) whereas specificity was [43% (40/93)] (**Table 1**). Sensitivity on sputum was lower than on isolates (**Supplementary Table 4**).

MTBDR*sl*: For direct testing, sensitivity was 82% (696/849) with culture as the reference standard, whereas specificity was 51% (47/93) (**Table 1**). Sensitivity on sputum was lower than on isolates (**Supplementary Table 4**).

Xpert and MDR detection

Of the Xpert rifampicin-resistant culture-positives, 12% (98/849) were MTBDR*plus* TUB-negative and 1% (5/849) MTBDR*plus* TUB-positive rifampicin-indeterminate. The PPV of Xpert-detected rifampicin resistance for MTBDR*plus*-defined MDR was 68% (509/746) [92% (686/746) and 70% (521/746) for MTBDR*plus*-defined rifampicin- and isoniazid-resistance, respectively].

Supplementary Table 1: Terms and definitions used in the interpretation of MTBDR*plus* and MTBDR*sl* results

Term[a]	Definition
TB detection	
TUB-band positive	A positive TUB-band indicates the presence of the members of *Mtb* complex. No DST pattern may be interpreted if not TUB-band positive.
TUB-band negative	The absence of a TUB-band indicates no *Mtb* complex detected.
Valid test	Presence of amplification control (AC) and conjugate control (CC) band. Banding intensity of the AC serves as the reference for further interpretation of drug locus control bands, wild type (WT) and mutation (MUT) bands.
Invalid test	Absent or faint AC and/or CC band, test should be repeated.
Uninterpretable	All banding patterns on strip are very light in comparison to AC band intensity or loci controls are absent with TUB band present or absent.
DST (Possible only if AC, CC band present and TB detection occurs; each result below applies to an individual drug class)	
Determinate	The AC, CC bands must be present first, presence of TUB-band and drug locus control band is present.
Indeterminate	An indeterminate result for a drug occurs if the corresponding locus control is missing while the AC, CC and TUB bands are present or absent.
Resistance	The absence of any WT bands with the presence of a MUT bands indicates resistance. The presence of a WT band with a mutant band indicates heteroresistance. The absence of a WT band and no MUT band implies resistance. For resistance determination, *rpoB*, *katG* and the *inhA* promoter region are amplified by MTBDR*plus* whereas *gyrA*, *gyrB*, *rrs*, and *eis* are amplified by MTBDR*sl*.
Susceptible	The presence of all WT bands to a specific gene and absence of a MUT band indicates susceptibility to a drug. This is, in this case, equivalent to "resistance not detected", however, we used "susceptible" as the study is programmatic, and this is the language the programme uses.
Overall (Applies to entire strip)	
Non-actionable	AC, CC present or absent, TUB negative or, if TUB-band positive, at least one indeterminate drug locus control.
Actionable	TUB positive and determinate for one or more drugs.
Operational sensitivity[b]	The number of samples which the LPA failed to detect *Mtb* resulting in an absence of the TUB-band and was missing one or more controls (AC, CC band)

[a] – *GLI Line probe assays for drug resistant tuberculosis detection et al, 2018 [8]*

[b] –*Intermediate, indeterminate and uninterpretable diagnostic test results, Dimel et al, 1987[9]*

Supplementary Table 2: Demographic and clinical characteristics showing no differences between patients who had phenotypically-defined second-line resistance and those that did not, nor between patients with different types of second-line resistance. Data are median (IQR) or n (%).

	All patients (n=849)	No second-line resistance (n=505)	Second-line drug resistance			
			Overall (n=103)	p-value*	Either FQ resistance/ SLID resistance (n=76)	Both FQ and SLID resistance (n=27)
Demographic						
Age, years	36 (28-45)	35 (28-44)	36 (24-45)	0.838	33 (27-43)	40 (29-50)
Men	517 (61)	319 (63)	62 (60)	0.335	46 (61)	16 (59)
Clinical						
HIV status						
Known (n=404)	404 (48)	233 (46)	49 (48)	0.879	37 (49)	12 (44)
Positive	203 (50)	112 (22)	23 (22)	0.506	18 (24)	5 (19)
Negative	201 (50)	121 (24)	26 (25)	0.633	19 (22)	7 (26)
Unknown (n=445)	445 (52)	272 (54)	54 (52)	0.879	30 (51)	15 (56)
Smear status						
Positive	373 (44)	263 (52)	56 (54)	0.731	43 (57)	13 (48)
Negative	476 (56)	242 (48)	47 (46)	0.731	25 (43)	14 (52)

*Comparisons between all patients with second-line drug resistant detected by phenotypic DST to those with no second-line phenotypic resistance detected.

Abbreviations: DST–drug sensitivity testing, FQ-fluoroquinolone, SLID–second-line injectable drugs.

Supplementary Table 3: *Mycobacterium tuberculosis* detection. MTBDR*sl* is likely to miss cases of direct detection of *M. tuberculosis* complex DNA (TUB-band positivity) in smear-negative sputum (low sensitivity) and both MTBDR*plus* and MTBDR*sl* are likely to misclassify *Mtb* cases as false positives due to reduced specificity and NPV estimates. Data are % (n/N) and 95% CI.

