The diagnostic accuracy of the GenoType® MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs (Review)

Theron G, Peter J, Richardson M, Barnard M, Donegan S, Warren R, Steingart KR, Dheda K

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[Diagnostic Test Accuracy Review]

The diagnostic accuracy of the GenoType® MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

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ABSTRACT

Background

Accurate and rapid tests for tuberculosis (TB) drug resistance are critical for improving patient care and decreasing the transmission of drug-resistant TB. Genotype®MTBDR*sl* *(*MTBDR*sl*) is the only commercially-available molecular test for detecting resistance in TB to the fluoroquinolones (FQs; ofloxacin, moxifloxacin and levofloxacin) and the second-line injectable drugs (SLIDs; amikacin, kanamycin and capreomycin), which are used to treat patients with multidrug-resistant (MDR-)TB.

Objectives

To obtain summary estimates of the diagnostic accuracy of MTBDR*sl* for FQ resistance, SLID resistance and extensively drug-resistant TB (XDR-TB; defined as MDR-TB plus resistance to a FQ and a SLID) when performed (1) indirectly (ie on culture isolates confirmed as TB positive) and (2) directly (ie on smear-positive sputum specimens).

To compare summary estimates of the diagnostic accuracy of MTBDR*sl* for FQ resistance, SLID resistance and XDR-TB by type of testing (indirect versus direct testing).

The populations of interest were adults with drug-susceptible TB or drug-resistant TB. The settings of interest were intermediate and central laboratories.

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Search methods

We searched the following databases without any language restriction up to 30 January 2014: Cochrane Infectious Diseases Group Specialized Register; MEDLINE; EMBASE; ISI Web of Knowledge; MEDION; LILACS; BIOSIS; SCOPUS; the metaRegister of Controlled Trials; the search portal of the World Health Organization International Clinical Trials Registry Platform; and ProQuest Dissertations & Theses A&I.

Selection criteria

We included all studies that determined MTBDR*sl* accuracy against a defined reference standard (culture-based drug susceptibility testing (DST), genetic testing or both). We included cross-sectional and diagnostic case-control studies. We excluded unpublished data and conference proceedings.

Data collection and analysis

For each study, two review authors independently extracted data using a standardized form and assessed study quality using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. We performed meta-analyses to estimate the pooled sensitivity and specificity of MTBDR*sl* for FQ resistance, SLID resistance, and XDR-TB. We explored the influence of different reference standards. We performed the majority of analyses using a bivariate random-effects model against culture-based DST as the reference standard.

Main results

We included 21 unique studies: 14 studies reported the accuracy of MTBDR*sl* when done directly, five studies when done indirectly and two studies that did both. Of the 21 studies, 15 studies (71%) were cross-sectional and 11 studies (58%) were located in low-income or middle-income countries. All studies but two were written in English. Nine (43%) of the 21 included studies had a high risk of bias for patient selection. At least half of the studies had low risk of bias for the other QUADAS-2 domains.

As a test for FQ resistance measured against culture-based DST, the pooled sensitivity of MTBDR*sl* when performed indirectly was 83.1% (95% confidence interval (CI) 78.7% to 86.7%) and the pooled specificity was 97.7% (95% CI 94.3% to 99.1%), respectively (16 studies, 1766 participants; 610 confirmed cases of FQ-resistant TB; *moderate quality evidence*). When performed directly, the pooled sensitivity was 85.1% (95% CI 71.9% to 92.7%) and the pooled specificity was 98.2% (95% CI 96.8% to 99.0%), respectively (seven studies, 1033 participants; 230 confirmed cases of FQ-resistant TB; *moderate quality evidence*). For indirect testing for FQ resistance, four (0.2%) of 1766 MTBDR*sl* results were indeterminate, whereas for direct testing 20 (1.9%) of 1033 were MTBDR*sl* indeterminate ($P < 0.001$).

As a test for SLID resistance measured against culture-based DST, the pooled sensitivity of MTBDR*sl* when performed indirectly was 76.9% (95% CI 61.1% to 87.6%) and the pooled specificity was 99.5% (95% CI 97.1% to 99.9%), respectively (14 studies, 1637 participants; 414 confirmed cases of SLID-resistant TB; *moderate quality evidence*). For amikacin resistance, the pooled sensitivity and specificity were 87.9% (95% CI 82.1% to 92.0%) and 99.5% (95% CI 97.5% to 99.9%), respectively. For kanamycin resistance, the pooled sensitivity and specificity were 66.9% (95% CI 44.1% to 83.8%) and 98.6% (95% CI 96.1% to 99.5%), respectively. For capreomycin resistance, the pooled sensitivity and specificity were 79.5% (95% CI 58.3% to 91.4%) and 95.8% (95% CI 93.4% to 97.3%), respectively. When performed directly, the pooled sensitivity for SLID resistance was 94.4% (95% CI 25.2% to 99.9%) and the pooled specificity was 98.2% (95% CI 88.9% to 99.7%), respectively (six studies, 947 participants; 207 confirmed cases of SLID-resistant TB, 740 SLID susceptible cases of TB; *very low quality evidence*). For indirect testing for SLID resistance, three (0.4%) of 774 MTBDR*sl* results were indeterminate, whereas for direct testing 53 (6.1%) of 873 were MTBDR*sl* indeterminate ($P < 0.001$).

As a test for XDR-TB measured against culture-based DST, the pooled sensitivity of MTBDR*sl* when performed indirectly was 70.9% (95% CI 42.9% to 88.8%) and the pooled specificity was 98.8% (95% CI 96.1% to 99.6%), respectively (eight studies, 880 participants; 173 confirmed cases of XDR-TB; *low quality evidence*).

Authors' conclusions

In adults with TB, a positive MTBDR*sl* result for FQ resistance, SLID resistance, or XDR-TB can be treated with confidence. However, MTBDR*sl* does not detect approximately one in five cases of FQ-resistant TB, and does not detect approximately one in four cases of SLID-resistant TB. Of the three SLIDs, MTBDR*sl* has the poorest sensitivity for kanamycin resistance. MTBDR*sl* will miss between one in four and one in three cases of XDR-TB. The diagnostic accuracy of MTBDR*sl* is similar when done using either culture isolates or smear-positive sputum. As the location of the resistance causing mutations can vary on a strain-by-strain basis, further research is required on test accuracy in different settings and, if genetic sequencing is used as a reference standard, it should examine all resistance-determining regions. Given the confidence one can have in a positive result, and the ability of the test to provide results within a matter

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of days, MTBDR*sl* may be used as an initial test for second-line drug resistance. However, when the test reports a negative result, clinicians may still wish to carry out conventional testing.

PLAIN LANGUAGE SUMMARY

The rapid test GenoType® MTBDR*sl* for testing resistance to second-line TB drugs

Background

Different drugs are available to treat people with tuberculosis (TB), but resistance to these drugs is a growing problem. People with drug-resistant TB are more likely to die than people with drug-susceptible TB. People with drug-resistant TB require "second-line" TB drugs that, compared with "first-line" TB drugs used to treat drug-susceptible TB, cause more side effects and must be taken for longer. Extensively drug-resistant TB (XDR-TB) is a type of TB that is resistant to almost all TB drugs. A rapid and accurate test could identify people with drug-resistant TB, likely improve patient care, and reduce the spread of drug-resistant TB.

Test evaluated by this review

GenoType® MTBDR*sl* (MTBDR*sl*) is the only rapid test that detects resistance to second-line fluoroquinolone drugs and the second-line injectable drugs. The test also detects XDR-TB. MTBDR*sl* can be performed on TB bacteria grown by culture from sputum, which takes a long time (indirect testing), or immediately on sputum (direct testing).

Main results

We examined evidence available up to 30 January 2014 and included 21 studies, 11 of which were in low-income or middle-income countries.

What do these results mean?

Fluoroquinolone drugs

By indirect testing, the test detected 83% of people with fluoroquinolone resistance and rarely gave a positive result for people without resistance. In a population of 1000 people, where 170 have fluoroquinolone resistance, MTBDR*sl* will correctly identify 141 people with fluoroquinolone resistance and miss 29 people. In this same population of 1000 people, where 830 people do not have fluoroquinolone resistance, the test will correctly classify 811 people as not having fluoroquinolone resistance and misclassify 19 people as having resistance (*moderate quality evidence*).

By direct testing, the test detected 85% of people with fluoroquinolone resistance and rarely gave a positive result for people without resistance (*moderate quality evidence*).

Second-line injectable drugs

By indirect testing, the test detected 77% of people with second-line injectable drug resistance and rarely gave a positive result for people without resistance. In a population of 1000 people, where 230 have second-line injectable drug resistance, MTBDR*sl* will correctly identify 177 people with second-line injectable drug resistance and miss 53 people. In this same population of 1000 people, where 770 do not have second-line injectable drug resistance, the test will correctly classify 766 people as not having second-line injectable drug resistance and misclassify four people as having resistance (*moderate quality evidence*).

By direct testing, the test detected 94% of people with second-line injectable drug resistance and rarely gave a positive result for people without resistance (*very low quality evidence*).

XDR-TB

By indirect testing, the test detected 71% of people with XDR-TB and rarely gave a positive result for people without XDR-TB. In a population of 1000 people, where 80 have XDR-TB, MTBDR*sl* will correctly identify 57 people with XDR-TB and miss 23 people. In this same population of 1000 people, where 920 do not have XDR-TB, the test will correctly classify 909 people as not having XDR-TB and misclassify 11 people as having XDR-TB (*low quality evidence*).

There was insufficient evidence to determine the accuracy of MTBDR*sl* by direct testing for XDR-TB.

Conclusions

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The results show that a positive MTBDR*sl* result for resistance to the fluoroquinolone drugs or the second-line injectable drugs is reliable evidence that the person has drug-resistant TB and further conventional drug-resistance testing is not required. However, when the test reports a negative result, clinicians may still wish to carry out conventional testing.

BACKGROUND

Tuberculosis (TB) is an infectious airborne disease caused by *Mycobacterium tuberculosis* bacteria and is the second most common cause of death from an infectious disease in adults (HIV/AIDS being first). TB predominantly affects the lungs (pulmonary TB) but can affect other parts of the body, such as the brain or the spine. Active TB disease is confirmed by the presence of viable TB bacilli. The symptoms of pulmonary TB include a persistent cough (for at least two weeks), fever, night sweats, weight loss, chills, haemoptysis and fatigue. In 2012, an estimated 8.6 million people developed TB and 1.3 million people died from TB (WHO 2013a). TB that is drug sensitive (also referred to as drug-susceptible TB) is the most common type of TB and may be effectively treated with a standardized regimen of first-line anti-TB drugs (WHO 2013a). However, TB bacilli may become drug resistant, meaning that first-line anti-TB drugs can no longer kill the bacilli. Drug resistance usually develops because of inappropriate or incorrect use of first-line drugs but new cases are increasingly caused by person-to-person transmission (Streicher 2011; Zhao 2012).

The emergence of drug-resistant TB (DR-TB) threatens to destabilise global TB control. In 2012, approximately 4% of new TB cases were multidrug resistant (WHO 2013a). Therapy for DR-TB requires treatment for more than 12 months and is toxic and expensive. In South Africa in 2011, the treatment of approximately 8000 cases of DR-TB, which comprised only 2.2% of the total TB burden, consumed 32% of the country's annual national TB budget of US$218 million (Pooran 2013). Fifty percent to 75% of patients experience unfavourable outcomes, such as death, treatment failure, or adverse drug reactions (Dheda 2010a; Dheda 2010b). There are two standardized definitions of DR-TB: multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). MDR-TB is caused by *M. tuberculosis* which, when tested microbiologically in the laboratory, is resistant to rifampicin and isoniazid. These drugs are two of the most effective and widely-used anti-TB drugs that form part of the standardized first-line regimen for drug-susceptible TB. Patients with MDR-TB are commonly treated with drugs belonging to the fluoroquinolone (FQ) and second-line injectable anti-TB drug (SLID) classes. The FQ drugs include ofloxacin and moxifloxacin and the SLIDs include amikacin and kanamycin (two aminoglycoside drugs) and capreomycin (a cyclic peptide drug). XDR-TB is caused by *M. tuberculosis* resistant to isoniazid, rifampicin, plus any FQ and at least one of the three SLIDs (amikacin, kanamycin or capreomycin). Hence, patients with XDR-TB are resistant to both first-line and second-line drugs.

In South Africa, 80% of MDR-TB is thought to be spread via person-to-person transmission (Streicher 2011) and the same is likely true of MDR-TB and XDR-TB in China (Zhao 2012). Modelling studies (Basu 2007; Basu 2009; Dowdy 2008) have shown that, through the expansion of capacity to rapidly diagnose DR-TB, patient cure rates will be improved through the earlier initiation of appropriate and effective TB treatment. Importantly, once a patient is placed on effective treatment, their infectiousness dramatically declines within one to two weeks (Menzies 1997). However, the exact "infectiousness period" for DR-TB remains unclear. Early treatment initiation may therefore help curtail the spread of DR-TB through the disruption of person-to-person transmission. Thus, there is an urgent need for rapid tests that allow the early detection of drug resistance and the selection of appropriate TB drugs.

Conventional tests for detecting TB drug resistance, referred to as drug susceptibility testing (DST), are traditionally 'phenotypic', in that bacteria in biological fluid from the patient (usually sputum) is inoculated into a culture medium containing the drug of interest and the presence (indicating resistance) or absence (indicating susceptibility) of *M. tuberculosis* growth is detected (Heysell 2012). Such testing is commonly performed indirectly, in that the pure bacterial culture or isolate grown from the original patient specimen is re-inoculated into drug-containing media. As the growth of *M. tuberculosis* typically takes between two to six weeks for the initial culture, there is often a significant time delay (two to six months) associated with the diagnosis of DR-TB, especially if re-inoculation is required. These delays are often further exacerbated by the technical and infrastructure requirements of testing, a lack of standardised methodologies for certain drugs (which cause unclear results that require repeating) (Richter 2009), as well as patient-associated difficulties, such as loss to follow-up. Recently, new tests for drug resistance such as the Genotype® MTBDR*sl* test (henceforth called MTBDR*sl*) that are rapid (potentially offering a turn-around time of one to two days) and 'genotypic' (as they detect the presence of specific mutations known to be associated with drug resistance) have offered considerable promise for the diagnosis of DR-TB.

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One of the challenges in this Cochrane Review is the choice of the reference standard used to determine the presence or absence of the target conditions (described below). Phenotypic culture-based DST is the most widely used reference standard for drug resistance and is recommended by the WHO (WHO 2007). However, phenotypic culture-based DST is acknowledged to be imperfect and the results are dependent on the concentration of drug used. Genetic sequencing is widely considered to be the best reference standard for testing for the presence of drug resistance; but due to the technical aspects, costs and time associated with this method, it is rarely feasible to perform it on all samples suspected of DR-TB or in all regions of the TB genome that might be associated with resistance. Furthermore, not all genetic determinants or mechanisms of resistance may be known for a particular drug. We discuss the strengths and limitations of the different reference standards further below.

Target condition being diagnosed

We considered the following three target conditions: resistance of *M. tuberculosis* to FQs; resistance of *M. tuberculosis* to SLIDs; and XDR-TB.

Index test(s)

The GenoType® MTBDR*sl* assay (MTBDR*sl*, Hain Life Sciences) detects mutations in the *gyrA* gene (encoding the A-subunit of DNA gyrase), the *rrs* gene (encoding the 16S rRNA complex) and the *embB* gene (which, together with the genes *embA* and *embC*, codes for arabinosyltransferase) of the TB-causing *M. tuberculosis* complex species (which includes *M. tuberculosis*,*M. africanum*,*M. bovis* subsp. *bovis*,*M. bovis* subsp. *caprae*,*M. bovis* subsp. *BCG*,*M. microti*,*M. canetti* and*M. pinnipedii*) (Hain Life Sciences 2012a). The presence of mutations in these genes is associated with resistance to the FQs (including ofloxacin and levofloxacin), SLIDs (including kanamycin, amikacin and capreomycin) and ethambutol, respectively. Since ethambutol is a first-line TB drug, we did not determine the accuracy of MTBDR*sl* assay for ethambutol resistance in this review.

The assay can be performed either on a patient specimen (direct testing) or on a culture grown from the patient specimen (indirect testing). The type of testing, direct or indirect, is dependent on the quantity of TB in the patient specimen. The manufacturer recommends that the assay is performed directly on the specimen if the specimen contains bacilli that can be seen using a light microscope and an acid-fast stain (smear-positive) (Figure 1).

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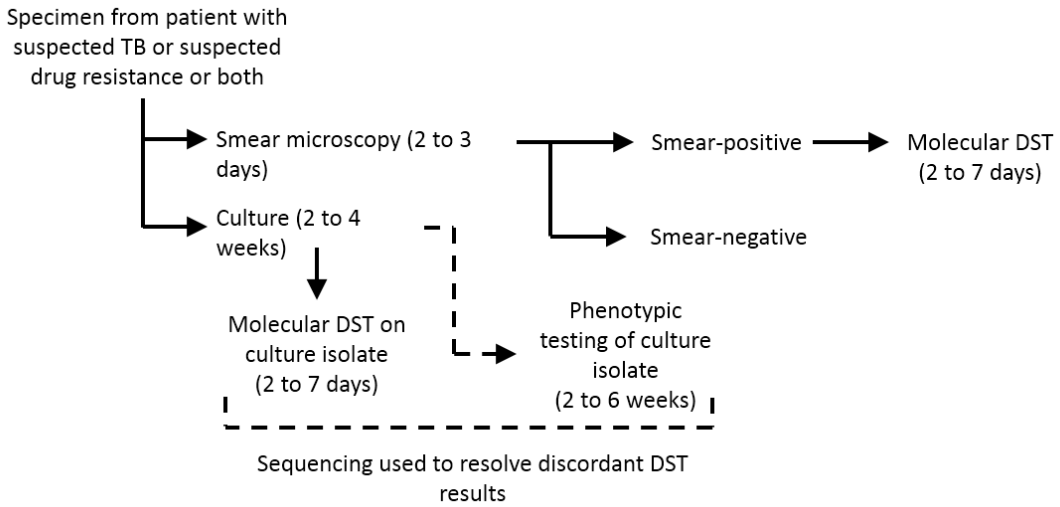
Figure 1. Clinical pathway diagram showing how molecular drug susceptibility testing (DST), which may use the MTBDRsl assay, is applied. A patient with suspected TB or suspected drug-resistant TB supplies a biological specimen (usually sputum), which is examined by smear microscopy and cultured. If acid-fast bacilli are observed under the microscope (smear-positive), a molecular DST can be performed directly on the specimen. If acid-fast bacilli are not observed (smear-negative), molecular DST can only be performed with acceptable accuracy on the culture isolate grown from the specimen. A molecular test for first-line drug resistance (for example, the MTBDRplus assay) is performed first and, only if resistance to the first-line drugs is indicated, the specimen is tested further for resistance to the second-line drugs using the MTBDRsl assay. Where molecular testing is not available, phenotypic testing for drug resistance may be performed on culture-positive isolates. Although phenotypic testing is being replaced by molecular-based methods in some settings, it is still usually performed in research studies seeking to measure the accuracy of the molecular test. Furthermore, some research studies also use gene sequencing as a reference standard or any specimens with discordant molecular DST-culture results.



The assay procedure is comprised of three sequential steps when using direct decontaminated patient material (decontaminated using the standard N-acetyl-cysteine and sodium hydroxide (NALC/NaOH) method), culture isolates in liquid media or when picking colonies from solid media. These steps are: (1) mycobacterial genomic DNA is extracted from the patient specimen or culture isolate; (2) regions within the *gyrA, rrs* and *embB* genes are selectively amplified using a multiplex polymerase chain reaction (PCR) assay; and (3) the amplification products are detected on a nitrocellulose membrane strip by reverse hybridisation and visualised using a streptavidin-conjugated alkaline phosphatase colour reaction. The observed bands, each corresponding to a specific probe, can be used to determine the drug susceptibility profile of the analysed specimen (an example is shown in Figure 2). The extraction can also be done indirectly on blood cultures, where a Middlebrook slant is inoculated prior to picking the colonies from the agar after incubation for a period of time.

