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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.

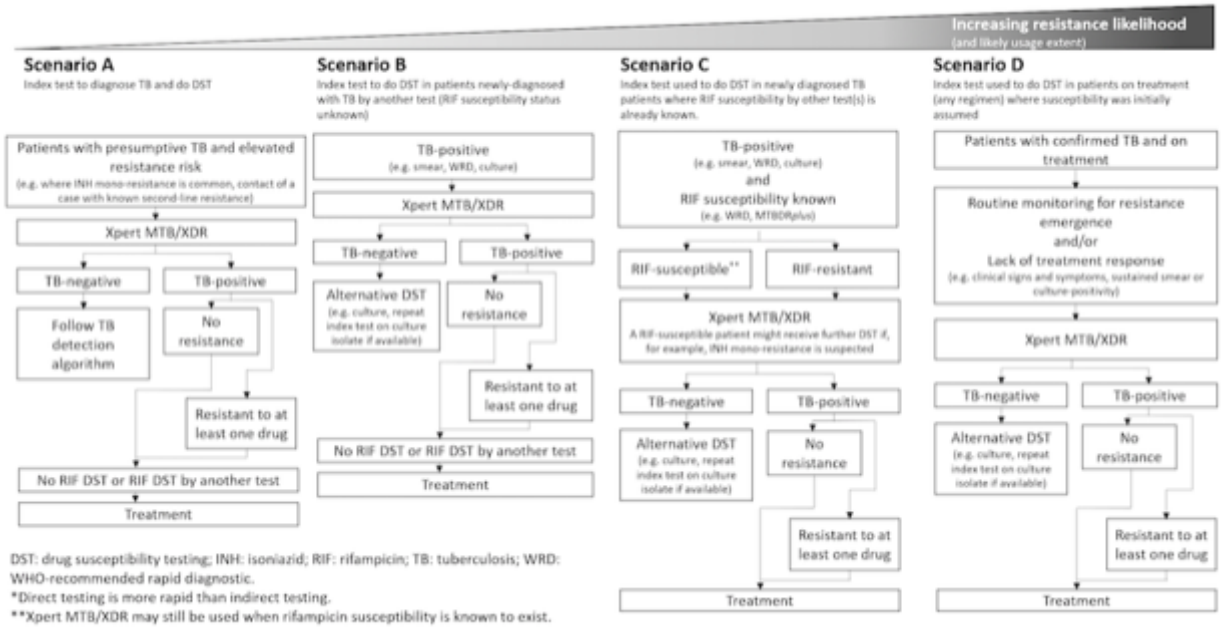
AMK: amikacin; CAP: capreomycin; ETH: ethionamide; FLQ: fluoroquinolone; INH: isoniazid; KAN: kanamycin; MTB: *Mycobacterium tuberculosis*.

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)



DST: drug susceptibility testing; INH: isoniazid; RIF: rifampicin; TB: tuberculosis; WRD: WHO-recommended rapid diagnostic.
*Direct testing is more rapid than indirect testing.
**Xpert MTB/XDR may still be used when rifampicin susceptibility is known to exist.

- 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:

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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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