	MTBDR*plus* (n=849)			MTBDR*sl* (n=849)		
	Overall (n=849)	Smear-positive (n=373)	Smear-negative (n=476)	Overall (n=849)	Smear-positive (n=373)	Smear-negative (n=476)
Proportion of culture-positives missed (operational sensitivity)						
AC and/or CC missing TUB negative	0 (0/849)	0 (0/373)	0 (0/476)	3 (28/849) 2-4	1 (2/373) 0-2	5 (26/476) 3-7 †p<0.001
TUB-negative but AC and CC present	12 (98/849) 9-13	3 (10/373) 1-4	18 (88/476) 15-22 †p<0.001	18 (125/849) 16-21 *p<0.001	7 (24/373) 4-10 *p<0.001	27 (101/476) 23-32 †p<0.001 *p<0.001
TUB positive but missing all gene loci	1 (5/849)	0 (1/373)	1 (4/476)	6 (48/849)	3 (10/373)	8 (38/476)
Total non-actionable	12 (103/849) 9-13	3 (11/373) 1-4	19 (92/476) 15-22 †p<0.001	24 (201/849) 15-20 *p<0.001	10 (36/373) 5-10 *p=0.006	35 (165/476) 23-31 †p<0.001 *p=0.002
Actionable result rates						
Actionable	88 (746/849) 86-90	97 (362/373) 94-98	81 (384/476) 77-84 †p<0.001	82 (696/849) 79-84 *p=0.002	93 (347/373) 90-95 *p=0.063	73 (349/476) 69-77 †p<0.001 *p=0.025
Diagnostic accuracy						
Sensitivity	88 (751/849) 86-90	97 (363/373) 94-98	82 (388/476) 77-84 †p<0.001	82 (696/849) 79-84 *p<0.001	93 (347/373) 90-95 *p=0.006	73 (349/476) 69-77 †p<0.001 *p=0.002
Specificity	43 (40/93) 32-53	36 (4/11) 10-69	44 (36/82) 32-54 †p=0.635	51 (47/93) 40-61 *p=0.303	73 (8/11) 39-93 *p=0.086	48 (39/82) 36-58 †p=0.117 *p<0.001
PPV	93 (784/840) 92-94	98 (396/404) 96-99	88 (388/436) 85-91 †p=0.001	94 (696/742) 93-96 *p=0.705	98 (347/350) 97-99 *p=0.199	89 (349/392) 86-92 †p<0.001 *p=0.985
NPV	28 (40/145) 23-35	26 (4/15) 7-55	27 (36/130) 20-36 †p>0.999	24 (47/200) 15-28 *p=0.388	23 (8/34) 10-41 *p=0.813	23 (39/166) 18-28 †p=0.996 *p=0.409

*Comparisons within rows and between columns (MTBDR*plus* vs MTBDR*sl*)

†Comparisons within rows and between columns by different smear status

Abbreviations: AC-amplification control, CC-conjugate control, PPV-positive predictive value, NPV-negative predictive value

Supplementary Table 4: MTBDRplus and MTBDRsl results from culture isolates. Sensitivities were high and exceeded direct testing values. Data are % (n/N) and 95% CI.

		Sensitivity	Specificity
MTBDR*plus*	TB	97 (151/155) 93-99	Non-calculable as all culture-positive
MTBDR*sl*	TB	100 (224/224) 98-100	Non-calculable as all culture-positive
	Fluoroquinolones	92 (12/13) 51-99	100 (211/211) 95-100
	Second-line injectables drugs	100 (6/6) 54-100	100 (218/218) 95-100
	Fluoroquinolone and second-line injectable drugs	100 (2/2) 15-100	100 (207/207) 95-100

Abbreviation: TB-tuberculosis

Supplementary Table 5: Re-classification of discrepant results showed an increase in sensitivity and specificity estimates especially in fluoroquinolones as most results were in favour of MTBDR*s/* assay and second-line injectables estimates did improve moderately. Data are % (n/N) and 95% CI.

		Overall		Smear-positive		Smear-negative	
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
MTBDRs/	Fluoroquinolone	100 (82/82) 95-100 †p<0.001	97 (311/321) 94-98 †p=0.013	100 (45/45) 92-100 †p=0.021	97 (188/194) 93-98 †p=0.044	100 (37/37) 90-100 †p=0.010	94 (119/126) 88-97 *p=0.275 †p=0.605
	Second-line injectable drug	92 (35/38) 68-93 †p=0.286	99 (338/339) 98-99 †p<0.001	95 (21/22) 66-97 †p=0.294	99 (211/212) 94-100 †p=0.322	94 (15/16) 54-95 *p=0.816 †p=0.285	95 (121/127) 85-96 *p=0.007 †p=0.040
	Fluoroquinolone and second-line injectable drugs	81 (22/27) 69-98 †p=0.339	97 (257/264) 94-98	100 (13/13) 71-100 †p=0.141	97 (165/169) 94-99	64 (9/14) 44-97 *p=0.026 †p=0.698	98 (92/95) 91-99 *p=0.701

*Comparisons between smear statuses
†Comparisons before and after discrepant analysis

Supplementary Table 6: Sequencing of discrepant fluoroquinolones between MTBDR*sl* and phenotypic drug susceptibility illustrate MTBDR*sl*'s diagnostic ability to detect "true drug resistance" is highly accurate as mutational patterns and probe detection correspond with sequencing, reducing so-called erroneous MTBDR*sl* resistance calls.