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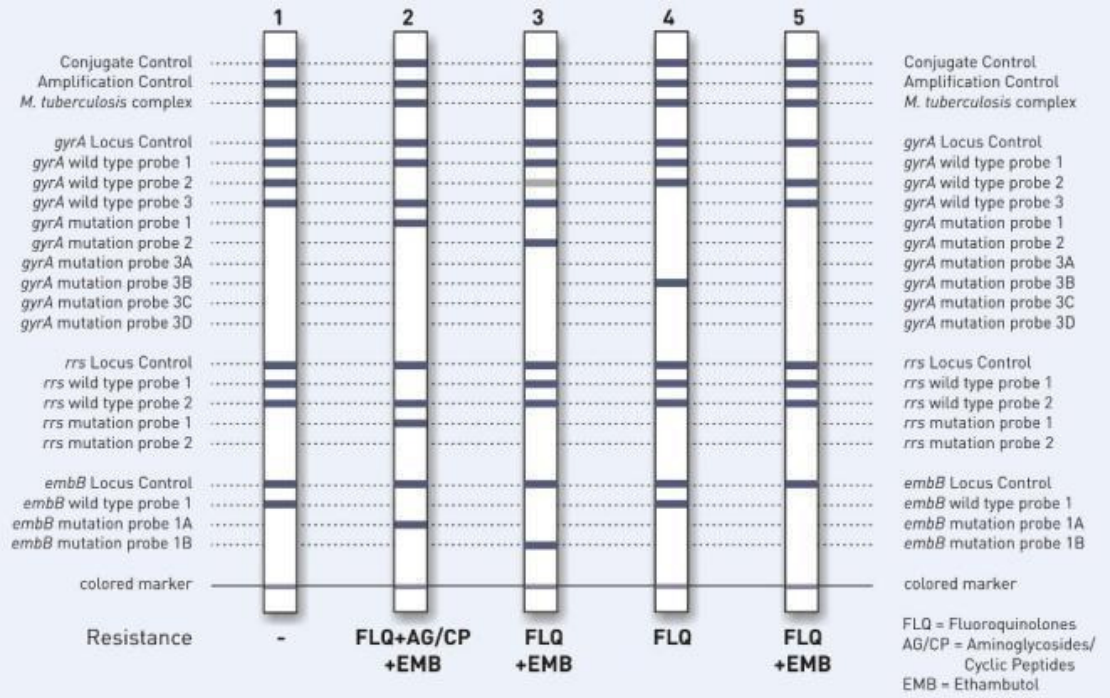
Figure 2. Examples of different GenoType® MTBDRsl strip readouts (Hain Life Sciences 2012b).



A template is supplied by the manufacturer to help read the strips Appendix 1 where the banding patterns are scored by eye, transcribed and manually fed into the Laboratory Information System (LIS). In high-volume settings, the GenoScan®, an automated reader, can be incorporated to interpret the banding patterns automatically and give a suggested interpretation (an example output of the machine is shown in Appendix 2. If the operator agrees with the interpretation, the results are automatically downloaded into the LIS, thus eliminating possible transcription errors. It is important to note that the automated reader only provides a suggested result and requires manual confirmation of the result after the operator has visually inspected the banding pattern. Nonetheless, the test manual provides fairly straightforward instruction with little room for variation in interpretation, even human interpretation. The entire assay procedure can be completed in five hours. The assay can also be performed on DNA from pure isolates taken from cultured patient specimens. Once a diagnosis of MDR-TB has been established, the MTBDR*sl* can also be used to confirm a diagnosis of XDR-TB.

Figure 2 shows an example of different MTBDR*sl* results. The assay consists of two internal controls (a conjugate control for confirmation of the colorimetric reaction used to visualise bands and an amplification control to ensure that nucleic acid amplification reaction has occurred) plus a control for each gene locus (*gyrA*, *rrs*, *embB*). The two internal controls plus the locus control for the gene of interest should always be positive; otherwise the assay cannot be evaluated for that particular drug. Of note is that a result can be indeterminate for one gene but valid for another (on the basis of only the gene-specific locus control failing). A band for the detection of the *M. tuberculosis* complex (the "TUB" band) is included. Should the wild-type or mutant probes appear whilst the locus control for a specific gene is less intense than that of the amplification control band (AC band) and the TUB band is interpretable, the locus probes should be considered secondary to that of the other probes for the gene in question and can thus be considered for interpretation.

An earlier version of the MTBDR*sl* manual (version 1) stated that if the locus band was absent but other non-control bands were present (even together with their accompanying gene locus control bands) the assay should be considered non-evaluable (Hain Life Sciences 2012a). However, the most recent version of the manual (version 2; Hain Life Sciences 2012b) states: "in rare cases the TUB zone may be negative while an evaluable resistance pattern is developed. If so, the presence of a strain belonging to the MTB complex must be suspected and the assay should be repeated". Upon inspection, most of these are nontuberculous mycobacteria and thus if the TUB band is not present, it is suggested to use the GenoType® CM/AS kit for the identification of other common mycobacteria, or additional species should the GenoType® CM/

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AS kit fail to produce a positive identification for any of the 17 species covered by the GenoType® CM/AS kit (Hain Life Sciences 2012b).

Clinical pathway

Figure 1 illustrates the clinical pathway. Depending on the setting, DST is either performed on all patients with confirmed TB or only on patients who are clinically suspected of having DR-TB (for example, if the patient's symptoms have failed to improved on first-line therapy, or if they still have viable bacilli in their sputum after an extended period of treatment. As mentioned above, the manufacturer recommends that if the patient specimen (usually sputum) is smear-positive the assay be performed directly on the specimen (direct testing). If smear-negative, it is recommended that the assay be performed on the culture isolate grown from the patient specimen (indirect testing). DST for resistance to the second-line drugs is only performed if resistance to the first-line drugs is confirmed. Where routine molecular (genotypic) testing is well established, phenotypic DST is not usually performed. However, we expected research studies evaluating the accuracy of molecular DSTs, such as the MTBDR*sl* assay, to almost always include phenotypic DST as a reference standard. Furthermore, we also expected some studies to use genetic sequencing to resolve any discordant index test-reference standard results.

Prior test(s)

As detailed in Figure 1, patients who received MTBDR*sl* testing will first have received (i) smear microscopy, (ii) liquid culture (if smear-negative), and (iii) phenotypic or genotypic DST for resistance to first-line drugs.

Role of index test(s)

MTBDR*sl* would be used as an initial test replacing phenotypic culture-based DST as the initial test.

Rationale

Second-line TB drugs are used to treat patients with TB that is resistant to the most effective and widely used first-line drugs. To ensure that the most appropriate and least toxic drugs are provided to patients as quickly as possible, it is critical to know whether a patient has resistance to FQs alone, resistance to SLIDs alone, or resistance to both FQs and SLIDs (XDR-TB) as this will guide the selection of drugs. In addition, the presence of XDR-TB has major prognostic implications for the patient and for infection control. The conventional method for the diagnosis of drug resistance (phenotypic culture-based testing) is vulnerable to contamination and the culture can lose viability, meaning it cannot be tested. This method is also slow and can take several months. The resulting diagnostic delay results in unnecessary morbidity, mortality and increased transmission, which is a major driver of new TB cases. There is a need for rapid assays to improve time-to-diagnosis and new molecular assays, such as the MTBDR*sl* assay, present a promising potential solution.

OBJECTIVES

- To assess and compare the diagnostic accuracy of MTBDR*sl* for the detection of resistance to FQs in patient specimens (using direct testing) and culture isolates (using indirect testing) confirmed as TB positive.

- To assess and compare the diagnostic accuracy of MTBDR*sl* for the detection of resistance to SLIDs in patient specimens (using direct testing) and culture isolates (using indirect testing) confirmed as TB positive.

- To assess and compare the diagnostic accuracy of MTBDR*sl* for the detection of XDR-TB in patient specimens (using direct testing) and culture isolates (using indirect testing) confirmed as TB positive.

Secondary objectives

We planned to investigate heterogeneity in relation to the reference standard (culture-based DST compared with (1) genetic sequencing, (2) culture-based DST and genetic sequencing, and (3) culture-based DST followed by genetic sequencing with discordant results) and individual drugs within a drug class (for example, ofloxacin and moxifloxacin within the FQ class). We also prespecified in the protocol investigations of heterogeneity in relation to HIV status, condition of the specimens (fresh or frozen, volume of specimen), patient population (patients suspected of having MDR-TB or XDR-TB) and whether WHO-recommended critical drug concentrations were used for culture-based reference testing.

METHODS

Criteria for considering studies for this review

Types of studies

We included all studies that determined the diagnostic accuracy of the index test in comparison with a defined reference standard, including case-control designs, in which cases and controls were sampled from the same patient population. We only included studies from which data could be extracted for true positives (TP), true

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negatives (TN), false positives (FP) and false negatives (FN). We excluded unpublished studies reported only in abstracts.

Participants

We included patients and specimens from patients of any age who were thought to have resistance to any of the second-line TB drugs, as well as patients and patient specimens with confirmed MDR-TB from all settings, irrespective of background burden and patient population.

Index tests

We included studies that evaluated the MTBDR*sl* assay.

Target conditions

We considered three target conditions:

1. Resistance to any of the FQs. The FQs include ofloxacin, levofloxacin and moxifloxacin. We excluded ciprofloxacin because this drug is infrequently used in DST.
2. Resistance to any of the SLIDs. The SLIDs include two aminoglycosides, kanamycin and amikacin, and one cyclic peptide, capreomycin.
3. XDR-TB.

For the FQs, the presence of mutations in each of the genes probed by the MTBDR*sl* assay has very high concordance with resistance to all drugs within that drug class. For example, a mutation in the *gyrA* usually means a strain is resistant to each of the FQs: ofloxacin, levofloxacin and moxifloxacin (Sirgel 2012a). The same holds true for the *rrs* gene and the two aminoglycosides, kanamycin and amikacin (Sirgel 2012b). Evidence is mixed regarding the level of concordance between resistance to the two aminoglycosides and capreomycin arising from mutations in the *rrs* gene. We acknowledge that determining resistance to all three SLIDs together, and thus including capreomycin with the aminoglycosides, may be a limitation. However, the index test results are reported in this manner. We discuss this issue further in the Discussion.

Reference standards

The following reference standards were used to define the target conditions:

1. Phenotypic culture-based DST: solid culture or a commercial liquid culture system (BACTEC 460, MGIT 960 and MGIT Manual System, Becton Dickinson, USA) incorporating the drug of interest.
2. Genetic sequencing of the *gyrA* or *rrs* genes, or both.
3. Two reference standards used together: phenotypic culture-based DST and genetic sequencing of the same samples. If a specimen was resistant according to phenotypic culture-based DST or had a mutation in the *gyrA* or *rrs* genes, the specimen was classified as having the target condition. If both phenotypic culture-based DST and genetic sequencing indicated susceptibility, the specimen was classified as not having the target condition.
4. Two reference standards used sequentially: phenotypic culture-based DST followed by selective testing by genetic sequencing of samples with discordant results (also referred to as discrepant analysis). Discordant results may be either index test positive/phenotypic culture-based DST negative or index test negative/phenotypic culture-based DST positive.

There are strengths and limitations to each of the reference standards. As mentioned, phenotypic culture-based DST is the conventional reference standard, but it is considered to be imperfect and is dependent on the drug concentration threshold used to define resistance. Genetic sequencing is considered to be more accurate than phenotypic culture-based DST; however, this is only if it targets all known resistance determining regions, which are not completely defined for the FQs and the SLIDs. Therefore, genetic sequencing can miss mutations that may cause drug resistance which fall outside of the targeted genes. Furthermore, genetic sequencing is usually applied only to culture isolates when results for the index test and the culture-based reference test do not agree. In this latter situation, there is potential for verification bias because the same reference standard is not being used to verify all index test results.

We carried out separate analyses for the different reference standards, described below. In our primary analysis we used culture-based DST as the reference standard. We expected all or nearly all included studies to report results using this reference standard.

Search methods for identification of studies

We attempted to identify all relevant studies regardless of language and publication status (published, unpublished, in press and ongoing). We searched for unpublished data as a means of ensuring the sensitivity of the search for published literature. Unpublished data in this field may provide misleading results as the data set is incomplete. While unpublished sources were searched, we did not include unpublished data in the review. We did not apply date restrictions to the searches.

Electronic searches

Vittoria Lutje (VL), the Information Specialist for the Cochrane Infectious Diseases Group, performed literature searching up to 30 January 2014. To identify all relevant studies, she searched the following databases using the search terms and strategy described in Appendix 3: Cochrane Infectious Diseases Group Specialized Register; MEDLINE (Pubmed, 1966 to January 2014); EMBASE OVID (1980 to January 2014); ISI Web of Knowledge (Science Citation Index - Expanded (1900 to present), Conference Proceedings Citation Index- Science (CPCI-S) (1990 to present) and BIOSIS Previews (1926 to January 2014)); MEDION (http://

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www.mediondatabase.nl/); LILACS (http://lilacs.bvsalud.org/en/ ; 1982 to January 2014); and SCOPUS (1995 to January 2014). VL also searched the metaRegister of Controlled Trials (mRCT; http://www.controlled-trials.com/) and the search portal of the World Health Organization (WHO) International Clinical Trials Registry Platform (www.who.int/trialsearch), to identify ongoing trials, and ProQuest Dissertations & Theses A&I to identify relevant dissertations.

Searching other resources

We reviewed reference lists of included articles and any relevant review articles identified through the above methods. We contacted the assay manufacturer (Hain Life Sciences) to identify unpublished studies. We contacted researchers at the Foundation for Innovative New Diagnostics (FIND), members of the StopTB Partnership's New Diagnostics Working Group and other experts in the field of TB diagnostics for information on ongoing or unpublished studies.

Data collection and analysis

Selection of studies

Two review authors (GT and JP) independently scrutinized titles and abstracts identified by electronic literature searching to identify potentially eligible studies. We selected all citations identified as suitable during this screen for full-text review. The same two review authors then independently reviewed full-text papers for study eligibility using the predefined inclusion and exclusion criteria. For full text articles, we resolved any discrepancies by discussion with a third review author (KRS). We maintained a list of excluded studies and their reasons for exclusion.

Data extraction and management

Two review authors (GT and JP) independently extracted a set of data from each study using a piloted data extraction form. We resolved any discrepancies by discussion. Based on the pilot, we finalized the data extraction form. We then independently extracted data on the following characteristics:

- Details of study: first author; publication year; country where testing was performed; setting (primary care laboratory, hospital laboratory, reference laboratory); study design; manner of participant selection; number of participants enrolled; number of participants for whom results available; industry sponsorship.
- Characteristics of participants: age (mean, SD; median, interquartile range; age range); HIV status; smear status; history of TB; known MDR-TB, pre-XDR-TB or XDR-TB status.
- Target conditions: resistance to FQs; resistance to SLIDs; XDR-TB.
- Reference standards: name and manufacturer; type; percentage of patients whose reference standard was 'uninterpretable' (for example, contaminated, sequencing failed).
- Details of specimen: type (such as expectorated sputum, induced sputum or culture isolate); condition (fresh or frozen); definition of a positive smear; type of testing (direct testing or indirect testing).
- Details of outcomes: the number of TP, TN, FP and FN results; number of indeterminate assay results.
- Time to treatment initiation: defined as the time from specimen collection until patient starts treatment.
- Time to diagnosis: defined as the time from specimen collection until there is an available TB result in lab or clinic, if the assay was performed in a clinic.

We assigned country income status (high-income or low- and middle-income) as classified by the World Bank List of Economies (World Bank 2014). We contacted authors of primary studies for missing data or clarifications. We entered all data into a database manager (Microsoft Excel 2012).

For one study that tested the same panel of TB isolates in multiple centres, we selected one centre that provided results in the middle range (neither the best nor the worst results).

Whenever possible, we extracted data that used a single patient as the unit of analysis (one MTBDR*sl* result per one specimen from one patient).

When culture-based DST was performed using more than one drug from the FQs (ofloxacin, moxifloxacin or levofloxacin) or SLIDs (amikacin, kanamycin or capreomycin), we extracted data (TP, TN, FP, FN) for each drug and for each class overall. We also extracted data for the SLIDs as a class overall if culture-based DST was performed using only one drug.

No studies reported on the number of 'no TB' or 'no result' results obtained from MTBDR*sl,* therefore we only reported the proportion of 'indeterminate' results.

In the 2 x 2 tables of TP, FP, FN and TN, we based the results of the index test on categorical assay results defined by the visual readout of the MTBDR*sl* strip.

Possible results for the Genotype® MTBDR*sl* assay (as defined by the product manual)

1. Sensitive to either FQs or SLIDs (referred to as 'aminoglycosides/cyclic peptides'), or both (conjugation and amplification bands present; TUB band present; gene locus band present; all wild type (*wt*) bands for each gene present; no mutation bands present). In the case of susceptibility to both drug classes, the test would indicate susceptibility for each, rather than having a single composite readout specifying XDR-TB.
2. Resistant to either FQs or SLIDs, or both (conjugation and amplification bands present; TUB band present; gene locus band present; all, none or some *wt* bands for each gene present; all, none or some mutation bands present with similar intensity to

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amplification control). In the case of resistance to both drug classes, the test would indicate resistance for each, rather than having a composite readout.

3. Indeterminate (faint bands) or no result (no conjugation or amplification bands present, no locus band present for the gene of interest).

4. No TB (negative for MTB complex irrespective of locus control band).

5. No result (failure of any one of the control bands, as well as the TUB band).

Assignment of results to the fluoroquinolones, second-line injectable drugs or both categories

MTBDR*sl* detects the presence of mutations in genes that cause drug resistance for drug classes (ie FQs, SLIDs or both), not to individual drugs within these classes (ofloxacin, moxifloxacin and levofloxacin in the case of the FQs; amikacin, kanamycin and capreomycin in the case of SLIDs). Thus, one study might use phenotypic DST for detection of kanamycin resistance and another study might use phenotypic DST for detection of amikacin resistance as reference standards to confirm SLID resistance. In such a scenario, if the phenotypic DST was positive for resistance and the MTBDR*sl* result was concordant, we classified the index case result as true-positive. We adopted the same approach for the FQs. Similarly, if the index tests reported resistance to a SLID and, in the case of genetic sequencing being used as a reference standard, the presence of mutations known to be associated with drug resistance to the SLIDs was confirmed, we recorded this as a concordant result positive for resistance to SLIDs. A similar approach was used for the FQs that used genetic sequencing as a reference standard.

Assessment of methodological quality

We appraised the quality of the included studies with the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool (Whiting 2011; Appendix 4). QUADAS-2 consists of four domains: patient selection, index test, reference standard, and flow and timing. We assessed all domains for the potential for risk of bias and the first three domains for concerns regarding applicability. We used signalling questions in each domain to form judgments about the risk of bias. One review author (GT) piloted the tool with two included studies and finalized the tool based on experience gained from the pilot testing. Two review authors then independently assessed methodological quality of included studies with the finalized tool.

Statistical analysis and data synthesis

We performed descriptive analysis for key variables (such as country income status and number of study participants) of the primary studies using Stata version 12.0 and displayed key study characteristics in Characteristics of included studies.