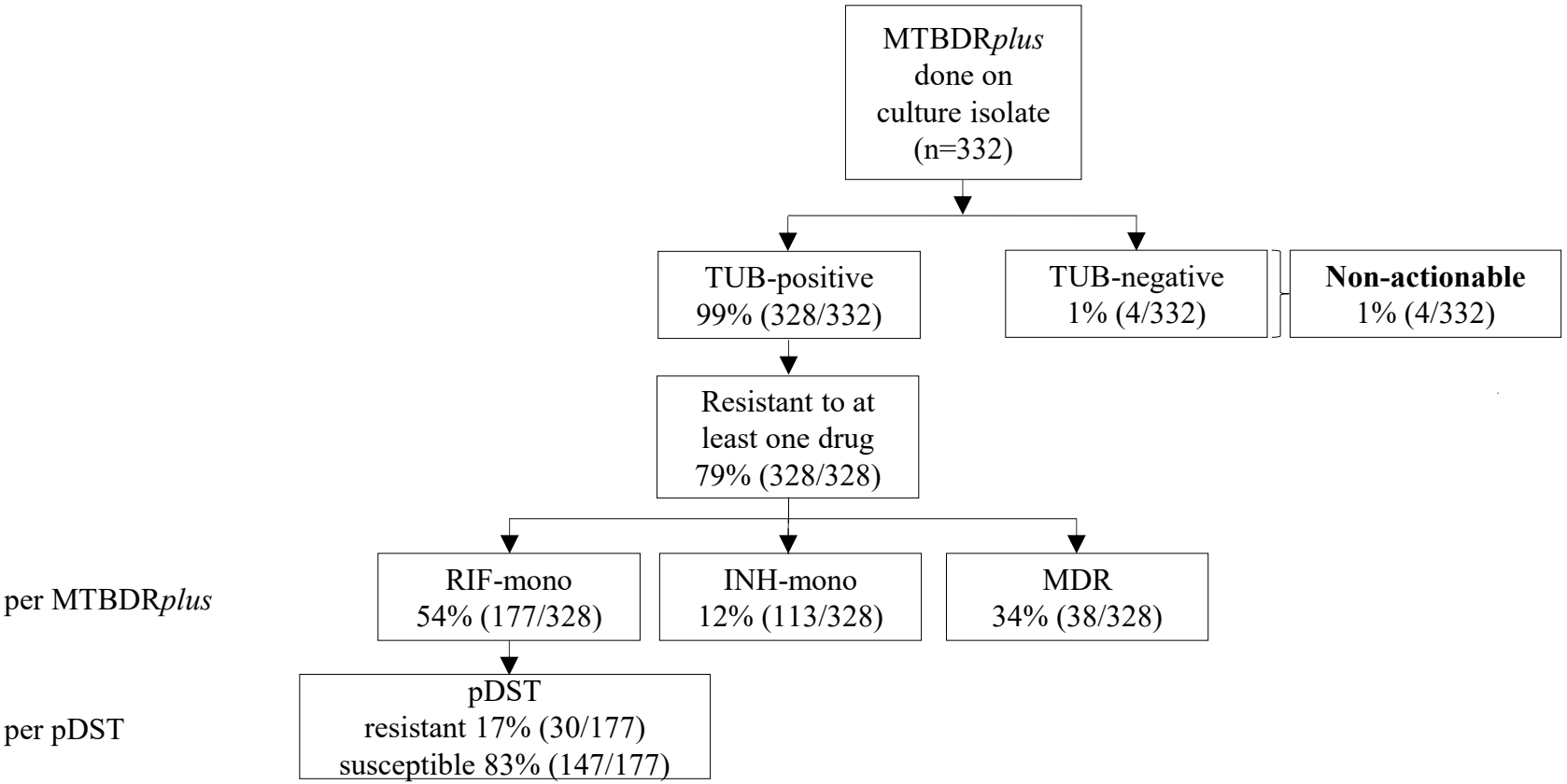
	MTBDR*sl*		Phenotypic drug susceptibility testing		Sequencing	
Sample code	*WT probe*	*Mutation Probe*	*Resistant (R)*	*Sensitive (S)*	*WT*	*Mutation*
			gyrA			
1	WT		R		WT	
2	WT		R		WT	
3	WT		R			88 CGG-TGG C88S, A88T
4	WT		R		WT	
5	WT		R		WT	
6	WT		R		WT	
7	WT		R		WT	
8	WT		R		WT	
9	WT		R		WT	
10	WT		R		WT	
11	WT		R			81 CAT-CCT
12	DWT2	MUT1 (A90V)		S		90 GCG-GTG (A90V)
13	DWT1	No MUT		S		89 GGC-GCC (D89N)
14	DWT3	MUT3A (D94A)		S	NR	
15	DWT2	MUT1 (A90V)		S	NR	
16	DWT3	MUT3C (D94G)		S		95 GAC-GGC
17	DWT3	MUT3B (D94N, D94Y)		S	WT	
18	DWT3	MUT1 (A90V)		S	WT	
19	DWT2	MUT1 (A90V)		S		90 CGC-GTG (A90V)
20	DWT2	MUT2 (S91P)		S		91 TCG-CCG (S91P)
21	DWT3	No MUT		S		94 GAC-TAC (D94G)
22	DWT2	MUT1 (A90V)		S		90 GCG-GTG (A90V)
23	DWT3	MUT3C (D94G)		S	WT	
24	DWT2	MUT1 (A90V)		S	NR	
25	DWT3	MUT3C D94G		S		95 GAC-GGC
26	DWT3	MUT3C (D94G)		S		94 GAC-AAC (D94G)
27	DWT1	No MUT		S		88 CGG-TGG (C88S, A88T)
28	DWT1	No MUT		S		86 ACA-ATA
29	WT	MUT3C (D94G)		S	NR	
30	DWT3	MUT3A,3C (D94A, D94G)		S	WT	
31	DWT2	MUT1 (A90V)		S		90 GCG-GTG (A90V)
32	DWT2	No MUT		S	WT	
33	WT	MUT3B (D94N, D94Y)		S	NR	
34	DWT2	MUT2 (S91P)		S	WT	
35	DWT1	No MUT		S	NR	

Abbreviation: WT-wild-type, DWT-deletion wild-type, MUT-mutation, NR-no result.

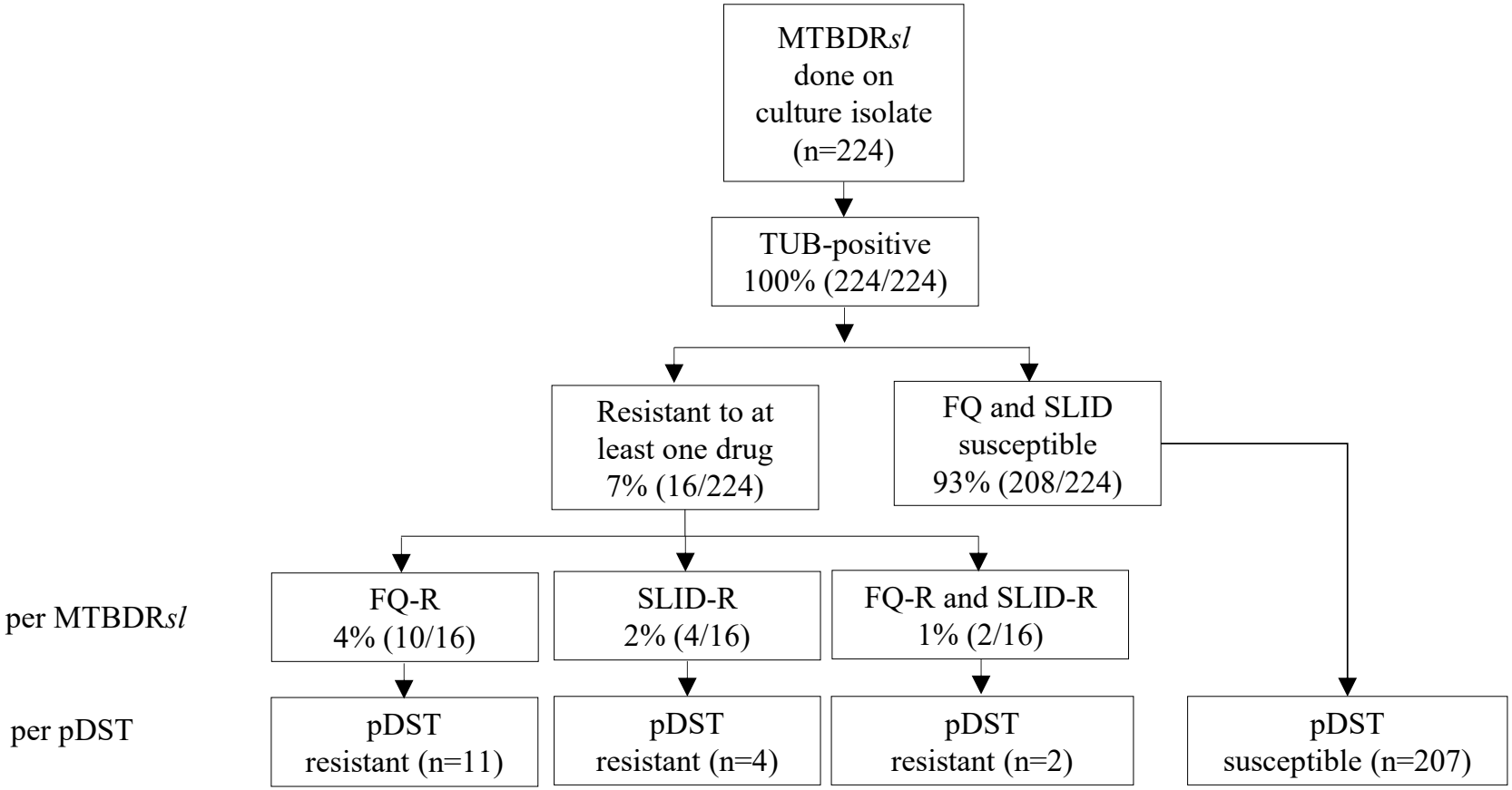
Supplementary Table 7: Sequencing of discrepant second-line injectables illustrate that MTBDR*sl*'s diagnostic ability to detect "true drug resistance" is sub-optimal and requires phenotypic testing as an additional tool to confirm resistance.

	MTBDR*sl*		pDST		Sequencing	
Number of samples	*WT probe*	*Mutation Probe*	*Resistant (R)*	*Sensitive (S)*	*WT*	*Mutation*
Genotype *rrs*						
1	WT		R		WT	
2	WT		R		NR	
3	WT		R		WT	
4	WT		R		NR	
5	WT		R		NR	
6	WT		R		WT	
7	WT	MUT1 A1401G		S		A1401G
8	DWT1	MUT1 A1401G		S	NR	
9	DWT1	MUT1 A1401G		S	NR	
10	DWT1	No MUT		S	NR	
11	DWT1	No MUT		S	WT	
12	DWT1,2	No MUT		S	WT	
13	DWT1	No MUT		S	WT	
14	WT	MUT2 G1484T		S	WT	
15	DWT1,2	MUT1 A1401G		S	WT	
16	DWT1	MUT1 A1401G		S	NR	
17	DWT1	MUT1 A1401G		S	NR	
18	DWT1	MUT1 A1401G		S	NR	
19	WT	MUT2 G1484T		S	NR	
20	DWT1	MUT1 A1401G		S	WT	
21	DWT1	MUT1 A1401G		S	WT	
22	DWT1	MUT1 A1401G		S	NR	
23	DWT1	No MUT		S	NR	
24	DWT1	No MUT		S	WT	
25	DWT1	No MUT		S	NR	
26	DWT1	No MUT		S	NR	
27	DWT1	No MUT		S	NR	
28	DWT1	No MUT		S	NR	

Abbreviation: WT-wild-type, DWT-deletion wild-type, MUT-mutation, NR-no result, pDST-phenotypic drug susceptibility testing.



Supplementary Figure 1. MTBDRplus indirect testing performance on culture isolates shows high numbers of actionable results in contrast to direct testing. Reasons for indirect MTBDRplus testing include direct MTBDRplus isoniazid-susceptible (n=225) or non-actionable (n=103) (see Methods for more information on the isoniazid DST algorithm).
Abbreviations: n-number, TUB-positive-TUB band positive, TUB-negative-TUB band negative, RIF-mono-rifampicin mono-resistant, INH-mono-isoniazid mono-resistant, MDR-multi-drug resistant, pDST-phenotypic drug susceptibility testing.



Supplementary Figure 2. **MTBDR*sl* performance indirectly shows culture isolates produce higher actionable results in contrast to direct testing.** Abbreviations: n-number, TUB-positive-TUB band positive, FQ-R-fluoroquinolone resistant, SLID-R-second-line injectable drug resistant, pDST-phenotypic drug susceptibility testing.