We used the reference standard 'culture' in our primary analyses. We stratified these analyses first by target condition (FQ resistance, SLID resistance or XDR-TB) and second by type of MTBDR*sl* testing (indirect testing or direct testing). Within each stratum (for example, FQ resistance by indirect testing), we plotted estimates of the studies' observed sensitivities and specificities in forest plots with 95% confidence intervals (CI) and in receiver-operating characteristic (ROC) space using Review Manager (RevMan). Where adequate data were available, we combined data using meta-analysis. We performed the majority of meta-analyses by fitting the bivariate random-effects model (Macaskill 2010; Reitsma 2005) using Stata version 11 with the metandi and xtmelogit commands. We compared models with separate and identical variance terms using likelihood ratio tests to determine the best fitting model. In situations in which there were fewer than four studies, we determined summary estimates of sensitivity and specificity by simplifying the bivariate model to two univariate random-effects logistic regression models. When it was not possible to fit the model and we observed little heterogeneity, we determined summary estimates of sensitivity and specificity separately using a fixed-effect model (Zamora 2006). We presented meta-analysis summaries in tables and ROC space.

We compared results from studies of direct testing with results from studies of indirect testing by adding a covariate for the type of testing to the model. We assessed the significance of the difference in test accuracy between studies using direct testing and studies using indirect testing by a likelihood ratio test comparing models with and without covariate terms. For these comparative analyses, we first included all studies with relevant data and then included only those studies that made direct comparisons between direct and indirect testing with the same participants, where such studies existed. We present the results according to the stated objectives, under the appropriate subheadings in the Results section for each condition: Estimates of the diagnostic accuracy of MTBDR*sl* using phenotypic culture-based DST as a reference standard, and Investigations of heterogeneity for each testing method.

Approach to uninterpretable (indeterminate) MTBDR*sl* results

We excluded indeterminate test results from the analyses for determination of sensitivity and specificity. We determined the proportion of indeterminate MTBDR*sl* results among the primary studies for each target condition and provided results separately for indirect and direct testing.

Investigations of heterogeneity

Within each stratum (for example SLID resistance), we investigated heterogeneity through visual examination of forest plots of

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sensitivity and specificity. Then, if sufficient studies were available, we explored the possible influence of the following pre-specified categorical covariates: reference standard (culture, genetic sequencing, culture and genetic sequencing, culture followed by genetic sequencing) and individual drug (amikacin, kanamycin and capreomycin). We determined variation in sensitivity and specificity by adding covariate terms to the meta-analysis models described above. The significance of the difference in test accuracy (for example, between studies using culture versus those using genetic sequencing as the reference standard) was assessed by a likelihood ratio test comparing models with and without covariate terms.

We had also planned to investigate the effect of HIV status, the condition of the specimen (fresh or frozen), sample volume, the drug concentration used for culture-based DST (WHO-recommended or not) and patient population (patients thought to have MDR-TB or XDR-TB) on summary estimates of sensitivity and specificity in a meta-regression analysis by adding covariate terms to the bivariate model. However, there were insufficient data for these additional analyses.

Sensitivity analyses

For our primary analysis using the culture-based DST reference standard, we performed sensitivity analyses for four QUADAS-2 signalling questions to explore whether the results we found were robust with respect to the methodological quality of the studies. We used the following questions:

- Was a consecutive or random sample of patients/specimens enrolled?
- Was a case-control design avoided?
- Were the reference standard results interpreted without knowledge of the results of the index test?
- Were the index test results interpreted without knowledge of the results of the reference standard?

We did not exclude any studies based on these answers.

Assessment of reporting bias

We did not undertake a formal assessment of publication bias of data included in this review using methods such as funnel plots or regression tests because such techniques have not been found to be helpful for determining publication bias within diagnostic test accuracy studies (Macaskill 2010; Tatsioni 2005).

Other analyses

We had intended to summarize two patient outcomes, time-to-diagnosis and time-to-treatment initiation; however time-to-diagnosis was the only one described in the included studies.

RESULTS

Results of the search

Our search identified 630 titles (Table 1; Table 2; Figure 3). We did not add any additional titles after reference review or contact with experts. After we removed duplicates, 262 titles remained of which we excluded 140 titles based on a review of title, or abstract, or both. We retrieved full text articles for 41 citations, of which we excluded 20, leaving 21 unique studies included in the review and meta-analysis (Figure 3). We have listed the reasons for exclusion of studies in the Characteristics of excluded studies section. One of the 21 studies (Ignatyeva 2012) evaluated a panel of isolates at four different sites in Eastern Europe and we extracted data for the one site that neither performed the best or the worst.

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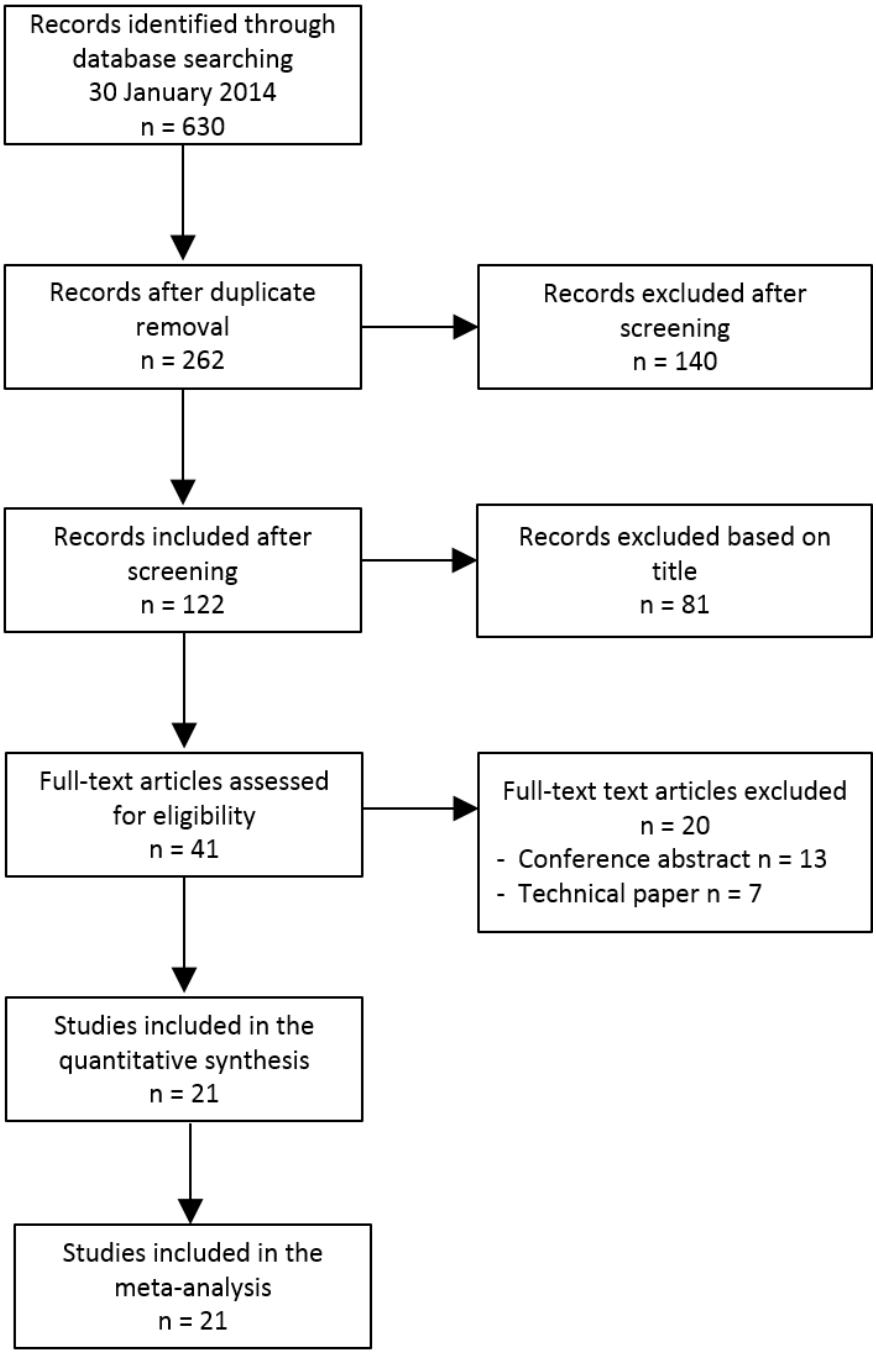
Figure 3. Study flow diagram.



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Methodological quality of included studies

Figure 4 and Figure 5 show the quality assessment of the 21 included studies. In the patient selection domain, we considered 10 studies (48%) to be at low risk of bias because participants were enrolled consecutively or randomly and the study design was cross-sectional. We considered nine studies to be at high risk of bias because (1) there was a case-control design (five studies: Brossier 2010; Hillemann 2009; Ignatyeva 2012; Kiet 2010; Miotto 2012); (2) enrolment was by convenience (three studies: Barnard 2012; Lacoma 2012; Lopez-Roa 2012); or (3) the study had both a case-control design and convenience sampling (one study: van Ingen 2010). We considered two studies to have unclear risk of bias because it was unclear how patients were selected (Chikamatsu 2012; Fan 2011). With regard to applicability (patient characteristics and setting), we judged 15 studies (71%) to include the appropriate patients and settings to address the review question and six studies to have a high concern about applicability (Brossier 2010; Hillemann 2009; Ignatyeva 2012; Kiet 2010; Miotto 2012; van Ingen 2010). In the index test domain, we considered two studies at high risk of bias as the index test results were not interpreted without knowledge of the results of the reference standard (Chikamatsu 2012; Kiet 2010) and seven studies at unknown risk of bias because information about blinding was unavailable (Brossier 2010; Fan 2011; Ferro 2013; Hillemann 2009; Lopez-Roa 2012; Surcouf 2011; Tukvadze 2014). In all but two studies (Brossier 2010; Tukvadze 2014), the use, conduct and interpretation of the index test was considered applicable. In the reference standard domain, we judged eleven studies (52%) to be at low risk of bias because the reference standard was appropriate and the results were interpreted without knowledge of the results of the MTBDR*sl* assay (Ajbani 2012; Barnard 2012; Chikamatsu 2012; Hillemann 2009; Huang 2011; Ignatyeva 2012;Jin 2013; Lopez-Roa 2012 Miotto 2012; Tukvadze 2014; Zivanovic 2012. We judged applicability to be of low concern for all studies in the reference standard domain. In the flow and timing domain, we considered 16 studies (76%) to be of low concern for risk of bias because all patients were accounted for in the analysis, information about uninterpretable results was provided and all patients had the same reference tests performed. We considered five studies to have unclear risk of bias in the flow and timing domain because discrepant analysis was performed (Ajbani 2012; Barnard 2012; Kiet 2010; Lacoma 2012; Lopez-Roa 2012) meaning that not all patients received culture-based and sequencing reference testing. Also, we considered one study (Ferro 2013) to have unclear risk of bias because not all patients were accounted for in the analyses. We noted industry involvement in seven (33%) studies and this included: i) donation of MTBDR*sl* tests (four studies: Hillemann 2009; Miotto 2012; Surcouf 2011; Ferro 2013); ii) preferred pricing of MTBDR*sl* tests (one study: Barnard 2012); iii) financial support for non-test related study costs (one study: Said 2012); and iv) involvement in the design, analysis or manuscript production (one study: Ajbani 2012).

Figure 4. Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies.

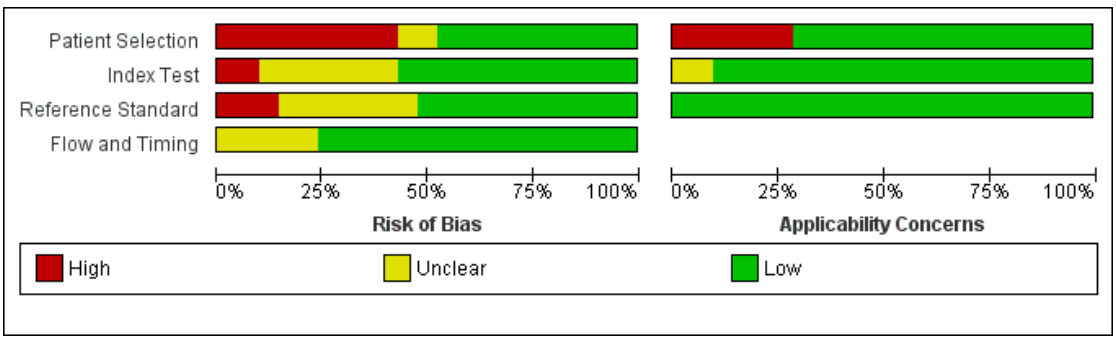

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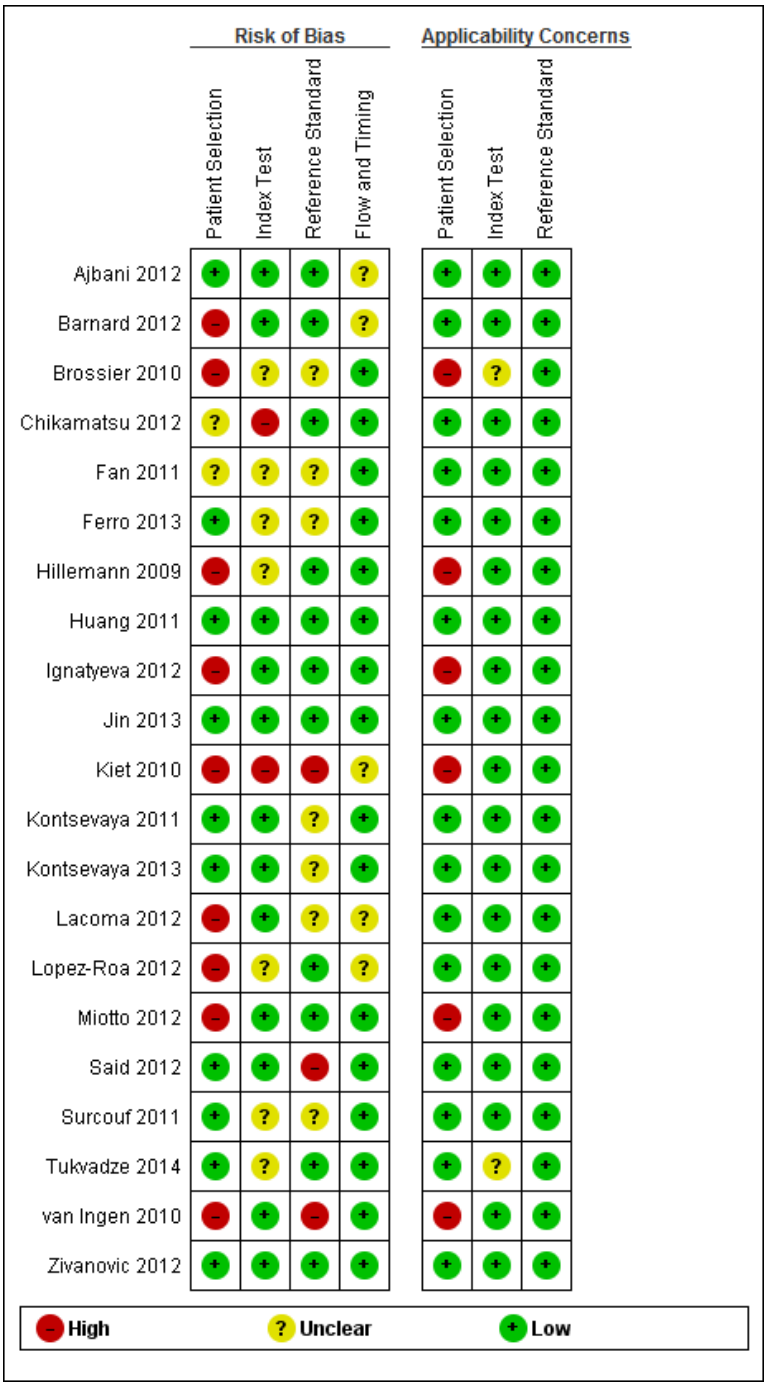
Figure 5. Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study.



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Findings

Of the 21 included studies, eight reported on MTBDR*sl* testing for resistance to FQs, SLIDs and XDR-TB, 12 reported on testing for resistance to FQs and SLIDs, and one reported on testing for resistance to FQs only. Fourteen studies reported on MTBDR*sl* performance when done directly on patient specimens, five studies reported on MTBDR*sl* when performed indirectly on isolates grown from the specimens and a further two studies contained information on both testing methods. Of the 21 studies, 11 used only phenotypic culture-based DST, seven used sequencing and culture on all specimens, three used culture followed by the sequencing of discrepant results and one used sequencing alone. The median (interquartile range (IQR)) number of participants in each study was 100 (50.75, 229.5). The proportion of patients screened with resistance to a FQ, SLID, or XDR-TB (according to phenotypic culture-based testing) were 30% (95% CI 28 to 32), 32% (95% CI 30 to 34), or 15% (95% CI 13 to 17), respectively. We presented key characteristics for the 21 studies in the Characteristics of included studies section. The majority (15 studies, 71%) were of cross-sectional study design. One study (Barnard 2012) included extrapulmonary specimens that we excluded from the analysis. Eleven studies (58%) were located in low-income or middle-income countries. All studies but two (Fan 2011, written in Chinese, and Chikamatsu 2012, written in Japanese) were in English.

I. Fluoroquinolone resistance detection

A. Estimates of the diagnostic accuracy of MTBDR*sl* using phenotypic culture-based DST as a reference standard

1. Indirect testing

We present forest plots of MTBDR*sl* sensitivity and specificity when performed indirectly for the detection of FQ resistance for 16 studies (1766 participants) that used phenotypic culture-based DST as a reference standard in Figure 6. For individual studies, sensitivity estimates ranged from 57% to 100% and specificity estimates ranged from 77% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 83.1% (95% CI 78.7 to 86.7) and 97.7% (95% CI 94.3 to 99.1), respectively.

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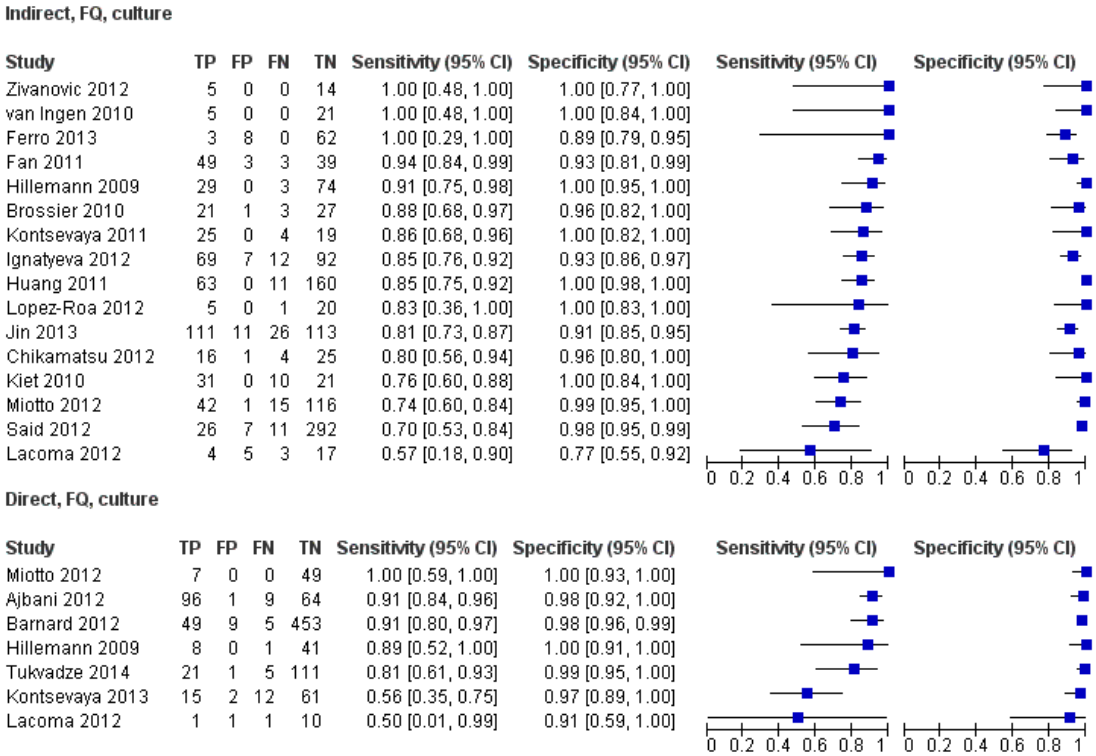
Figure 6. Forest plots of MTBDRsl sensitivity and specificity when performed indirectly or directly for FQ resistance detection and using phenotypic culture-based DST as a reference standard. The individual studies are ordered by decreasing sensitivity. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Values between brackets are the 95% CIs of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).