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Appendix IV

Chapter 3 Supplement

To test or not? Xpert MTB/RIF as an alternative to smear microscopy to guide line probe assay testing for drug-resistant tuberculosis

Supplementary Table of Contents

Figure 1. Predictive values (shaded areas 95% CI) of different sputum bacillary load readouts (smear grade, Xpert semi-quantitation category, C_{Tmin}) to classify MTBDR*plus* (orange) or MTBDR*sl* (blue) results as "likely non-actionable" or "likely actionable". Results presented overall (**A**-**D**, **G**-**H**) or restricted to smear-negative patients (**E**-**F**, **I**-**J**). With decreasing mycobacterial load, the proportion of specimens equal or below that threshold (above for C_{Tmin}) and hence flagged as likely non-actionable that are truly non-actionable increases (increasing PPV). In contrast, the proportion of specimens above the threshold (below for C_{Tmin}) and hence classified as "likely actionable" that are truly actionable slowly increases as bacterial load increases (NPV). PPVs for non-actionable MTBDR*sl* results were generally higher than those for MTBDR*plus* (due to MTBDRsl's increased pre-test probability of a non-actionable result), whereas NPVs were diminished. C_{Tmin} was obtained higher PPVs not possible with other readouts. On the bottom row above x-axes are the corresponding software assigned Xpert semiquantitation categories based on C_{Tmin} values. Abbreviations: C_{Tmin}-cycle threshold (minimum), Neg-negative, NPV-negative predictive value, P-positive, PPV-positive predictive value, SC-scanty, Xpert-Xpert MTB/RIF.

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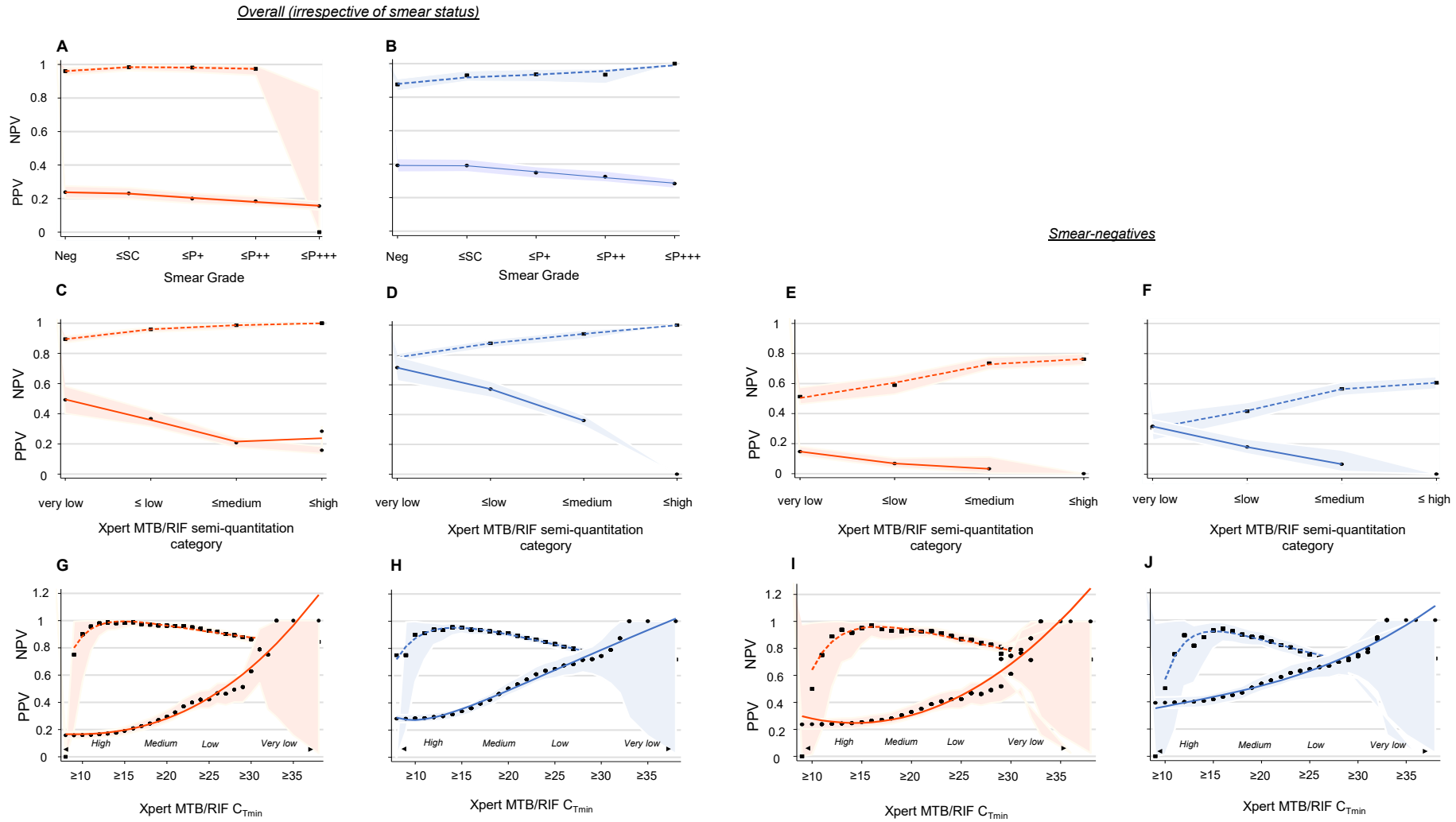

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Supplementary Table 1. Diagnostic accuracy of different measures of sputum mycobacterial load to identify if a direct MTBDR*plus* or MTBDR*sl* result will be "likely non-actionable" (direct LPA testing hence not useful) and the proportion of LPA-based resistance diagnoses (INH, FQ) that would be missed if that threshold to exclude "likely non-actionables" from LPAs is applied. Different use cases [rule-in (~95% specificity), rule-out (~95% sensitivity), Youden's index (highest sum of sensitivity and specificity)] for each measure are shown (all patients, smear-positives, smear-negatives). The rule-in use case would minimise patients falsely excluded from a rapid LPA, however, smear grade and Xpert semi-quantitation categories could not meet this threshold. Doing MTBDR*plus* and MTBDR*sl* in smear-negatives with C_{Tmin} <29 would permit reliable direct LPA testing (~95% of actionables correctly identified) and reduce non-actionable result rates by 18% and 13% respectively with low rates of missed resistance in patients with C_{Tmin} greater or equal to this threshold. Data are %, 95% CI, and n/N or % (n/N). Abbreviations: CI-confidence interval; C_{Tmin}-cycle threshold (minimum); INH-isoniazid; FQ-fluroquinolones; NPV-negative predictive value (proportion flagged as "likely actionable" truly actionable); P-positive; PPV-positive predictive value (proportion flagged as "likely non-actionable" truly non-actionable); SC-scanty.

	MTBDR*plus*						MTBDR*sl*					
Method, use case	**Threshold**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**INH^R diagnoses missed**	**Threshold**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**FQ^R diagnoses missed**
	All specimens irrespective of smear status											
Smear status												
N/A	Negative	90 (84-94) 133/148	54 (50-57) 426/792	27 (25-29) 133/469	97 (95-98) 426/441	49 264/537	Negative	83 (78-87) 220/266	50 (47-54) 339/674	40 (37-42) 220/555	88 (85-91) 339/385	42 44/104
Smear microscopy grade												
Rule-in	N/A (No cut-point approaches 95% sensitivity)						N/A (No cut-point approaches 95% sensitivity)					
Rule-out	≤SC	96 (92-99) 133/148	60 (56-63) 477/792	23 (20-27) 143/621	98 (96-99) 314/319	56 301/537	≤P+	95 (91-97) 252/266	70 (66-73) 468/674	35 (32-39) 252/721	94 (90-96) 205/219	64 67/104
Youden index	Same as rule-out						≤SC	92 (88-95) 244/266	56 (52-60) 378/674	39 (35-43) 244/623	93 (89-95) (35-43) 295/317	48 50/104
Xpert semi-quantitation category												
Rule-in	N/A (No cut-point approaches 95% sensitivity)						N/A (No cut-point approaches 95% sensitivity)					
Rule-out	N/A (No cut-point approaches 95% sensitivity)						≤medium	95 (91-97) 252/266	67 (63-70) 448/674	36 (32-40) 252/701	94 (90-97) 225/239	88 92/104
Youden index	≤high	100 (98-100) 148/148	100 (99-100) 792/792	15 (13-18) 148/940	0 (0-0)	35 178/537	≤low	72 (66-78) 193/266	22 (18-25) 144/674	57 (52-62) 193/338	88 (85-90) 529/602	97 101/104
Xpert C_{Tmin}												
Rule-in	≥29	30 (22-37) 44/148	95 (92-96) 750/792	51 (40-62) 44/86	88 (85-89) 750/854	4 21/537	≥28	34 (28-40) 90/266	95 (93-96) 638/674	71 (62-79) 90/126	78 (75-81) 638/814	3 3/104
Rule-out	≥17	95 (89-97) 140/148	39 (35-42) 311/792	22 (19-26) 140/621	98 (95-98) 311/319	58 310/537	≥16	94 (91-97) 251/266	34 (30-38) 229/674	36 (32-39) 251/696	94 (90-96) 229/244	63 65/104
Youden index	≥22	83 (76-89) 124/148	73 (70-76) 580/792	37 (31-42) 124/336	96 (94-97) 580/604	78 419/537	≥20	84 (78-88) 222/266	68 (64-71) 456/674	50 (45-55) 222/440	91 (88-93) 456/500	77 80/104
	Smear-positive specimens											
Smear microscopy grade												
Rule-in	N/A (No cut-point approaches 95% sensitivity)						N/A (No cut-point approaches 95% sensitivity)					
Rule-out	N/A (No cut-point approaches 95% sensitivity)						N/A (No cut-point approaches 95% sensitivity)					
Youden index	≤SC	86 (82-89) 314/366	100 (98-100) 15/15	100 (98-100) 314/314	22 (13-34) 15/67	14 37/273	≤SC	88 (84-92) 295/334	53 (38-67) 22/47	93 (90-96) 295/317	39 (27-52) 25/64	10 6/60
Xpert semi-quantitation category												
Rule-in	N/A (No cut-point approaches 95% sensitivity)						N/A (No cut-point approaches 95% sensitivity)					
Rule-out	N/A (No cut-point approaches 95% sensitivity)						N/A (No cut-point approaches 95% sensitivity)					
Youden index	≤low	92 (88-94) 335/366	67 (38-88) 10/15	99 (97-99) 335/340	24 (12-40) 10/41	99 270/273	≤low	94 (91-96) 314/335	43 (28-58) 20/46	92 (89-95) 314/340	49 (33-65) 20/41	100 60/60