2. Direct testing

In Figure 6 we show forest plots of MTBDR*sl* sensitivity and specificity when performed directly for the detection of resistance to FQs for seven studies (1033 participants) that used phenotypic culture-based DST as a reference standard. For individual studies, sensitivity estimates ranged from 50% to 100% and specificity estimates ranged from 91% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 85.1% (95% CI 71.9 to 92.7) and 98.2% (95% CI 96.8 to 99.0), respectively.

3. Comparison of indirect versus direct testing

(i) Diagnostic accuracy

We present results comparing indirect and direct MTBDR*sl* testing for detection of FQ resistance in Table 3, Table 4 and Figure 7. There was no evidence of a statistically significant difference in MTBDR*sl* accuracy between indirect and direct testing and using culture-based DST as a reference standard when the test was performed in different populations (indirect comparison, P = 0.549). Direct comparisons within the same population were not possible because no studies performed direct and indirect MTBDR*sl* testing on specimens or isolates from the same patients.

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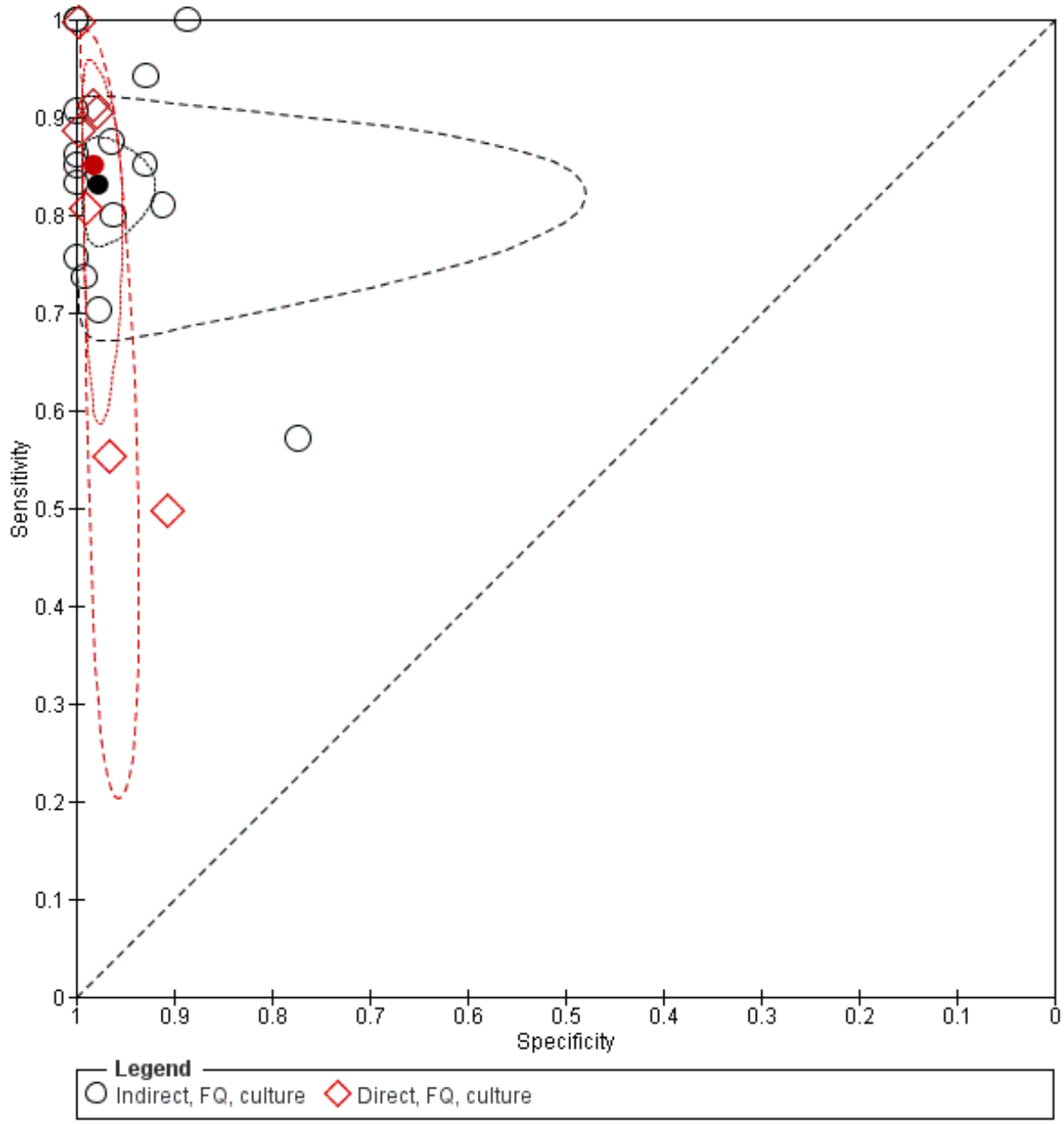
Figure 7. Summary plots of MTBDRsl sensitivity and specificity comparing detection of fluoroquinolone resistance by indirect and direct testing. The solid circles correspond to the summary estimates of sensitivity and specificity and are shown with 95% confidence regions (dotted lines) and 95% prediction regions (dashed lines).



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(ii) Indeterminate rates

For indirect testing for FQ resistance, four (0.2%) of 1766 MTBDR*sl* results were indeterminate (three culture DST resistant and one culture DST sensitive), whereas for direct testing 20 (1.9%) of 1033 were MTBDR*sl* indeterminate (P < 0.001; 14 were culture DST-sensitive and six did not report a culture-based DST result).

B. Investigations of heterogeneity

1. Indirect testing

(i) Type of reference standard

We present MTBDR*sl* accuracy estimates for detection of FQ resistance against different reference standards in Table 3 and Appendix 5.

Reference standard is genetic sequencing:

For individual studies (seven in total), sensitivity estimates ranged from 85% to 100% and specificity estimates ranged from 92% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 99.3% (95% CI 85.9 to 100.0) and 99.7% (95% CI 92.0 to 100.0), respectively. The accuracy using this reference standard was higher than when culture-based DST was used (P < 0.001 for indirect statistical comparisons, Table 3; P < 0.001 for direct statistical comparisons, Table 4). Five studies sequenced the *gyrA* gene and two sequenced *gyrA* and *gyrB*.

Reference standard is culture-based DST and genetic sequencing (ie both investigations performed in all isolates):

For individual studies (seven in total), sensitivity estimates ranged from 74% to 91% and specificity estimates ranged from 99% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 82.0% (95% CI 77.7 to 85.6) and 99.8% (95% CI 98.5 to 100.0), respectively. The accuracy using this reference standard was higher than when culture-based DST was used (P < 0.001 for indirect comparisons, Table 3; P < 0.001 for direct comparisons, Table 4).

Reference standard is culture-based DST followed by genetic sequencing of discrepant index test-culture-based DST results:

For individual studies (three in total), sensitivity estimates ranged from 73% to 100% and specificity estimates ranged from 94% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 83.7% (95% CI 74.2 to 90.8) and 99.7% (95% CI 98.4 to 100.0), respectively. Comparisons between accuracy estimates using this reference standard and culture-based DST were not possible given the small number of studies in the former group.

(ii) Drugs used in the culture-based DST

We present MTBDR*sl* accuracy estimates for detection of resistance to ofloxacin and moxifloxacin against a phenotypic culture-based reference standard in Table 3, Table 4 and Appendix 6. For ofloxacin resistance, sensitivity estimates ranged from 70% to 100% and specificity estimates ranged from 91% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 82.9% (95% CI 79.5 to 87.1) and 98.2% (95% CI 96.1 to 99.1), respectively. For moxifloxacin resistance, sensitivity estimates ranged from 57% to 100% and specificity estimates from 77% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 91.4% (95% CI 64.7 to 98.4) and 90.6% (95% CI 79.3 to 96.1), respectively. The accuracy of MTBDR*sl* when performed indirectly was not different for ofloxacin versus moxifloxacin (indirect comparison, P = 0.091). Appendix 7 presents a summary ROC plot of sensitivity versus specificity comparing test performance for detection of resistance to the individual FQ drugs.

(iii) Drug concentration used in culture-based DST

Nine studies used the WHO-recommended critical concentration of ofloxacin, whereas two did not (Jin 2013; Kiet 2010). Ferro 2013 used the WHO-recommended critical concentration for low level moxifloxacin resistance whereas Lacoma 2012 used the concentration recommended for high level resistance. Two studies (Fan 2011; van Ingen 2010) did not used the recommended critical concentration of moxifloxacin. Comparisons between accuracy estimates for each drug according to concentration were not possible given the small number of studies.

2. Direct testing

(i) Type of reference standard

Reference standard is genetic sequencing:

No studies performed direct MTBDR*sl* testing and used genetic sequencing as a reference standard.

Reference standard is culture-based DST and genetic sequencing (ie both investigations performed in all isolates):

No studies performed direct MTBDR*sl* testing and used both phenotypic culture-based DST and genetic sequencing (performed in all isolates) as a reference standard.

Reference standard is culture-based DST followed by genetic sequencing of discrepant index test-culture-based DST results:

Two studies reported MTBDR*sl* sensitivity and specificity when performed directly for the detection of resistance to FQs, with

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phenotypic culture-based DST and genetic testing performed only on discrepant results as a reference standard. The reported sensitivities were 91% and 96% and the reported specificities were 98% and 99%.

(ii) Drugs used in the culture-based DST

Sensitivity estimates for MTBDR*sl* for ofloxacin resistance by direct testing against a phenotypic culture-based reference standard for three studies ranged from 89% to 100%. Specificity estimates from 98% to 100%. No studies performed MTBDR*sl* by direct testing for moxifloxacin resistance.

(iii) Drug concentration used in culture-based DST

All three studies in this category used the WHO-recommended critical concentration for ofloxacin.

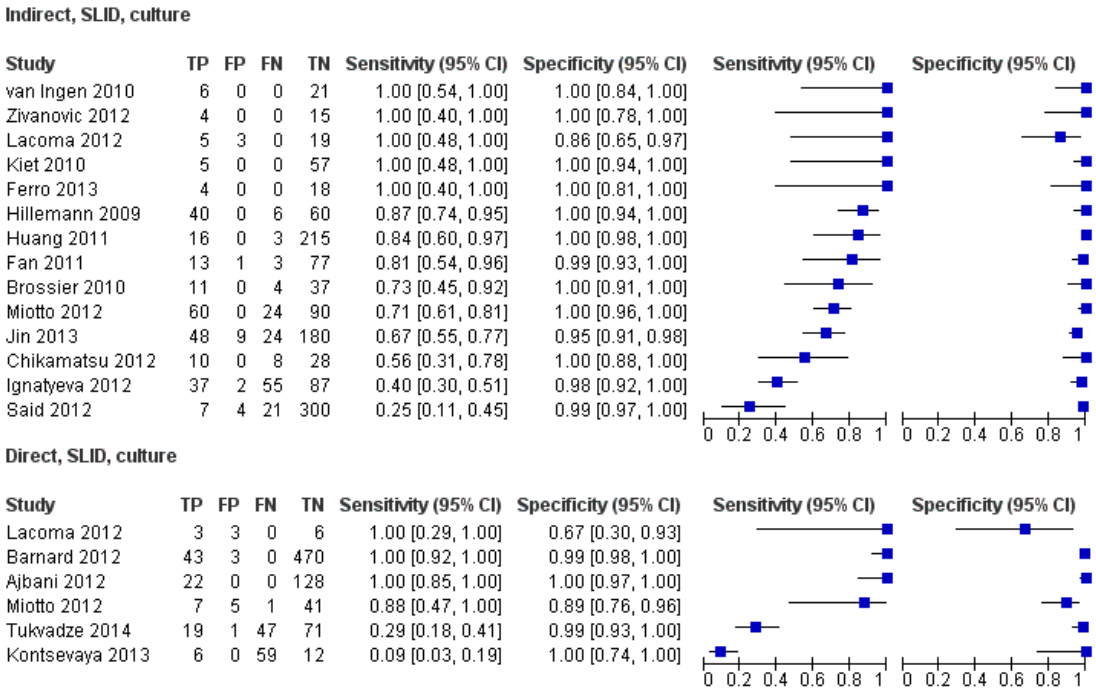
II. SLID resistance detection

A. Estimates of the diagnostic accuracy of MTBDR*sl* using phenotypic culture-based DST as a reference standard

1. Indirect testing

We present forest plots of MTBDR*sl* sensitivity and specificity when performed indirectly for the detection of resistance to SLIDs for 14 studies (1637 participants) that used phenotypic culture-based DST as a reference standard in Figure 8. For individual studies, sensitivity estimates ranged from 25% to 100% and specificity estimates ranged from 86% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 76.9% (95% CI 61.1 to 87.6) and 99.5% (95% CI 97.1 to 99.9), respectively.

Figure 8. Forest plots of MTBDRsl sensitivity and specificity for SLID resistance detection when performed indirectly or directly and using phenotypic culture-based DST as a reference standard. The individual studies are ordered by decreasing sensitivity. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Values between brackets are the 95% CIs of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line)



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2. Direct testing

In Figure 8 we show forest plots of MTBDR*sl* sensitivity and specificity when performed directly for the detection of resistance to SLIDs for six studies (947 participants) that used phenotypic culture-based DST as a reference standard. For individual studies, sensitivity estimates ranged from 9% to 100%, with one study from Eastern Europe reporting low sensitivity (Kontsevaya 2013). Specificity estimates ranged from 67% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 94.4% (95% CI 25.2 to 99.9) and 98.2% (95% CI 88.9 to 99.7), respectively. When the study from Eastern Europe that reported low sensitivity (Kontsevaya 2013) was removed (Appendix 8), the pooled sensitivity increased to 98.0% (95% CI 39.6 to 100.0), while the pooled specificity decreased to 97.8% (95% CI 86.4 to 99.7).

3. Comparison of indirect versus direct testing

(i) Diagnostic accuracy

We present results comparing indirect and direct MTBDR*sl* testing for SLID resistance in Table 3, Table 4 and Figure 9. The pooled sensitivity for direct testing (94.4%, 95% CI 25.2 to 99.9) was similar to the pooled estimate for indirect testing (76.9%, 95% CI 61.1 to 87.6) when the test was performed in different populations using all studies (indirect comparisons, P = 0.451). The pooled specificity was lower (indirect comparisons, P = 0.005) for direct testing (98.2%, 95% CI 88.9 to 99.7) when compared to indirect testing (99.5%, 95% CI 97.1 to 99.9) .

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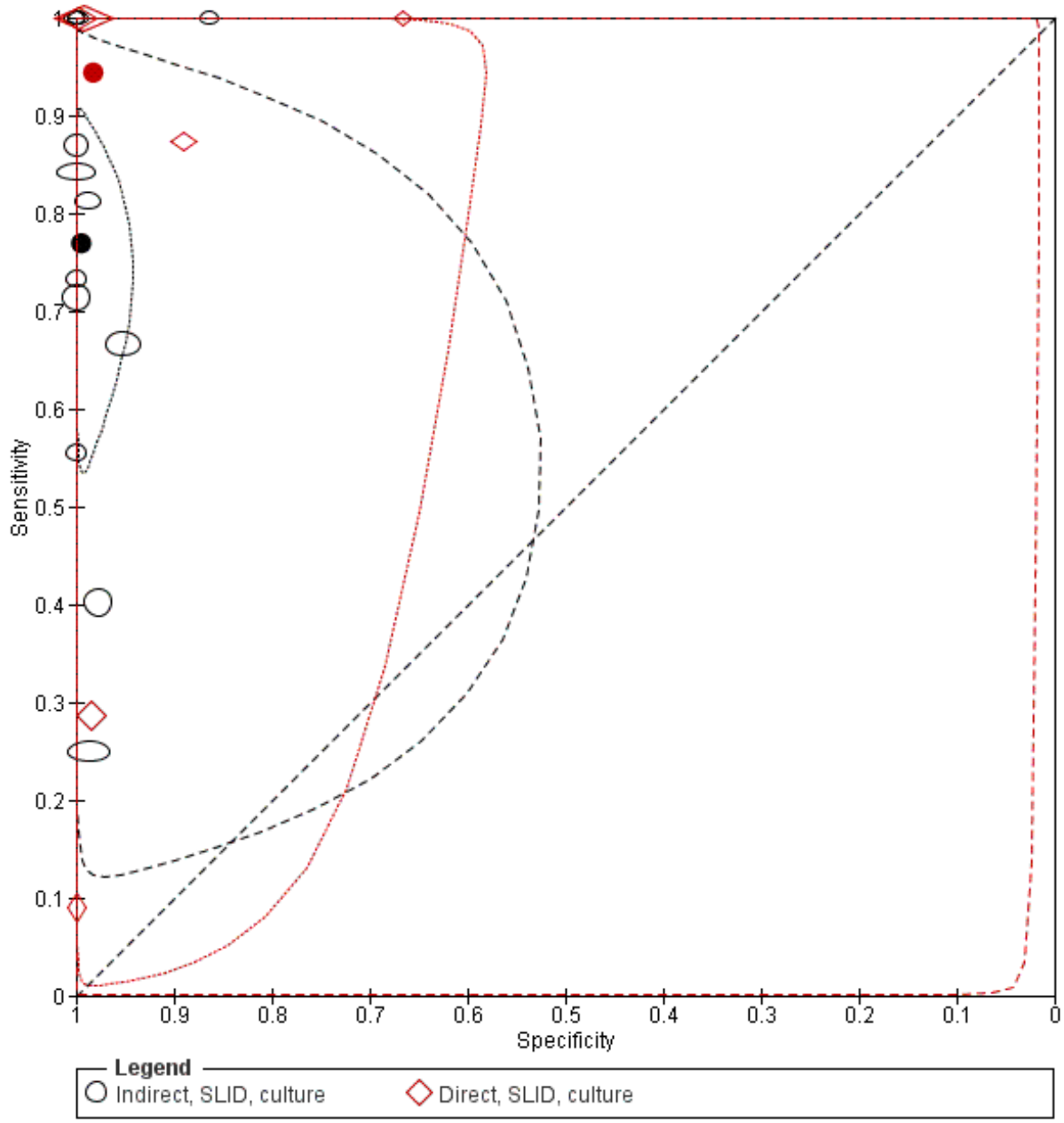
Figure 9. Summary plots of MTBDRsl sensitivity and specificity comparing detection of resistance for second-line injectable drugs by indirect and direct testing. The solid circles correspond to the summary estimates of sensitivity and specificity and are shown with 95% confidence regions (dotted lines) and 95% prediction regions (dashed lines).



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(ii) Indeterminate rates

For indirect testing for SLID resistance, three (0.4%) of 774 MTBDR*sl* results were indeterminate (one culture DST resistant and two culture DST sensitive; three studies did not report these), whereas for direct testing 53 (6.1%) of 873 were MTBDR*sl* indeterminate (four were culture DST resistant, 22 were culture DST susceptible and 27 did not have a culture-based DST result; one study did not report indeterminate results) (P < 0.001).

B. Investigations of heterogeneity

1. Indirect testing

(i) Type of reference standard

We present MTBDR*sl* accuracy estimates for detection of SLID resistance against different reference standards in Table 3 and Appendix 9.

Reference standard is genetic sequencing:

For individual studies (six in total), sensitivity estimates ranged from 62% to 100% and specificity estimates ranged from 96% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 97.8% (95% CI 77.0 to 99.7) and 99.5% (95% CI 94.5 to 100.0), respectively. The accuracy using this reference standard was higher than when culture-based DST was used (P = 0.017 for indirect statistical comparisons, Table 3; P = 0.045 for direct statistical comparisons, Table 4). All six studies sequenced only the *rrs* gene.