Xpert C_{Tmin}												
Rule-in	≥23	60 (32-83) 9/15	95 (93-97) 346/366	31 (15-50) 9/29	98 (96-99) 346/352	5 15/273	≥23	37 (23-52) 17/46	96 (93-98) 323/335	58 (38-76) 17/29	91 (88-94) 323/352	2 1/60
Rule-out	N/A (No cut-point approaches 95% sensitivity)						≥14	95 (85-99) 44/46	25 (20-30) 84/335	14 (11-19) 44/295	97 (91-99) 84/86	78 47/60
Youden index	≥22	66 (38-88) 10/15	91 (88-94) 336/366	25 (12-41) 10/40	98 (96-99) 336/341	92 251/273	≥20	58 (43-73) 27/46	85 (81-89) 288/335	36 (25-48) 27/74	93 (90-96) 288/307	90 54/60
Smear-negative specimens												
Xpert semi-quantitation category												
Rule-in	N/A (No cut-point approaches 95% sensitivity)						N/A (No cut-point approaches 95% sensitivity)					
Rule-out	N/A (No cut-point approaches 95% sensitivity)						N/A (No cut-point approaches 95% sensitivity)					
Youden index	≤low	85 (78-91) 114/133	57 (53-62) 243/426	58 (52-63) 114/297	82 (76-86) 243/262	90 237/264	≤low	78 (72-83) 173/220	63 (57-68) 215/339	58 (52-63) 173/297	82 (76-86) 215/262	93 41/44
Xpert C_{Tmin}												
Rule-in	≥29	18 (12-26) 25/133	96 (93-97) 410/426	61 (44-75) 25/41	79 (75-82) 410/518	8 20/264	≥29	13 (9-18) 30/220	96 (93-97) 328/339	73 (57-85) 30/41	63 (59-67) 328/518	7 3/44
Rule-out	≥18	94 (88-97) 125/133	25 (20-29) 106/426	28 (24-32) 125/445	93 (86-96) 106/114	73 193/264	≥18	95 (90-97) 208/220	30 (25-35) 102/339	46 (42-51) 208/445	89 (82-94) 102/114	68 30/44
Youden index	≥22	81 (80-87) 107/133	63 (58-67) 270/426	41 (34-46) 107/263	91 (87-94) 270/296	64 168/264	≤23	73 (66-78) 161/220	69 (64-74) 237/339	61 (55-67) 161/263	80 (75-84) 237/296	82 36/44

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Appendix V

Chapter 5 Supplement

Melting the *eis*: Non-detection of kanamycin resistance markers by routine diagnostic tests and identification of new *eis* promoter variants

Supplementary Material

Methods

Sanger sequencing and GenoType MTBDR*s*/ VER 2.0

All isolates to be Sanger sequenced were re-cultured from frozen bacterial stock cultures. For isolates of sample set 1, the sequence of the *eis*-promoter region in each isolate was determined by PCR amplification of thermal lysates followed by Sanger sequencing. Each isolate of sample set 1 for which Sanger sequencing detected an *eis*-promoter mutation, Sanger sequencing was repeated once to confirm the result. Isolates from sample set 2 were Sanger sequenced to confirm WGS results, using the same DNA that was used for WGS (see below). Briefly, the PCR reaction mix contained the following final concentrations of: 1x HotStartTaq *Plus* Master Mix (Qiagen, San Diego, CA, USA), 500nM of each primer (forward 5' CCATGGGACCGGTACTTGCT 3', reverse 5' ACTTCACCAGGCACCGTCAA 3'), and 1x SYTO 9 Green Fluorescent Nucleic Acid Stain (Thermo Fisher Scientific). As template, 1ul of thermal lysate (sample set 1) or purified DNA (sample set 2) was added to the reaction mix. Amplification of the *eis*-promoter region of the selected isolates was carried out using a CFX96^{TM} Real-Time System C1000 Touch Thermal Cycler (BioRad) running the following thermocycling protocol: Initial denaturation at 95°C for 5min, followed by 40 cycles of 95°C for 1min, annealing at 62°C for 1min and elongation at 72°C for 1min, followed by a final elongation at 72°C for 10min. Successful amplification was confirmed by a high-resolution melt from 80°C – 95°C with an increment of 0.5°C, each increment temperature held for 5 seconds.

Isolates which repeatedly failed to amplify were excluded from further analyses. Successfully amplified PCR products were sent to the Central DNA Sequencing Facilities of Stellenbosch University for targeted Sanger sequencing using the forward PCR Primer. The resulting chromatographs were analyzed using

BioEdit Sequence Alignment Editor v. 7.2.5 (1) comparing them to the *Mycobacterium tuberculosis* H37Rv reference genome (Accession number: AL123456).

The GenoType MTBDR*sl* VER 2.0 (MTBDR*sl*) assay was done according to the manufacturer's protocol using the same DNA used for WGS (if not indicated otherwise). Failing assays (*e.g.,* complete gene locus control or conjugate control band missing; defined as per manufacturer's protocol) were repeated once. The analytical sensitivity (limit of detection) of the MTBDR*sl* assay is 1.65 x 10^5 bacteria/ml for culture samples and 150 bacteria/ml for clinical samples (2).

Phenotypic Drug Susceptibility Testing and Minimum Inhibitory Concentration Determination

All isolates used in this study (sample sets 1 and 2), were initially subjected to routine pDST on solid Löwenstein Jensen medium against INH, RIF, AMK and ofloxacin (OFX). Susceptibility was determined according to the 1% proportion method at clinical breakpoints of 0.2ug/ml for INH, 40.0ug/ml for RIF, 30ug/ml for AMK and 2ug/ml for OFX (3, 4). MICs for KAN were subsequently determined for isolates with an *eis*-promoter mutation missed by the MTBDR*sl* (sample set 1) and for representatives of each additional (combination of) *eis*-promoter mutation(s) (sample set 2). These MICs were done using two-fold serial dilutions ranging from 10.0ug/ml to 1.25ug/ml using the BACTEC MGIT 960 system with the TB eXiST module of the EpiCentre software at Stellenbosch University (5). Susceptibility to KAN and AMK (*i.e.,* pDST at Stellenbosch University) was based on a clinical breakpoint of 2.5ug/ml for KAN and 30ug/ml for AMK as per the 1% proportion method, defined as the lowest drug concentration that inhibits > 99% of growth. One isolate showed intermediate growth (*i.e.,* growth of > 100 growth units [GU] within seven days after the growth control reached a GU of 400) at all measured drug concentrations. The bacteria that grew under KAN pressure (intermediate growth < 1%; bacteria from the 10ug/ml drug containing tube) were re-grown in KAN containing medium (*i.e.,* selective sub-culturing), and pDST and subsequent Sanger sequencing were repeated following the procedures described above.