Reference standard is culture-based DST and genetic sequencing (ie both investigations performed in all isolates):

For individual studies (seven in total), sensitivity estimates ranged from 30% to 85% and specificity estimates ranged from 99% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 56.7% (95% CI 40.8 to 71.3) and 99.9% (95% CI 99.2 to 100.0), respectively. The accuracy using this reference standard was higher than when culture-based DST was used (P = 0.008 for indirect comparisons, Table 3).

Reference standard is culture-based DST followed by genetic sequencing of discrepant index test-culture-based DST results:

For individual studies (three in total), sensitivity estimates ranged from 34% to 100% and specificity estimates ranged from 95% to 100%. We did not determine summary estimates because there were only three studies and the sensitivity was variable.

(ii) Drugs used in the culture-based DST

We present MTBDR*sl* accuracy estimates for detection of resistance to amikacin, kanamycin and capreomycin by indirect testing against a phenotypic culture-based reference standard in Table 3 and Figure 10. For amikacin resistance, sensitivity estimates ranged from 80% to 100% and specificity estimates ranged from 97% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 87.9% (95% CI 82.1 to 92.0) and 99.5% (95% CI 97.5 to 99.9), respectively. For kanamycin resistance, sensitivity estimates ranged from 25% to 100% and specificity estimates from 86% to 100%. The pooled sensitivity and specificity were 66.9% (95% CI 44.1 to 83.8) and 98.6% (95% CI 96.1 to 99.5). For capreomycin resistance, sensitivity estimates ranged from 21% to 100% and specificity estimates from 86% to 100%. The pooled sensitivity and specificity were 79.5% (95% CI 58.3 to 91.4) and 95.8% (95% CI 93.4 to 97.3). Figure 11 presents a summary ROC plot of sensitivity versus specificity comparing test performance for detection of resistance to the individual SLIDs by indirect testing.

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Figure 10. Forest plots of MTBDRsl sensitivity and specificity when performed indirectly for the detection of resistance to amikacin (Ak), kanamycin (Kn) and capreomycin (Cm) using culture as a reference standard. The individual studies are ordered by decreasing sensitivity. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Values between brackets are the 95% CIs of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).

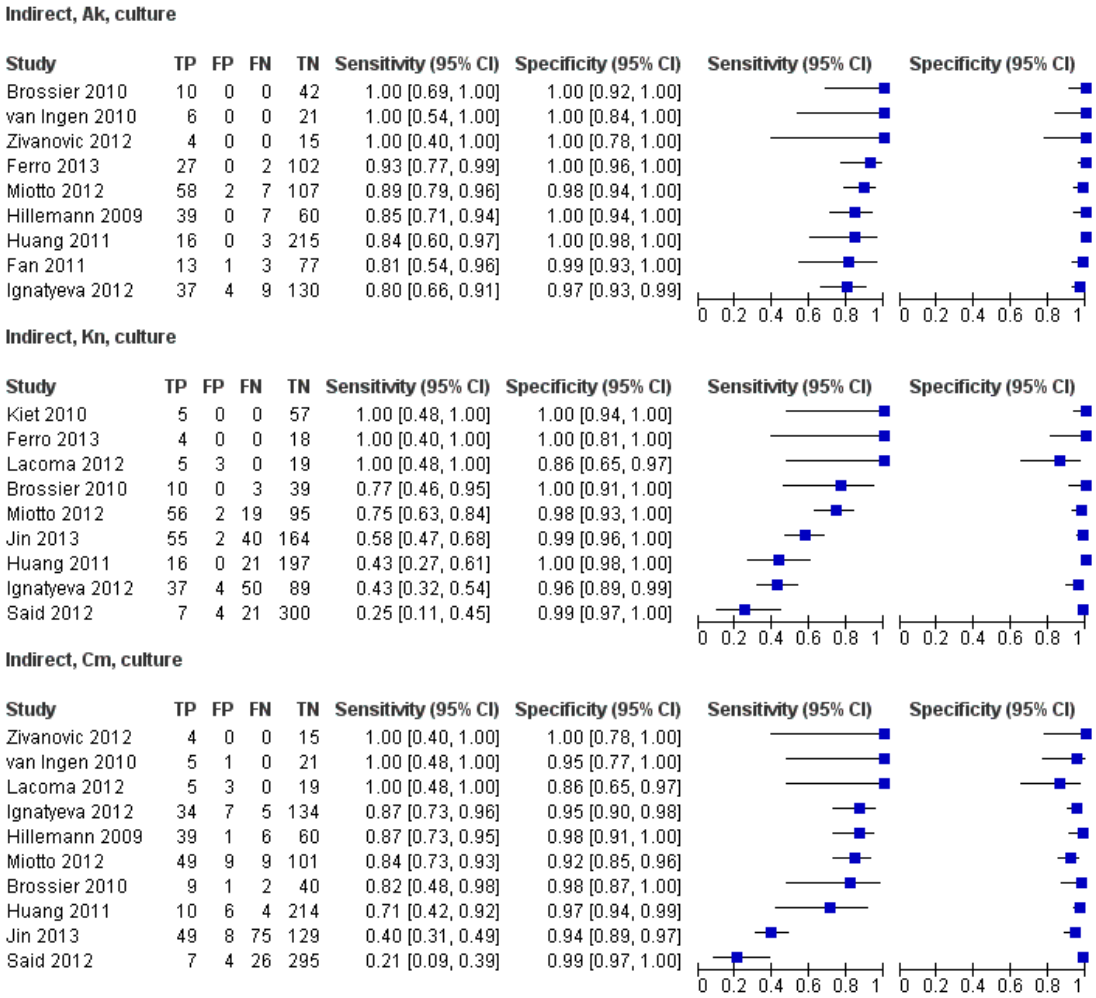

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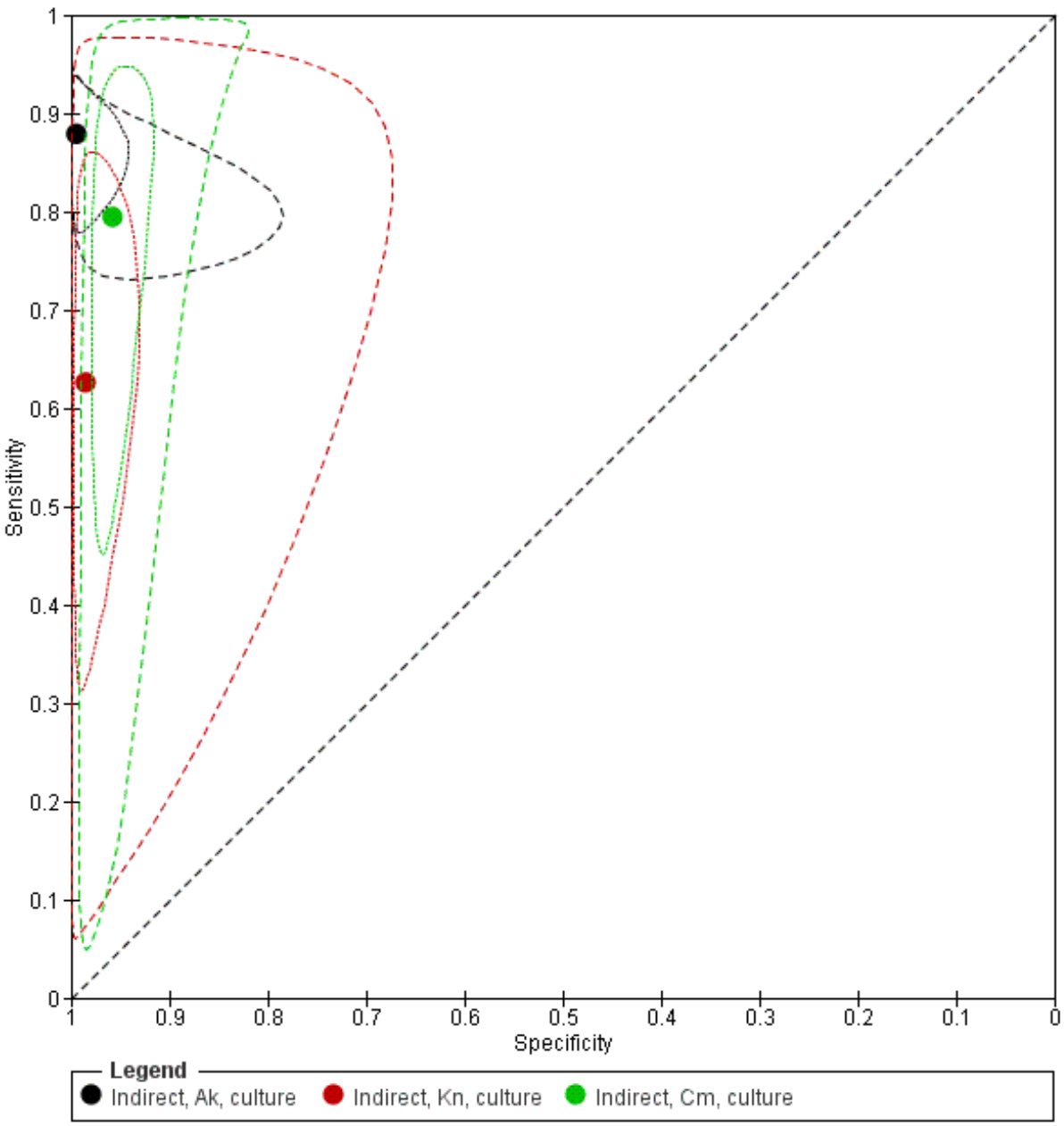
Figure 11. Summary plots of MTBDRsl sensitivity and specificity comparing indirect detection of resistance for amikacin (Ak), kanamycin (Kn) and capreomycin (Cm) using culture as a reference standard. The solid circles correspond to the summary estimates of sensitivity and specificity and are shown with 95% confidence regions (dotted lines) and 95% prediction regions (dashed lines).



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(iii) Drug concentration used in culture-based DST

Five studies used the WHO-recommended critical concentration of amikacin, whereas four did not. Two studies (Huang 2011; Ferro 2013) used the WHO-recommended critical concentration of kanamycin, whereas seven did not. Seven studies used the WHO-recommended critical concentration of capreomycin, two did not (Brossier 2010; Huang 2011) and one (Jin 2013) did not report the critical concentration used. Comparisons between accuracy estimates according to drug concentration were not possible given the small number of studies and participants.

2. Direct testing

(i) Type of reference standard

Reference standard is genetic sequencing:

No studies performed direct MTBDR*sl* testing and used genetic sequencing as a reference standard.

Reference standard is culture-based DST and genetic sequencing (ie both investigations performed in all isolates):

No studies performed direct MTBDR*sl* testing and used both phenotypic culture-based DST and genetic sequencing (performed in all isolates) as a reference standard.

Reference standard is culture-based DST followed by genetic sequencing of discrepant index test culture-based DST results:

We found two studies, both of which reported perfect sensitivity and specificity: 100% (95% CI 85 to 100) and 100% (95% CI 97 to 100) for Ajbani 2012 and 100% (95% CI 92 to 100) and 100% (95% CI 98 to 100) for Barnard 2012, respectively.

(ii) Drugs used in the culture-based DST

We present MTBDR*sl* accuracy estimates for detection of resistance to amikacin, kanamycin and capreomycin by direct testing against a phenotypic culture-based reference standard in Figure 12. For amikacin resistance, sensitivity estimates ranged from 64% to 100% and specificity estimates ranged from 89% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 97.3% (95% CI 55.1 to 99.9) and 99.3% (95% CI 92.3 to 99.9), respectively. For kanamycin resistance, sensitivity estimates ranged from 9% to 100% and specificity estimates from 91% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 72.1% (95% CI 9.5 to 98.5) and 98.8% (95% CI 89.3 to 99.9), respectively. For capreomycin resistance, sensitivity estimates ranged from 57% to 100% and specificity estimates from 90% to 100%. The pooled sensitivity and specificity were 68.7% (95% CI 55.4 to 79.5) and 97.0% (95% CI 89.6 to 99.2). Figure 13 presents a summary ROC plot of sensitivity versus specificity comparing test performance for detection of resistance to the individual SLIDs by direct testing.

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Figure 12. Forest plots of MTBDRsl sensitivity and specificity when performed directly for the detection of resistance to amikacin (Ak), kanamycin (Kn) and capreomycin (Cm) using culture as a reference standard. The individual studies are ordered by decreasing sensitivity. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Between brackets are the 95% CI of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).

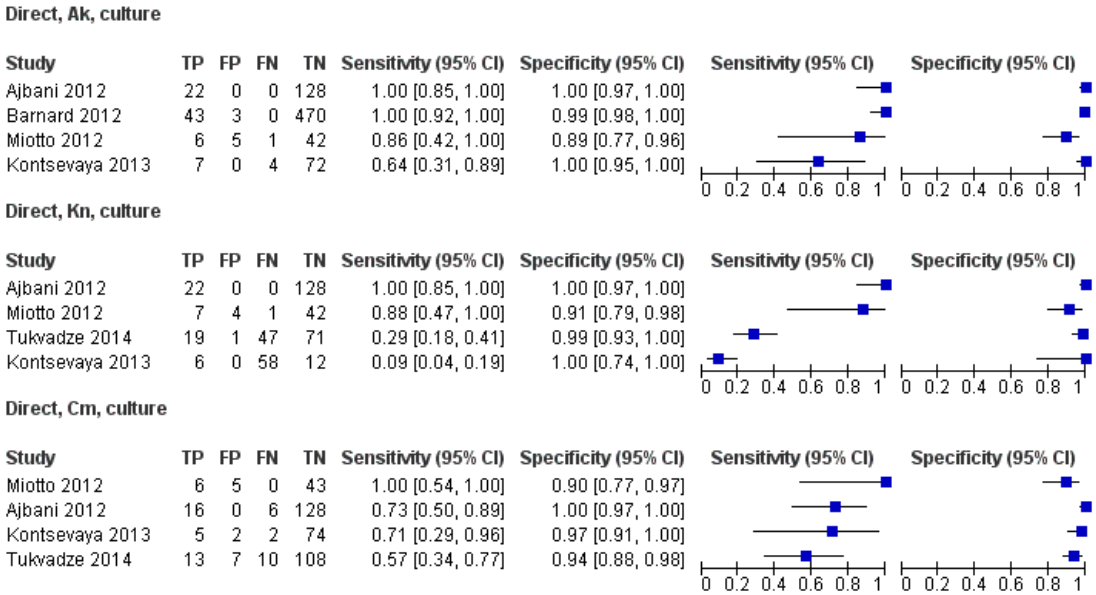

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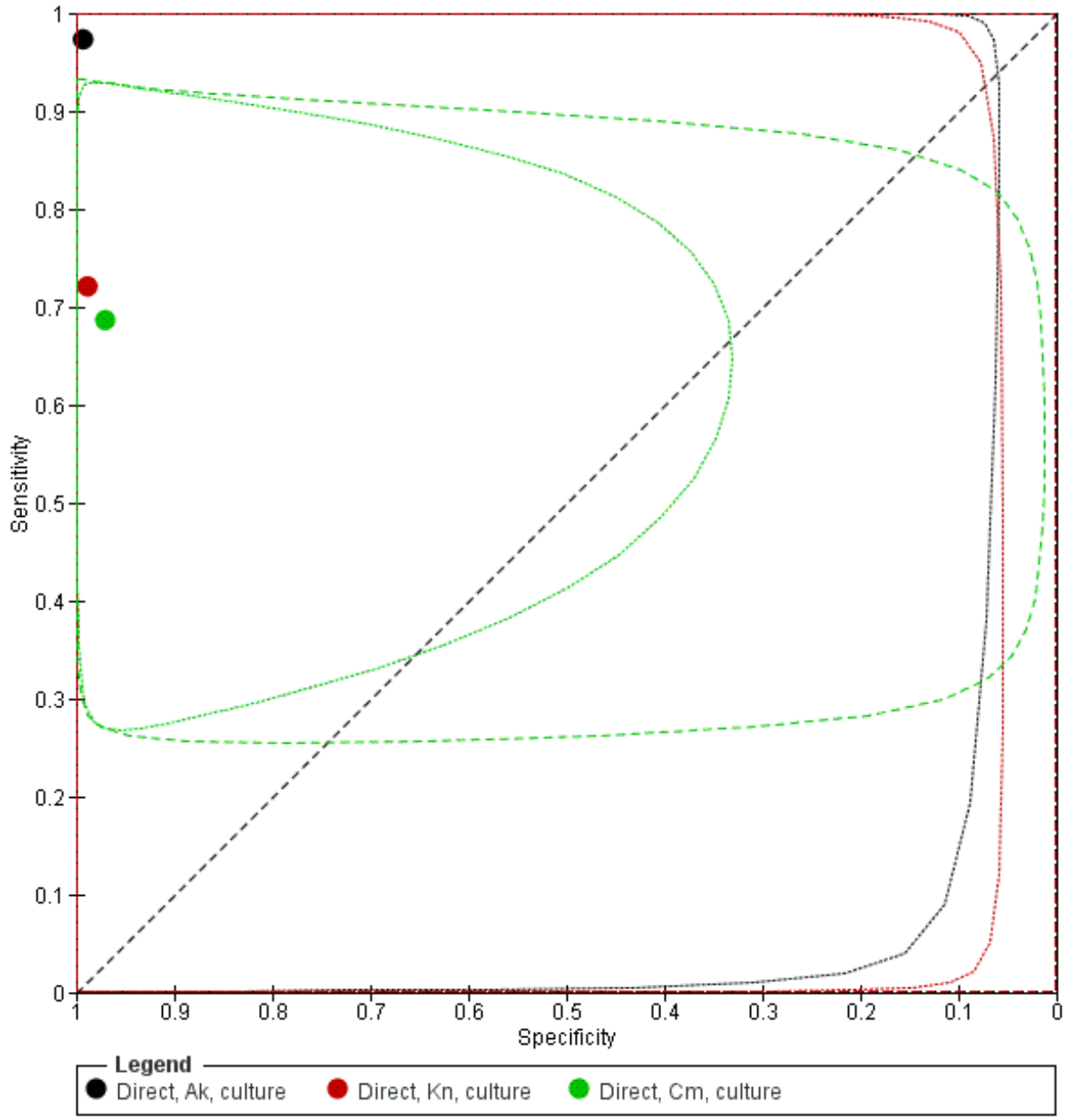
Figure 13. Summary plots of MTBDRsl sensitivity and specificity comparing direct detection of resistance for amikacin (Ak), kanamycin (Kn) and capreomycin (Cm) using culture as a reference standard. The solid circles correspond to the summary estimates of sensitivity and specificity and are shown with 95% confidence regions (dotted lines) and 95% prediction regions (dashed lines).



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(iii) Drug concentration used in culture-based DST

Three studies in this category used the WHO-recommended critical concentration for amikacin. One study (Barnard 2012) used a method (culture on Middlebrook 7H11 media) for which the WHO does not recommend a critical concentration. Two studies used the WHO-recommended critical concentration for kanamycin (Ajbani 2012; Tukvadze 2014) and two (Kontsevaya 2013; Miotto 2012) did not. All four studies used the WHO-recommended critical concentration for capreomycin.

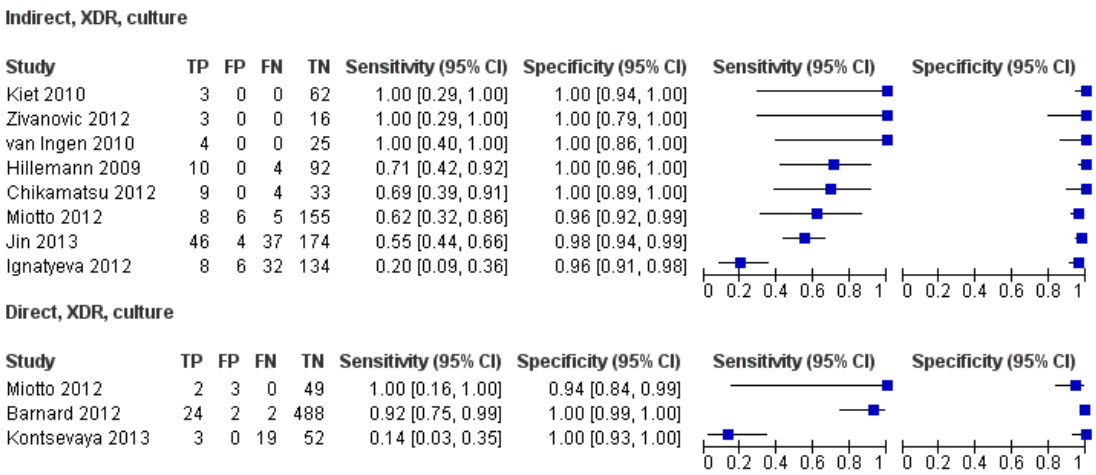
III. XDR-TB detection

A. Estimates of the diagnostic accuracy of MTBDR*sl* using phenotypic culture-based DST as a reference standard

1. Indirect testing

In Figure 14 we present forest plots of MTBDR*sl* sensitivity and specificity for XDR-TB for eight studies (880 participants) that used phenotypic culture-based DST as a reference standard. For individual studies, sensitivity estimates ranged from 20% to 100% and specificity estimates ranged from 96% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 70.9% (95% CI 42.9 to 88.8) and 98.8% (95% CI 96.1 to 99.6), respectively.

Figure 14. Forest plots of MTBDRsl sensitivity and specificity when performed indirectly and directly for the detection of XDR-TB using phenotypic culture-based DST as a reference standard. The individual studies are ordered by decreasing sensitivity. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Between brackets are the 95% CI of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).



2. Direct testing

We show forest plots of MTBDR*sl* sensitivity and specificity for XDR-TB for three studies (664 participants) that used phenotypic culture-based DST as a reference standard in Figure 14. We observed considerable heterogeneity and did not calculate pooled estimates. The test yielded sensitivities and specificities of 92% (95% CI 75 to 99) and 100% (95% CI 99 to 100) for Barnard 2012, 14% (95% CI 5 to 35) and 100% (95% CI 93 to 100) for Kontsevaya 2013 and 100% (95% CI 16 to 100) and 94% (95% CI 84 to 99) for Miotto 2012.

3. Comparison of indirect versus direct testing

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(i) Diagnostic accuracy

We present results for indirect MTBDR*sl* testing for XDR-TB in Table 5. The pooled sensitivity was 70.9% (95% CI 42.9 to 88.8) and the pooled specificity was 98.8% (95% CI 96.1 to 99.6). We were unable to compare these estimates with those for direct testing, because we did not calculate pooled estimates for direct testing as there were only three studies with considerable heterogeneity.

(ii) Indeterminate rates

For indirect testing for XDR-TB, one (0.1%) of 880 MTBDR*sl* results was indeterminate (one culture DST sensitive), whereas for direct testing 12 (1.8%) of 644 were MTBDR*sl* indeterminate (five were culture DST susceptible and seven did not report a culture-based DST result) (P < 0.001).

B. Investigations of heterogeneity

1. Indirect testing

We present MTBDR*sl* accuracy estimates for detection of XDR-TB against different reference standards in Table 5.

(i) Type of reference standard

Reference standard is genetic sequencing:
For individual studies (three in total), sensitivity estimates were all 100% and specificity estimates ranged from 95% to 100%. In the meta-analysis, the pooled sensitivity and specificity were 100% (95% CI 94.6 to 100) and 97.5% (95% CI 95.6 to 98.7), respectively.
Reference standard is culture-based DST and genetic sequencing (ie both investigations performed in all isolates):
We found two studies. Sensitivity and specificity estimates for Jin 2013 were 56% (95% CI 45 to 67) and 99% (95% CI 96 to 100), respectively, and 71% (95% CI 44 to 90) and 99% (95% CI 95 to 100), respectively for Miotto 2012. The pooled sensitivity and specificity were 58.8% (95% CI 49.1 to 67.9) and 98.8% (95% CI 96.8 to 99.5), respectively.
Reference standard is culture-based DST followed by genetic sequencing of discrepant index test-culture-based DST results:
No studies performed indirect MTBDR*sl* testing when performed indirectly for XDR-TB and used phenotypic culture-based DST and genetic sequencing for discordant analysis as a reference standard.

(ii) Drugs used in the culture-based DST

One (Kiet 2010) of the eight studies that performed indirect testing for XDR-TB and used cultured-based DST as a reference standard used ofloxacin and kanamycin. Two studies (Hillemann 2009; Zivanovic 2012) used ofloxacin, amikacin and capreomycin. One study (Miotto 2012) used ofloxacin, amikacin and kanamycin. One study (Chikamatsu 2012) used levofloxacin, amikacin, kanamycin and capreomycin. One study (Ignatyeva 2012) used ofloxacin, amikacin, kanamycin and capreomycin. One study (Jin 2013) used ofloxacin, kanamycin and capreomycin. One study (van Ingen 2010) used moxifloxacin, amikacin and ofloxacin. As all but two studies used a different combination of drugs, we did not compare test performance according to drugs used in the culture-based DST.

(iii) Drug concentration used in culture-based DST

Four studies in this category used the WHO-recommended critical concentration for ofloxacin (Hillemann 2009; Ignatyeva 2012; Miotto 2012; Zivanovic 2012) and two did not (Jin 2013; Kiet 2010). van Ingen 2010 used moxifloxacin but did not use the WHO-recommended critical concentration. For the study that used levofloxacin (Chikamatsu 2012) the WHO does not recommend a critical concentration for the type of culture used (Ogawa culture). For the six studies that used amikacin, four used the WHO-recommended critical concentration (Hillemann 2009; Ignatyeva 2012; Miotto 2012; Zivanovic 2012), one did not report the concentration used (Chikamatsu 2012) and one used a type of culture-based testing (Middlebrook 7H10 media) for which the WHO did not specify a recommended critical concentration (van Ingen 2010). Of the five studies that used kanamycin, three did not use the WHO-recommended critical concentration (Jin 2013; Kiet 2010; Miotto 2012), one did not report the concentration used (Chikamatsu 2012) and one used a type of culture-based testing (MGIT 960) for which the WHO did not specify a recommended critical concentration (Ignatyeva 2012). Of the six studies that used capreomycin, five used the WHO-recommended critical concentration (Hillemann 2009; Ignatyeva 2012; Miotto 2012; van Ingen 2010; Zivanovic 2012) and two did not report the concentration used (Chikamatsu 2012; Jin 2013).

2. Direct testing

(i) Type of reference standard

Reference standard is genetic sequencing:
No studies performed direct MTBDR*sl* testing for XDR-TB and used genetic sequencing as a reference standard.
Reference standard is culture-based DST and genetic sequencing (ie both investigations performed in all isolates):

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No studies performed direct MTBDR*sl* testing and used both phenotypic culture-based DST and genetic sequencing (performed in all isolates) as a reference standard.

Reference standard is culture-based DST followed by genetic sequencing of discrepant index test-culture-based DST results:

We found a single study (Miotto 2012) that used phenotypic culture-based DST and performed genetic testing only on discrepant results. This study reported a sensitivity of 92% (95% CI 75 to 99) and a specificity of 100% (95% CI 99 to 100).

Sensitivity analyses

We undertook sensitivity analyses by limiting inclusion in the meta-analyses to: studies with consecutive or random selection of samples, studies with cross-sectional design, studies where index test results were blinded to reference standard results, and studies where reference standard results were blinded to index test results. Table 6 contains sensitivity analyses for the FQs. For the SLIDs (Table 7), using culture-based DST as the reference standard and direct testing, the pooled sensitivity estimate was lower when we dropped studies that enrolled patients by convenience. However, in all the analyses for the detection SLID resistance by direct testing, we found wide 95% CIs suggesting less precision around the pooled estimates. The other sensitivity analyses made no difference to any of the findings.

Other analyses

Only four studies described the effect of MTBDR*sl* on time-to-diagnosis. Lopez-Roa 2012 reported it to have a time-to-diagnosis of eight hours, compared to DST using the agar proportion method (21 days) or the MGIT 960 method (eight days). Said 2012 stated that MTBDR*sl* had a median time-to-diagnosis of two days, compared to 11 days for the agar proportion method. Tukvadze 2014 noted a median time-to-diagnosis using MTBDR*sl* of 10 days, versus 70 to 104 days for culture-based DST. Barnard 2012 reported it to have a median turn-around-time of one day (after the diagnosis of first-line resistance), whereas the median turn-around-time for phenotypic culture-based DST was 31 days.

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Summary of findings

Patients	Patients or specimens of any age presumed to have resistance to any of the second-line TB drugs and those with confirmed MDR-TB
Prior testing	Patients who received MTBDR*sl* testing will first have received smear microscopy or culture (if smear-negative) or both for the detection of TB and phenotypic or genotypic DST for resistance to first-line TB drugs
Settings	Intermediate or central level laboratories
Index (new) test	MTBDR*sl* assay
Reference standard	Culture-based DST
Studies	Cross-sectional and case control studies in which cases and controls were sampled from the same patient population

A. MTBDR*sl* for fluoroquinolones by indirect testing

Prevalence	Sensitivity (95% CI) 83.1% (78.7 to 86.7)	Specificity (95% CI) 97.7% (94.3 to 99.1)	Number of participants (studies)	Quality of the evidence (GRADE)*
12%	A diagnostic test does not always accurately detect all of the people who actually have the disease or condition in question 120 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 100 people will be correctly identified as having resistance (TPs) . However, 20 people with resistance will remain undetected; their ' ' negative" test results will be incorrect (FNs)	A diagnostic test does not always accurately identify all of the people who do not have the disease or condition in question 880 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test, 860 of these people will be correctly identified as not having resistance (TNs). However, 20 people will be incorrectly identified; their ' ' positive" test results will suggest they have resistance (FPs)	1766 (16 studies)	Quality of the evidence indicates how likely it is that the accuracy of the test will be substantially different from what the research found Moderate ⊕⊕⊕o
17%	170 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 141 people will be correctly identified as having resistance (TPs) . However, 29 people with resistance will remain undetected; their ' ' negative"	830 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test, 811 of these people will be correctly identified as not having resistance (TNs). However, 19 people will be incorrectly identified; their ' ' positive" test		

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	test results will be incorrect (FNs)	results will suggest they have resistance (FPs)		
21%	210 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 175 people will be correctly identified as having resistance (TPs) . However, 35 people with resistance will remain undetected; their ' ' negative" test results will be incorrect (FNs)	790 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test. 772 of these people will be correctly identified as not having resistance (TNs). However, 18 people will be incorrectly identified; their ' ' positive" test results will suggest they have resistance (FPs)		

B. MTBDR*sl* for fluoroquinolones by direct testing

Prevalence	Sensitivity (95% CI) 85.1% (71.9 to 92.7)	Specificity (95% CI) 98.2% (96.8 to 99.0)	Number of participants (studies)	Quality of the evidence (GRADE)*
12%	120 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 102 people will be correctly identified as having resistance (TPs) . However, 18 people with resistance will remain undetected; their ' ' negative" test results will be incorrect (FNs)	880 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test,864 of these people will be correctly identified as not having resistance (TNs). However, 16 people will be incorrectly identified; their ' ' positive" test results will suggest they have resistance (FPs)	1033 (7 studies)	Quality of the evidence indicates how likely it is that the accuracy of the test will be substantially different from what the research found Moderate ⊕⊕⊕o
17%	170 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 145 people will be correctly identified as having resistance (TPs) . However, 25 people with resistance will remain undetected; their ' ' negative" test results will be incorrect (FNs)	830 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test, 815 of these people will be correctly identified as not having resistance (TNs). However, 15 people will be incorrectly identified; their ' ' positive" test results will suggest they have resistance (FPs)		

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21%	210 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 179 people will be correctly identified as having resistance (TPs). However, 31 people with resistance will remain undetected; their ' ' negative" test results will be incorrect (FNs)	790 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test. 776 of these people will be correctly identified as not having resistance (TNs). However, 14 people will be incorrectly identified; their ' ' positive" test results will suggest they have resistance (FPs)	

*We deducted one point for limitations. We did not deduct points for indirectness; however we consider sensitivity and specificity to be surrogates for patient-important outcomes and high accuracy does not mean that patients will get better.

DST = drug susceptibility testing; TP = true positive; FP = false positive; TN = true negative; FN = false negative.

Patients	Patients or specimens of any age presumed to have resistance to any of the second-line TB drugs and those with confirmed MDR-TB
Prior testing	Patients who received MTBDR*sl* testing will first have received smear microscopy or culture (if smear-negative) or both for the detection of TB and phenotypic or genotypic DST for resistance to first-line TB drugs
Settings	Intermediate or central level laboratories
Index (new) test	MTBDR*sl* assay
Reference standard	Culture-based DST
Studies	Cross-sectional and case control studies in which cases and controls were sampled from the same patient population
A. MTBDR*sl* for second-line injectable drugs by indirect testing	

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Prevalence	Sensitivity (95% CI) 76.9% (61.1 to 87.6)	Specificity (95% CI) 99.5% (97.1 to 99.9)	Number of participants (studies)	Quality of the evidence (GRADE)*	
15%	A diagnostic test does not always accurately detect all of the people who actually have the disease or condition in question 150 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 115 people will be correctly identified as having resistance (TPs) . However, 35 people with resistance will remain undetected; their ' ' negative" test results will be incorrect (FNs)	A diagnostic test does not always accurately identify all of the people who do not have the disease or condition in question 850 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test, 846 of these people will be correctly identified as not having resistance (TNs) . However, 4 people will be incorrectly identified; their ' ' positive" test results will suggest they have resistance (FPs)	1637 (14 studies)	Quality of the evidence indicates how likely it is that the accuracy of the test will be substantially different from what the research found Moderate ⊕⊕⊕o	
23%	230 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 177 people will be correctly identified as having resistance (TPs) . However, 53 people with resistance will remain undetected; their ' ' negative" test results will be incorrect (FNs)	770 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test, 766 of these people will be correctly identified as not having resistance (TNs) . However, 4 people will be incorrectly identified; their ' ' positive" test results will suggest they have resistance (FPs)			
30%	300 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 231 people will be correctly identified as having resistance (TPs) . However, 69 people with resistance will remain undetected;	700 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test. 696 of these people will be correctly identified as not having resistance (TNs) . However, 4 people will be incorrectly identified; their ' ' pos-			

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	their ‘ ‘ negative” test results will be incorrect (FNs)	itive” test results will suggest they have resistance (FPs)			

B. MTBDR*sl* for second-line injectable drugs by direct testing

Prevalence	Sensitivity (95% CI) 94.4% (25.2 to 99.9)	Specificity (95% CI) 98.2% (88.9 to 99.7)	Number of participants (studies)	Quality of the evidence (GRADE)*	
15%	A diagnostic test does not always accurately detect all of the people who actually have the disease or condition in question 150 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 142 people will be correctly identified as having resistance (TPs) . However, 8 people with resistance will remain undetected; their ‘ ‘ negative” test results will be incorrect (FNs). There is considerable uncertainty in these results. If the CIs are taken into account, then between 0 and 112 people might be missed (FNs)	A diagnostic test does not always accurately identify all of the people who do not have the disease or condition in question 850 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test, 835 of these people will be correctly identified as not having resistance (TNs). However, 15 people will be incorrectly identified; their ‘ ‘ positive” test results will suggest they have resistance (FPs) . There is considerable uncertainty in these results. If the CIs are taken into account, then between 3 and 94 people might be misclassified as positive (FPs)	947 (6 studies)	Quality of the evidence indicates how likely it is that the accuracy of the test will be substantially different from what the research found Very low ⊕ooo	
23%	230 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 217 people will be correctly identified as having resistance (TPs) . However, 13 people with resistance will remain undetected; their ‘ ‘ negative” test results will	770 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test, 756 of these people will be correctly identified as not having resistance (TNs). However, 14 people will be incorrectly identified; their “positive” test results will sug-			

	be incorrect (FNs). There is considerable uncertainty in these results. If the CIs are taken into account, then between 0 and 172 people might be missed (FNs)	gest they have resistance (FPs) . There is considerable uncertainty in these results. If the CIs are taken into account, then between 2 and 85 people might be misclassified as positive (FPs)		
30%	300 people (out of 1000 people) have (as yet undetected) resistance. Of the 1000 people who take the MTBDR*sl* test, 283 people will be correctly identified as having resistance (TPs) . However, 17 people with resistance will remain undetected; their ' ' negative" test results will be incorrect (FNs). There is considerable uncertainty in these results. If the CIs are taken into account, then between 0 and 224 people might be missed (FNs)	700 people (out of 1000 people) do not have resistance. Of the 1000 people who take the MTBDR*sl* test. 687 of these people will be correctly identified as not having resistance (TNs). However, 13 people will be incorrectly identified; their ' ' positive" test results will suggest they have resistance (FPs) . There is considerable uncertainty in these results. If the CIs are taken into account, then between 2 and 78 people might be misclassified as positive (FPs)		

*We deducted one point for limitations and, for direct testing, two additional points for imprecision (considering the very wide 95% CI for pooled sensitivity). We did not deduct points for indirectness; however we consider sensitivity and specificity to be surrogates for patient-important outcomes and high accuracy does not mean that patients will get better.

DST = drug susceptibility testing; TP = true positive; FP = false positive; TN = true negative; FN = false negative.

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Patients	Patients or specimens of any age presumed to have resistance to any of the second-line TB drugs and those with confirmed MDR-TB
Prior testing	Patients who received MTBDR*sl* testing will first have received smear microscopy or culture (if smear-negative) or both for the detection of TB and phenotypic or genotypic DST for resistance to first-line TB drugs
Settings	Intermediate or central level laboratories
Index (new) test	MTBDR*sl* assay
Reference standard	Culture-based DST
Studies	Cross-sectional and case control studies in which cases and controls were sampled from the same patient population

A. MTBDR*sl* for XDR-TB by indirect testing

Prevalence	Sensitivity (95% CI) 70.9% (42.9 to 88.8)	Specificity (95% CI) 98.8% (96.1 to 99.6)	Number of participants (studies)	Quality of the evidence (GRADE)*
8%	A diagnostic test does not always accurately detect all of the people who actually have the disease or condition in question 80 people (out of 1000 people) have (as yet undetected) XDR-TB. Of the 1000 people who take the MTBDR*sl* test, 57 people will be correctly identified as having XDR-TB (TPs). However, 23 people with XDR-TB will remain undetected; their ' ' negative" test results will be incorrect (FNs)	A diagnostic test does not always accurately identify all of the people who do not have the disease or condition in question 920 people (out of 1000 people) do not have XDR-TB. Of the 1000 people who take the MTBDR*sl* test, 909 of these people will be correctly identified as not having XDR-TB (TNs). However, 11 people will be incorrectly identified; their ' ' positive" test results will suggest they have XDR-TB (FPs)	880 (8 studies)	Quality of the evidence indicates how likely it is that the accuracy of the test will be substantially different from what the research found Low ⊕⊕oo
11%	110 people (out of 1000 people) have (as yet undetected) XDR-TB. Of the 1000 people who take the MTBDR*sl* test, 78 people will be correctly identified as having XDR-TB (TPs). However, 32 people with XDR-TB will remain undetected; their ' ' negative" test results will be incor-	890 people (out of 1000 people) do not have XDR-TB. Of the 1000 people who take the MTBDR*sl* test, 879 of these people will be correctly identified as not having XDR-TB (TNs). However, 11 people will be incorrectly identified; their ' ' positive" test results will suggest they have XDR-TB		

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	rect (FNs)		(FPs)		
B. MTBDR*sl* for XDR-TB, by direct testing, 644 participants (3 studies). There was considerable heterogeneity in accuracy estimates and we did not perform a meta-analysis					

*We deducted one point for limitations and one point for imprecision (considering the wide 95% CI for pooled sensitivity). We did not deduct points for indirectness; however we consider sensitivity and specificity to be surrogates for patient-important outcomes and high accuracy does not mean that patients will get better.