Whole genome sequencing

For WGS each isolate was re-cultured from culture stocks and DNA was extracted by standard procedures as previously described (6). Whole genome sequencing libraries had been prepared using the standard genomic DNA sample preparation kits from Illumina (Illumina, Inc, San Diego, CA), following the manufacturer's protocol. The libraries were sequenced on an llumina HiSeq or Illumina NextGen Seq platform. The resulting sequencing reads were mapped to the *Mtb* H37*Rv* reference strain (Accession No. AL123456). Variant calling and annotation were conducted using a within-house pipeline including 3 mappers (Burrows-Wheeler Alignment tool, NovoAlign, Smalt) (7–9) and 2 variant callers (GATK Gene Analysis Tool Kit, SAMtools) (7, 10) as previously described (11). Sequences with an average coverage below 20x for 2 or 3 of the mappers and/or with mapped reads <80% for 2 or 3 of the mappers were excluded, resulting in an average coverage (*i.e.,* average across the three mappers) of 35x to 443x per isolate. Only SNVs called from all 3 alignment bam files were considered high confidence SNVs. No frequency cut-off was applied, and variants detected at a frequency ≥ 95% were considered fixed. The genotypic drug resistance profile of each isolate was determined using markers defined by Coll *et al* and Miotto *et al* (12, 13). Artemis (14) was used to visually inspect sequencing reads. Based on this visual inspection of the reads and on variant frequency analysis it was determined that none of the isolates with more than one *eis*-promoter mutation had a double mutation, *i.e.,* the different mutations were always on different WGS reads, suggesting differently evolved sub-clones within the same patient.

Raw sequencing reads of the isolates listed in Table 1 have been deposited at the European Nucleotide Archive (PRJEB41458).

Limitations

The presented study made use of two different sample sets complementing each other but also baring limitations described below.

Not all isolates of data set 1 that were typed SLID susceptible by the MTBDR*s*l were available for Sanger sequencing due to insufficient sample volumes, contamination, or loss of viability. The proportion of missed *eis*-promoter mutations could therefore be higher.

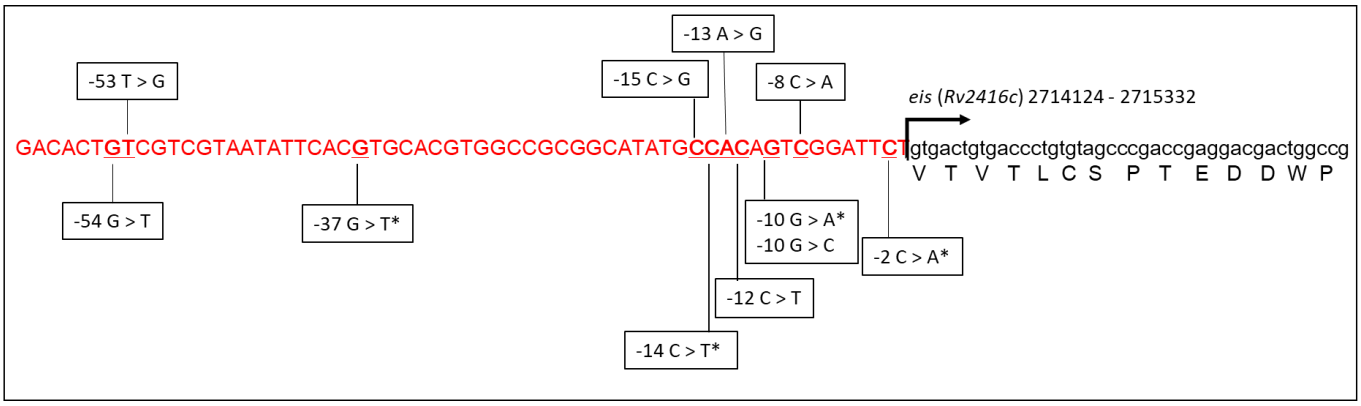
Despite analyzing data collected over the last 25 years, no conclusions about the prevalence of *eis*-promoter mutations across that period can be drawn, as sample set 2 of this study was a convenience sample of WGS isolates collected from several studies with different research questions. All WGS isolates irrespective of their phenotypic status were screened for *eis*-promoter mutations, but only representatives of those carrying such a mutation were further analyzed. However, in combination with data set 1 – which is surveillance data from one year of the rifampicin-resistant *Mtb* population in the WCP – our data provide insights on the type and frequency of *eis*-promoter mutations present in the WCP.

Unfortunately, no data on treatment outcome of the patients was available for analysis and no conclusions on the clinical impact of the detected *eis*-promoter mutations can be drawn. Similarly, sub-culturing is required to determine KAN MICs. As some of the isolates lost viability, the impact of these isolate's mutations on the MIC could not be determined.

In this study, the MIC was only determined once. For susceptible isolates with an MIC near the clinical breakpoint of 2.5ug/ml (*e.g.*, table 2) a repetition of the MIC may have resulted in a slightly elevated MIC and therefore in low-level KAN resistance.

Our results do not allow to make any conclusions on whether *eis*-promoter mutations act as steppingstones for the acquisition of high-level resistance for KAN (and/or AMK). To analyze this, further *in vitro* experiments, and analyses of serial samples would be required to investigate the development of resistance over time with and without drug pressure.

Supplementary Figures and Tables



Supplementary Figure 1: *Mycobacterium tuberculosis* H37Rv coding sequence of the gene *eis* (*Rv2416c;* black) and 60bp of its promoter region (red), with the known kanamycin resistance conferring mutations according to Coll *et al* and Miotto *et al* (12, 13). The mutations -15 C > G and -10 G > C are mutations disputed to confer resistance. The GenoType MTBDR*sl* VER 2.0 assay (Hain Lifescience, Germany) defines specific banding patterns for only the most common *eis*-promoter mutations (marked with *). The remaining *eis*-promoter mutations may however also cause a failing wild type band, which would then be interpreted as "undefined mutation detected" (2).

Supplementary Table 1: *Eis*-promoter mutations and their frequency in 2863 whole genome sequenced isolates

Supplementary Table 2: Additional information on the samples and patients of data set 2

M = male; F = female

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