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DISCUSSION

This systematic review found that, when used indirectly on culture isolates, MTBDR*sl* had higher pooled sensitivity for detection of FQ resistance (83.1%) than for detection of SLID resistance (76.9%). When used directly on smear-positive sputum specimens, MTBDR*sl* had lower pooled sensitivity for FQ resistance (85.1%) than for SLID resistance (94.4%); however the pooled sensitivity for detection of SLID resistance was imprecise (95% CI 25.2 to 99.9). When SLID resistance was analysed for individual drugs, the pooled sensitivity was highest for amikacin (87.9%). For detection of resistance to both FQs and SLIDs, pooled specificity was high (> 97%). For detection of XDR-TB by indirect testing, the pooled sensitivity of MTBDR*sl* was 70.9% and the pooled specificity was 98.9%. The average sensitivities and specificities of MTBDRsl for detection of resistance to FQs and SLIDs and XDR-TB included in the meta-analyses are given in the 'Summary of Findings' tables (Summary of findings 1; Summary of findings 2; Summary of findings 3) and Table 3 Table 4 and Table 5.

When MTBDR*sl* accuracy was compared according to whether the test was performed directly or indirectly, the sensitivities were similar for FQ resistance and SLID resistance. Indirect MTBDR*sl* testing for SLID resistance had slightly superior specificity compared to direct testing (99.5% versus 98.2%). Indirect testing for both FQ and SLID resistance had a lower rate of uninterpretable MTBDR*sl* results than direct testing.

We performed subgroup analyses in which we compared the accuracy of MTBDR*sl* against different reference standards comprised of phenotypic culture-based DST (the traditional gold standard) or genetic sequencing. We looked at MTBDR*sl* accuracy against each type of reference standard alone or in combination (where either all specimens received both culture-based DST and sequencing). When used indirectly on culture isolates for detection of FQ resistance, MTBDR*sl* had higher pooled sensitivity against genetic sequencing than against culture-based DST (99.3% versus 83.1%). This suggests that MTBDR*sl* is sensitive for detecting FQ resistance caused by mutations in *gyrA* (the only gene that is targeted by MTBDR*sl* for detection of FQ resistance). However, against culture-based DST, MTBDR*sl* sensitivity for FQ resistance was only 83.1% suggesting that just less than one in five cases may be caused by mutations outside of *gyrA*, such as in *gyrB*, a gene which is not targeted by MTBDR*sl*. Only two studies (Brossier 2010; Huang 2011) performed genetic sequencing for both *gyrA* and *gyrB* and they reported sensitivity estimates of 84.6% and 100.0%, respectively.

Similarly, we found higher pooled sensitivity for SLID resistance when MTBDR*sl* was evaluated against genetic sequencing rather than culture-based DST (97.0% versus 76.9%). In this case, both genetic sequencing and MTBDR*sl* only target the *rrs* gene for resistance to SLIDs. This approach can potentially miss mutations outside of this region that are responsible for SLID resistance. Using culture-based DST (sensitivity 76.9%), it appears that around one in four cases of SLID-resistant TB may be caused by mutations outside of *rrs*. The prevalence of these non-*rrs* mutations, which can occur in regions such as *tlyA*, *eis* and *gidB* (Georghiou 2012), appears to be most pronounced for kanamycin given the reduced sensitivity (66.9%) of MTBDR*sl* for resistance to this drug compared to the other SLIDs (sensitivity of 87.9% and 79.5% for amikacin and capreomycin, respectively, against culture-based DST). The sensitivity of MTBDR*sl* for SLID resistance, and in particular kanamycin resistance, is likely to vary according to the genetic background of TB strains, where some may have a greater frequency of resistance-causing mutations that fall outside of *rrs* and different levels of cross-resistance within the SLIDs. When we excluded a large study from Eastern Europe (Kontsevaya 2013), both the sensitivity of MTBDR*sl* and the precision of our pooled estimate improved.

We are aware of an unpublished Foundation for Innovative and New Diagnostics-sponsored evaluation of MTBDR*sl* at the University of Cape Town (K. Dheda, personal communication). For the direct detection of drug resistance compared to culture-based DST as a reference standard, this work reported a sensitivity and specificity of 79.2% (38/48) and 86.5% (45/52), respectively, for the detection of resistance to ofloxacin, and a sensitivity and specificity of 72.9% (35/48) and 94.2% (49/52) respectively for the detection of resistance to amikacin. When performed indirectly on culture isolates, the sensitivity and specificity for the detection of ofloxacin resistance were 72.3% (115/159) and 99.0% (100/101) respectively, and 76.6% (125/157) and 98.0% (99/101) respectively for amikacin resistance, when compared to culture-based DST as a reference standard.

MTBDR*sl* is the only commercially-available rapid molecular test for the detection of resistance to the FQs, SLIDs and XDR-TB. Alternative phenotypic methods of DST for TB may take several weeks (Barnard 2012; Lopez-Roa 2012) to several months (Said 2012; Tukvadze 2014). This lengthy turnaround time, during which the patient may be on ineffective therapy and contribute to ongoing TB transmission, is further exacerbated by the need to first grow a *M. tuberculosis* isolate (which itself may take two to six weeks). Two systematic reviews of MTBDR*sl* exist (Feng 2013; WHO 2013b). As in our review, WHO 2013b used a random-effects meta-analysis model and arrived at similar summary estimates, generally within three percentage points of those described in our review. Feng 2013 used a fixed-effects model and reported accuracy estimates for kanamycin and capreomycin resistance that were substantially lower than the ones we found. Our review included additional studies not included in these previous reviews. Key questions remain regarding test accuracy and potential sources of heterogeneity, including risk of bias assessment, type of testing (indirect versus direct testing) and reference standard (for example, culture-based DST versus genetic sequencing). We address several

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of these questions in this review. Although we intended to investigate whether the observed test accuracy varied between studies according to HIV infection, specimen condition (frozen versus fresh), specimen type (induced sputum or extrapulmonary specimen), the drug concentration used in culture-based DST (studies that used WHO-recommended concentrations versus those that did not) or population (patients suspected of having MDR-TB or XDR-TB), there were unfortunately insufficient data to perform these additional analyses for each target condition. We were also unable to examine sources of heterogeneity for detection of XDR-TB due to insufficient data. More comparative diagnostic accuracy data are needed from strains from different geographic regions (for example, Eastern Europe), where resistance-causing mutations that fall outside of the genes targeted by MTBDR*sl* are less common than in drug-resistant strains from South Africa, for example. Such future research should include genetic sequencing as a reference standard that targets all known resistance-determining mutations and not just those detectable using MTBDR*sl*.

Summary of main results

The main results are presented in the 'Summary of findings' tables:

- When used indirectly on culture isolates, MTBDR*sl* detected 83.1% of FQ-resistant cases with high specificity (97.7%) when culture-based DST was used as a reference standard. When evaluated against genetic testing as a reference standard, the sensitivity and specificity were 99.3% and 99.7%, respectively.
- When used directly on smear-positive sputum specimens, MTBDR*sl* detected 85.1% of FQ-resistant cases with high specificity (98.2%).
- When used indirectly on culture isolates, MTBDR*sl* detected 76.9% of SLID resistant cases with high specificity (99.5%) when culture-based DST was used as a reference standard. The pooled sensitivities for resistance to amikacin, kanamycin and capreomycin were 87.9%, 66.9% and 79.5%, respectively. The sensitivity and specificity for SLID resistance evaluated against genetic testing as a reference standard were 97.0% and 99.5%, respectively.
- When used directly on smear-positive sputum specimens, MTBDR*sl* detected 94.4% of SLID-resistant cases with high specificity (98.2%). The pooled sensitivities for resistance to amikacin, kanamycin and capreomycin were 97.3%, 72.1% and 68.7%, respectively.
- When used indirectly on culture isolates, MTBDR*sl* detected 70.9% of XDR-TB cases with high specificity (98.8%) when culture-based DST was used as a reference standard.

The proportion of indeterminate results was lower when MTBDR*sl* was performed indirectly rather than directly (0.2% versus 1.9% for FQ resistance, P < 0.001; 0.4% versus 6.1% for SLID resistance, P < 0.001; 0.1% versus 1.8% for XDR-TB resistance, P = 0.002).

Application of the meta-analysis to a hypothetical cohort

'Summary of findings' tables (Summary of findings 1; Summary of findings 2; Summary of findings 3) summarize the review findings by applying the results to a hypothetical cohort of 1000 individuals with MDR-TB thought to have resistance to a FQ, or SLID, or both. We present scenarios, based on WHO estimates (WHO 2013a), with the prevalence of FQ resistance varying from 12% to 17% to 21%, that of SLID resistance varying from 15% to 23% to 30%, and that of XDR-TB varying from 8% to 11%. The consequences of FP results are likely patient anxiety, morbidity from additional testing, possible delay in further diagnostic evaluation, and prolonged and unnecessary treatment with drugs that may have lower bacteriocidal activity than second-line regimens and often have serious side effects. The consequences of FN results are an increased risk of patient morbidity and mortality, and continued risk of community transmission of drug-resistant TB.

1A. Indirect testing for fluoroquinolone resistance, MTBDR*sl* performed on culture isolates

FQ resistance prevalence of 12%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 120 patients have FQ-resistant TB, then MTBDR*sl* would be expected to miss between 20 and 26 cases and falsely diagnose between eight and 50 cases.

FQ resistance prevalence of 17%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 170 patients have FQ-resistant TB, then MTBDR*sl* would be expected to miss 23 and 26 cases and falsely diagnose between seven and 47 cases.

FQ resistance prevalence of 21%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 210 patients have FQ-resistant TB, then MTBDR*sl* would be expected to miss between 28 and 45 cases and falsely diagnose between seven and 45 cases.

1B. Direct testing for fluoroquinolone resistance, MTBDR*sl* performed on sputum specimens

FQ resistance prevalence of 12%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 120 patients have FQ-resistant TB, then MTBDR*sl* would be expected to miss between nine and 34 cases and falsely diagnose between nine and 28 cases.

FQ resistance prevalence of 17%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 170 patients have FQ-resistant TB, then MTBDR*sl* would be expected to miss between 12 and 48 cases and falsely diagnose between eight and 27 cases.

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FQ resistance prevalence of 21%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 210 patients have FQ-resistant TB, then MTBDR*sl* would be expected to miss between 15 and 59 cases and falsely diagnose between eight and 25 cases.

2A. Indirect testing for SLID resistance, MTBDR*sl* performed on culture isolates

SLID resistance prevalence of 15%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 150 patients have SLID resistant TB, then MTBDR*sl* would be expected to miss between 19 and 58 cases and falsely diagnose between one and 25 cases.
SLID resistance prevalence of 23%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 230 patients have SLID resistant TB, then MTBDR*sl* would be expected to miss between 29 and 89 cases and falsely diagnose between one and 22 cases.
SLID resistance prevalence of 30%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 300 patients have SLID resistant TB, then MTBDR*sl* would be expected to miss between 37 and 117 cases and falsely diagnose between one and 20 cases.

2B. Direct testing for SLID resistance, MTBDR*sl* performed on sputum specimens

SLID resistance prevalence of 15%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 150 patients have SLID resistant TB, then MTBDR*sl* would be expected to miss between zero and 112 cases and falsely diagnose between three and 94 cases.
SLID resistance prevalence of 23%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 230 patients have SLID resistant TB, then MTBDR*sl* would be expected to miss between zero and 172 cases and falsely diagnose between two and 85 cases.
SLID resistance prevalence of 30%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 300 patients have SLID resistant TB, then MTBDR*sl* would be expected to miss between zero and 224 cases and falsely diagnose between two and 78 cases.

3A. Indirect testing for XDR-TB resistance, MTBDR*sl* performed on culture isolates

XDR-TB prevalence of 8%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 80 patients have XDR-TB, then MTBDR*sl* would be expected to miss between nine and 46 cases and falsely diagnose between four and 36 cases.
XDR-TB prevalence of 11%: if the pooled estimates for MTBDR*sl* are applied to a hypothetical cohort of 1000 patients with TB, where 110 patients have XDR-TB, then MTBDR*sl* would be expected to miss between 12 and 63 cases and falsely diagnose between four and 35 cases.

Strengths and weaknesses of the review

The results of this Cochrane Review are based on strict and careful searching, study inclusion and data extraction. The strength of this review is that it allows an assessment of different methods of testing (indirect versus direct) and different reference standards.

Completeness of evidence

This is a reasonably complete data set. We included any non-English studies we found. We excluded seven studies that were not diagnostic accuracy studies and 13 studies that were conference abstracts (however, several of these were included in the form of full-length published papers). We did not include unpublished data. Studies of diagnostic test accuracy tend to be poorly indexed (Whiting 2005) and we may therefore have missed some studies despite the comprehensive search.

Accuracy of the reference standards used

For our primary analysis, we used phenotypic culture-based DST. This was the most frequently deployed reference standard in the included studies. Although considered to be the 'gold standard' for drug-resistant TB, culture-based DST is not 100% accurate for detection of drug resistance, in particular with respect to detection of second-line drug resistance. We also determined MTBDR*sl* accuracy using genetic testing (gene sequencing of loci known to be associated with drug resistance) and both genetic testing and culture-based DST as reference standards. Many TB experts consider genetic sequencing to be the best available reference standard, provided it encompasses all the possible resistance determining regions. In addition, we determined the accuracy of MTBDR*sl* against a fourth reference standard, where genetic testing was only performed as part of a discrepant analysis in culture-DST-MTBDR*sl* discordant specimens. However, in most cases we were unable to determine summary estimates due to the small number of studies and therefore were unable to compare MTBDR*sl* accuracy estimates using this reference standard with those obtained using culture-based DST, or genetic sequencing, or both, as the reference standard.

Quality and quality of reporting of the included studies

We judged nine of the 21 included studies as having high risk of bias for patient selection (either due to a case-control design, or enrolment by convenience, or both). Otherwise, for the other QUADAS-2 domains, we considered at least half of the included studies to have low risk of bias. We noted that seven studies (33%)

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did not provide information about whether the MTBDR*sl* results were read in the absence of knowledge of the results of the reference standard. Overall, we had low concern about applicability. In addition, seven studies (33%) had industry involvement.

Interpretability of subgroup analyses

We performed subgroup analyses according to type of testing and reference standard used and for the individual drugs in the FQ and SLID drug classes. For comparing test accuracy, we only performed analyses that had at least four studies in a given subgroup. We performed statistical testing and provided P values where appropriate. We performed both indirect and direct comparisons.

Completeness and relevance of the review

There are no other commercially-available tests for resistance beyond MDR-TB. Several products, such as the GeneXpert® XDR-TB cartridge (Cepheid, USA), are expected to be commercially available in 2015. Our review is the most complete analysis of the diagnostic accuracy of the MTBDR*sl* test to date.

Unpublished data

We did not include unpublished studies in the review, though we regularly checked the TB literature to see if studies we identified as abstracts had been published. It is our experience that unpublished diagnostic accuracy data frequently changes after publication. In addition, primary study authors in our field rarely give permission to publish their unpublished data. We have nevertheless related our findings in the Discussion to those of the largest unpublished study that we are aware of.

Applicability of findings to the review question

We found MTBDR*sl* to have moderate sensitivity and excellent specificity for the detection of FQ resistance, SLID resistance and XDR-TB. The proportion of false-negative results is concerning and means that the test will likely only be usable in clinical practice as a "rule-in" test for drug resistance, with further DST being required in patients who have a susceptible MTBDR*sl* result. The local genetic background of drug-resistant strains (which, for example, may have a greater frequency of kanamycin-resistance causing mutations outside of *rrs*) also needs to be considered by test operators. In contrast, the proportion of false-positive results was small as the specificity was excellent. We found no significant differences in accuracy between indirect testing on isolates and direct testing on smear-positive sputum specimens. MTBDR*sl* accuracy was generally greater when measured against a reference standard that included genetic testing. However, such genetic testing was only limited to the genes the MTBDR*sl* targeted and did not detect mutations outside of these genes that may cause phenotypic drug resistance. For some subgroup analyses (for example, patient characteristics), there were insufficient data to analyse differences.

AUTHORS' CONCLUSIONS

Implications for practice

In adults presumed to have resistance to second-line TB drugs, MTBDR*sl* has moderate sensitivity and excellent specificity. Approximately 25% patients with XDR-TB will be missed by MTBDR*sl* and 0.01% of patients without XDR-TB will be falsely diagnosed as having XDR-TB. Where possible, MTBDR*sl* should be performed directly on smear-positive sputum, as the accuracy is similar to when it is performed indirectly (on culture isolates) and there is no need to wait several weeks for the culture to grow (although the rate of uninterpretable results is marginally higher when the test is performed directly). Therefore, given its rule-in value and rapidity, MTBDR*sl* may be used as an initial test for second-line drug resistance. However, phenotypic culture-based DST should still be used for the downstream investigation of patients who have susceptible MTBDR*sl* results. The use of MTBDR*sl* in routine care should improve the time to the diagnosis of drug-resistant TB and could thereby lead to the earlier initiation of appropriate patient therapy and improvements in patient health, provided the necessary accompanying improvements in capacity and infrastructure are made.

Implications for research

Future studies should assess the diagnostic accuracy of MTBDR*sl* when performed in different laboratory settings and patients (for example, in people living with HIV). The test's accuracy should be examined and compared using strains from different geographical regions, as these are likely to have different frequencies of resistance-causing mutations that fall outside of the genes targeted by MTBDR*sl* (and therefore MTBDR*sl* will likely having different sensitivities for each condition in these strains). Future molecular tests for FQ and SLID resistance should have more genetic targets than just *gyrA* and *rrs*. Studies are also needed to assess the effect of MTBDR*sl* implementation on time-to-treatment, patient health and cost-effectiveness.

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* *Indicates the major publication for the study*

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Ajbani 2012

Study characteristics			
Patient sampling	Cross-sectional design with consecutive enrolment of participants, prospective data collection		
Patient characteristics and setting	1. Country of origin: India 2. World Bank classification of country: middle/low 3. Type of lab: hospital 4. Type of patients: confirmed MDR-TB cases 5. Patients were smear-positive (n = 170)		
Index tests	1. Manufacturer involvement: yes, in design, analysis or manuscript production 2. Type of testing: direct 3. Type of specimens: smear-positive 4. Specimen treatment: NALC-NaOH 5. Specimen condition: frozen 6. Duration of freezing: < 1 year		
Target condition and reference standard(s)	1. Culture (Liquid; MGIT 960) used for FQ, SLID 2. FQ drugs: ofloxacin (2 µg/mL) and moxifloxacin (0.25 µg/mL) 3. SLIDs: amikacin (1 µg/mL), capreomycin (2.5 µg/mL) and kanamycin (2.5 µg/mL) 4. Discrepant analysis: yes, with sequencing		
Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes			
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low

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Ajbani 2012 (*Continued*)

DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		

Barnard 2012

Study characteristics	
Patient sampling	Cross-sectional design with convenience-based enrolment of participants, prospective data collection
Patient characteristics and setting	1. Country of origin: South Africa 2. World Bank classification of country: middle/low 3. Type of lab: reference 4. Type of patients: confirmed MDR-TB cases, confirmed RIF monoR, confirmed INH monoR

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	5. Patients were smear-positive (n = 516; excluding EPTB)
Index tests	1. Manufacturer involvement: yes, reduced price 2. Type of testing: direct 3. Type of specimens: smear-positive 4. Specimen treatment: NALC-NaOH 5. Specimen condition: fresh 6. Tested after storage at room temperature or refrigerated within 48 hours of collection
Target condition and reference standard(s)	1. Culture (solid; AP method on 7H11) used for FQ, SLID 2. FQ drugs: ofloxacin (2 µg/mL) 3. SLIDs: amikacin (4 µg/mL) 4. Discrepant analysis: yes, with sequencing
Flow and timing	Uninterpretable results reported: yes
Comparative	
Notes	• Reported performance on EPTB specimens • Reported on the utility of the index test on specimens that were culture-contaminated (and hence could not receive a phenotypic DST) • Reported on time-to-result

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		

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Barnard 2012 (*Continued*)

			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		

Brossier 2010

Study characteristics	
Patient sampling	Case-control design with unknown mechanism of enrolment of participants, prospective data collection
Patient characteristics and setting	1. Country of origin: France 2. World Bank classification of country: high 3. Type of lab: reference 4. Type of patients: confirmed MDR-TB cases, confirmed XDR-TB cases, confirmed DS-TB patients
Index tests	1. Manufacturer involvement: no 2. Type of testing: indirect
Target condition and reference standard(s)	1. Culture (Solid; agar proportion method on LJ) and sequencing used for FQ, SLID 2. FQ drugs: ofloxacin (2 µg/mL) 3. SLIDs: amikacin (20 µg/mL), kanamycin (20 µg/mL), capreomycin (20 µg/mL) 4. Genes sequenced for FQ: gyrA and gyrB

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	5. Genes sequenced for SLIDs: rrs 6. Discrepant analysis: no		
Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes			

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	No		
			High
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
			Unclear
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		

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Brossier 2010 (*Continued*)

			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

Chikamatsu 2012

Study characteristics	
Patient sampling	Cross-sectional design with unknown mechanism of enrolment of participants, unknown direction of data collection
Patient characteristics and setting	1. Country of origin: Japan 2. World Bank classification of country: high 3. Type of lab: reference 4. Type of patients: unknown
Index tests	1. Manufacturer involvement: no 2. Type of testing: indirect
Target condition and reference standard(s)	1. Culture (solid; Ogawa solid culture for FQs, unclear for SLIDs) and sequencing used for FQ, SLID 2. FQ drugs: levofloxacin (1 µg/mL) 3. SLIDs: amikacin (unknown concentration), kanamycin (unknown concentration), capreomycin (unknown concentration) 4. Genes sequenced for FQ: gyrA 5. Genes sequenced for SLIDs: rrs 6. Discrepant analysis: no
Flow and timing	Uninterpretable results reported: yes
Comparative	
Notes	
Methodological quality	

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Chikamatsu 2012 (*Continued*)

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	No		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		

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Chikamatsu 2012 (*Continued*)

Were all patients included in the analysis?	Yes		

Fan 2011

Study characteristics			
Patient sampling	Cross-sectional design with convenience-based enrolment of participants, prospective data collection		
Patient characteristics and setting	1. Country of origin: China 2. World Bank classification of country: middle/low 3. Type of lab: research 4. Type of patients: confirmed MDR-TB patients and confirmed XDR-TB patients		
Index tests	1. Manufacturer involvement: no 2. Type of testing: indirect		
Target condition and reference standard(s)	1. Culture (liquid; MGIT960) 2. FQ drugs: ofloxacin (2 µg/mL), moxifloxacin (0.25 µg/mL) 3. SLIDs: amikacin (1 µg/mL) 4. Discrepant analysis: no		
Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes			
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			

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Fan 2011 (*Continued*)

Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

Ferro 2013

Study characteristics	
Patient sampling	Cross-sectional design with random enrolment of participants, prospective data collection
Patient characteristics and setting	1. Country of origin: Colombia 2. World Bank classification of country: middle/low 3. Type of lab: reference 4. Type of patients: confirmed DS-TB, MDR-TB, MDR-TB with some known second-line resistance and XDR-TB patients

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Index tests	1. Manufacturer involvement: yes 2. Type of testing: indirect
Target condition and reference standard(s)	1. Culture based DST (solid, 7h10) 2. FQ drugs: moxifloxacin 2 µg/mL 3. SLIDS: amikacin 5 µg/mL, kanamycin 5 µg/mL 4. No XDR information reported 5. There was no discrepant analysis
Flow and timing	Uninterpretable results reported: yes
Comparative	
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Unclear		
			Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		

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Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Unclear		

Hillemann 2009

Study characteristics	
Patient sampling	Case-control design with the random enrolment of participants, prospective data collection for clinical specimens, retrospective for culture isolates
Patient characteristics and setting	1. Country of origin: Germany 2. World Bank classification of country: high 3. Type of lab: reference 4. Type of patients: confirmed XDR-TB cases, confirmed DS-TB cases 5. The specimens tested were smear positive and smear negative
Index tests	1. Manufacturer involvement: yes, donation of tests 2. Type of testing: direct and indirect 3. Type of specimens: smear-positive 4. Specimen treatment: NALC-NaOH 5. Specimen condition: frozen 6. Duration of freezing: > 1 year
Target condition and reference standard(s)	1. Culture (liquid and solid; MGIT 960 and LJ) and sequencing used for FQ, SLID 2. FQ drugs: ofloxacin (2 µg/mL for liquid) 3. SLIDs: amikacin (1 µg/mL for liquid) and capreomycin (2.5 µg/mL for liquid) 4. Genes sequenced for FQ: gyrA 5. Genes sequenced for SLIDs: rrs 6. Discrepant analysis: no

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Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes			
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
			High
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
			Low
DOMAIN 4: Flow and Timing			

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Hillemann 2009 (*Continued*)

Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

Huang 2011

Study characteristics			
Patient sampling	Cross-sectional design with consecutive enrolment of participants, prospective data collection		
Patient characteristics and setting	1. Country of origin: China 2. World Bank classification of country: middle/low 3. Type of lab: reference 4. Type of patients: confirmed MDR-TB cases		
Index tests	1. Manufacturer involvement: no 2. Type of testing: indirect		
Target condition and reference standard(s)	1. Culture (solid; 7H11) and sequencing used for FQ, SLID 2. FQ drugs: ofloxacin (2 µg/mL) 3. SLIDs: amikacin (1 µg/mL), kanamycin (6 µg/mL) and capreomycin (10 µg/mL) 4. Genes sequenced for FQ: gyrA and gyrB 5. Genes sequenced for SLIDs: rrs and eis 6. Discrepant analysis: no		
Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes			
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		

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Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

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Ignatyeva 2012

Study characteristics			
Patient sampling	Case control design with consecutive enrolment of participants, prospective data collection		
Patient characteristics and setting	1. Country of origin: Estonia 2. World Bank classification of country: middle/low 3. Type of lab: reference 4. Type of patients: confirmed MDR-TB cases, confirmed XDR-TB cases and confirmed DS-TB cases		
Index tests	1. Manufacturer involvement: no 2. Type of testing: indirect		
Target condition and reference standard(s)	1. Culture (liquid; MGIT 960) used for FQ, SLID 2. FQ drugs: ofloxacin (2 µg/mL) 3. SLIDs: amikacin (1 µg/mL), kanamycin (5 µg/mL) and capreomycin (2.5 µg/mL)		
Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes	Other findings: the interpretability of the Genotype® MTBDR*sl* assay was high, varying between 98.0% and 100% for the first reading and between 95.5% and 100% for the second reading (Table 3)		
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
			High
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		

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If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

Jin 2013

Study characteristics	
Patient sampling	Cross-sectional design with consecutive enrolment of participants, prospective data collection
Patient characteristics and setting	1. Country of origin: China 2. World Bank classification of country: middle/low 3. Type of lab: reference 4. Type of patients: confirmed MDR-TB patients and confirmed XDR-TB patients
Index tests	1. Manufacturer involvement: no 2. Type of testing: indirect

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Jin 2013 (*Continued*)

Target condition and reference standard(s)	1. Culture (solid; LJ) and sequencing used for FQ, SLID 2. FQ drugs: ofloxacin (2 µg/mL) 3. SLIDs: kanamycin (10 µg/mL) 4. Genes sequenced for FQ: gyrA 5. Genes sequenced for SLIDs: rrs 6. Discrepant analysis: no
Flow and timing	Uninterpretable results reported: yes
Comparative	
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		

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Jin 2013 (*Continued*)

Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

Kiet 2010

Study characteristics	
Patient sampling	Case control design with consecutive enrolment of participants, prospective data collection
Patient characteristics and setting	1. Country of origin: Vietnam 2. World Bank classification of country: middle/low 3. Type of lab: reference 4. Type of patients: confirmed MDR-TB cases with FQ resistance, confirmed FQ monoresistant cases
Index tests	1. Manufacturer involvement: no 2. Type of testing: indirect
Target condition and reference standard(s)	1. Culture (solid; LJ) used for FQ, SLID 2. FQ drugs: ofloxacin (2 µg/mL) 3. SLIDs: kanamycin (20 µg/mL), not WHO recommended critical concentrations for LJ solid culture 4. Discrepant analysis: yes
Flow and timing	Uninterpretable results reported: yes
Comparative	
Notes	

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Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
			High
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	No		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		

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Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		

Kontsevaya 2011

Study characteristics			
Patient sampling	Cross sectional design with consecutive enrolment of participants, prospective data collection		
Patient characteristics and setting	1. Country of origin: United Kingdom 2. World Bank classification of country: high 3. Type of lab: reference 4. Type of patients: confirmed MDR-TB cases		
Index tests	1. Manufacturer involvement: no 2. Type of testing: indirect		
Target condition and reference standard(s)	1. Culture (liquid; MGIT960) used for FQ 2. FQ drugs: Ofloxacin (2 µg/mL), moxifloxacin (0.25 µg/mL) 3. Discrepant analysis: no		
Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes			
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low

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Kontsevaya 2011 (*Continued*)

DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

Kontsevaya 2013

Study characteristics	
Patient sampling	Cross sectional design with consecutive enrolment of participants, prospective data collection
Patient characteristics and setting	1. Country of origin: Russia 2. World Bank classification of country: Middle/low 3. Type of lab: unknown 4. Type of patients: confirmed MDR-TB cases

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	5. Median age: 35 6. All HIV-infected (n = 90) 7. Previous TB: 38/90 8. Male: 71/90
Index tests	1. Manufacturer involvement: no 2. Type of testing: direct 3. Type of specimens: smear-positive 4. Specimen treatment: unknown 5. Specimen condition: unknown 6. Duration of freezing: unknown
Target condition and reference standard(s)	1. Culture (Liquid; MGIT960) used for FQ, SLID 2. FQ drugs: ofloxacin (2 µg/mL) and moxifloxacin (0.25 µg/mL) 3. SLIDs: kanamycin (5 µg/mL), amikacin (1 µg/mL) and capreomycin (2.5 µg/mL) 4. Discrepant analysis: no
Flow and timing	Uninterpretable results reported: no
Comparative	
Notes	Other findings: analysis of test performance stratified according to sputum smear positivity showed that the test readability for individual drugs and their drug groups ranged from 80.0% to 100.0%, with the lowest for specimens graded 1 (Table 5). Within this group of specimens, lower readability rates were observed for the AG/CP group of drugs (n 3; 20.0% of tests failed), with higher readability rates for FQ and ethambutol. Similar trends were observed in specimens graded 2 and 3 (Fig. 1). Total agreement between the molecular assay and phenotypic DST was the highest (84.1%) for FQs and lowest (23.5%) for the injectable drugs

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			

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Kontsevaya 2013 (*Continued*)

Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

Lacoma 2012

Study characteristics	
Patient sampling	Cross-sectional design with convenience-based enrolment of participants, prospective data collection
Patient characteristics and setting	1. Country of origin: Spain 2. World Bank classification of country: high 3. Type of lab: hospital 4. Type of patients: confirmed MDR-TB cases 5. Smear-positive patients whose specimens were tested directed: 49/54

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Index tests	1. Manufacturer involvement: no 2. Type of testing: direct and indirect 3. Type of specimens: smear-positive and smear negative 4. Specimen treatment: NALC-NaOH 5. Specimen condition: frozen 6. Duration of freezing: > 1 year
Target condition and reference standard(s)	1. Culture (liquid; BACTEC460TB) used for FQ, SLID 2. FQ drugs: moxifloxacin (0.5 µg/mL) 3. SLIDs: kanamycin (5 µg/mL) and capreomycin (1.25 µg/mL) 4. Discrepant analysis: yes (for indirect testing only)
Flow and timing	Uninterpretable results reported: yes
Comparative	
Notes	

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			

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Lacoma 2012 (*Continued*)

Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		

Lopez-Roa 2012

Study characteristics	
Patient sampling	Cross-sectional design with convenience-based enrolment of participants, prospective data collection
Patient characteristics and setting	1. Country of origin: Spain 2. World Bank classification of country: high 3. Type of lab: hospital 4. Type of patients: confirmed MDR-TB cases
Index tests	1. Manufacturer involvement: no 2. Type of testing: indirect
Target condition and reference standard(s)	1. Culture (solid and liquid; 7H11 and MGIT 960) used for FQ, SLID 2. FQ drugs: ofloxacin (2 µg/mL) 3. SLIDs: amikacin (4 µg/mL) 4. Discrepant analysis: yes
Flow and timing	Uninterpretable results reported: yes
Comparative	

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Notes	Other findings: the turnaround time for agar proportion, MGIT 960 and GenoType® MTBDR*sl* were, respectively, 21 days, 8 days and 8 hours.		
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
			Low
DOMAIN 4: Flow and Timing			

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Lopez-Roa 2012 (*Continued*)

Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		

Miotto 2012

Study characteristics	
Patient sampling	Isolates: case-controlled design with consecutive enrolment of participants, prospective data collection Specimens: cross-sectional design with consecutive enrolment of participants, prospective data collection
Patient characteristics and setting	1. Country of origin: Italy 2. World Bank classification of country: high 3. Type of lab: hospital 4. Type of patients: confirmed MDR-TB cases, confirmed XDR-TB cases, confirmed MDR-TBs with some known 2nd line resistance
Index tests	1. Manufacturer involvement: yes, donation of test 2. Type of testing: direct and indirect 3. Type of specimens: smear-positive 4. Specimen treatment: NALC-NaOH 5. Specimen condition: frozen 6. Duration of freezing: > 1 year
Target condition and reference standard(s)	1. Culture (solid; 7H11) and sequencing used for FQ, SLID 2. FQ drugs: ofloxacin (2 µg/mL) 3. SLIDs: kanamycin (5 µg/mL) and capreomycin (10 µg/mL) 4. Discrepant analysis: no 5. Genes for FQ: gyrA 6. Genes for SLIDs: rrs
Flow and timing	Uninterpretable results reported: yes
Comparative	
Notes	Other findings: NPV for SLID is higher in Beijing strains

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Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
			High
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		

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Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

Said 2012

Study characteristics			
Patient sampling	Cross-sectional design with consecutive-based enrolment of participants, prospective data collection		
Patient characteristics and setting	1. Country of origin: South Africa 2. World Bank classification of country: middle/low 3. Type of lab: research 4. Type of patients: confirmed MDR-TB cases		
Index tests	1. Manufacturer involvement: yes, financial support. 2. Type of testing: indirect		
Target condition and reference standard(s)	1. Culture (solid; 7H11) 2. FQ drugs: ofloxacin (2 µg/mL) 3. SLIDs: kanamycin (5 µg/mL) and capreomycin (10 µg/mL). Not the WHO critical concentrations for SLIDs. 4. Discrepant analysis: no		
Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes	Other findings: turnaround times for DST ranged from 6 to 21 days (median 11) for the agar proportion method and from 2 to 3 days (median 2) for the MTBDR*sl* assay. DST results of the MTBDR*sl* assay as compared to the agar proportion method are shown in Table 2		
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		

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Said 2012 (*Continued*)

Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

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Surcouf 2011

Study characteristics			
Patient sampling	Cross-sectional design with consecutive-based enrolment of participants, prospective data collection		
Patient characteristics and setting	1. Country of origin: Cambodia 2. World Bank classification of country: middle/low 3. Type of lab: unknown 4. Type of patients: confirmed MDR-TB cases		
Index tests	1. Manufacturer involvement: yes, donation of tests 2. Type of testing: indirect		
Target condition and reference standard(s)	1. Sequencing used for reference standard 2. FQ genes: gyra 3. SLID genes: rrs 4. Discrepant analysis: no		
Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes	Other findings: spoligotyping results showed that the majority of MDR strains belonged to the Beijing family (57/101, 56%) or were Beijing like (2/101, 2%). This percentage is higher in MDR FQ-R strains (10/14, 71%). This confirms that Beijing strains are more prone to accumulate antibiotic resistances		
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		

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Surcouf 2011 (*Continued*)

If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

Tukvadze 2014

Study characteristics	
Patient sampling	Cross-sectional design with consecutive-based enrolment of participants, prospective data collection
Patient characteristics and setting	1. Country of origin: Georgia 2. World Bank classification of country: middle/low 3. Type of lab: reference 4. Type of patients: confirmed MDR-TB cases
Index tests	1. Manufacturer involvement: no 2. Type of testing: direct
Target condition and reference standard(s)	1. Culture based DST, LJ 2. FQ: ofloxacin 2 µg/mL

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	3. SLIDS: capreomycin 40 µg/mL; kanamycin 30 µg/mL 4. There was no discrepant analysis 5. All reported XDR resistance		
Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes			

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
			Unclear
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		

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Tukvadze 2014 (*Continued*)

			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

van Ingen 2010

Study characteristics			
Patient sampling	Cross-sectional design with convenience-based enrolment of participants, retrospective data collection		
Patient characteristics and setting	1. Country of origin: Netherlands 2. World Bank classification of country: high 3. Type of lab: reference 4. Type of patients: confirmed MDR-TB cases, confirmed MDR-TBs with some known second-line resistance		
Index tests	1. Manufacturer involvement: no 2. Type of testing: indirect		
Target condition and reference standard(s)	1. Culture (solid; 7H10) 2. FQ drugs: moxifloxacin (1 µg/mL) 3. SLIDs: amikacin (5 µg/mL) and capreomycin (10 µg/mL). WHO critical concentrations not used for 7H10 solid culture 4. Discrepant analysis: no		
Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes	Relevant clinical information? unclear		
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**

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van Ingen 2010 (*Continued*)

DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
			High
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

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Zivanovic 2012

Study characteristics			
Patient sampling	Cross-sectional design with consecutive-based enrolment of participants, prospective data collection		
Patient characteristics and setting	1. Country of origin: Serbia 2. World Bank classification of country: middle/low 3. Type of lab: reference 4. Type of patients: confirmed MDR-TB cases		
Index tests	1. Manufacturer involvement: no 2. Type of testing: indirect		
Target condition and reference standard(s)	1. Culture (solid and liquid; LJ and MGIT960) 2. FQ drugs: ofloxacin (2 µg/mL) 3. SLIDs: amikacin (1 µg/mL for MGIT) and capreomycin (2.5 µg/mL for MGIT) 4. Discrepant analysis: no		
Flow and timing	Uninterpretable results reported: yes		
Comparative			
Notes			
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
			Low
DOMAIN 2: Index Test All tests			

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Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
			Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
			Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Bantouna 2011	Conference abstract.
Bergvala 2010	Technical. Not a diagnostic accuracy study.
Brossier 2010a	Conference abstract.

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(*Continued*)

Choi 2010	Technical. Not a diagnostic accuracy study.
Fallico 2012	Conference abstract.
Felkel 2013	Technical. No diagnostic data for FQs, SLIDs or XDR-TB.
Festoso 2011	Conference abstract.
Gkaravela 2012	Conference abstract.
Iem 2013	Technical. Only 1 case of second-line resistance.
Jang 2011	Conference abstract.
Karabela 2007	Conference abstract.
Kontos 2011	Conference abstract.
Kontos 2012	Conference abstract.
Lemus 2011	Conference abstract.
López-Roa 2010	Conference abstract.
Singh 2013	Technical. No information on resistance to the pre-specified FQs and no cases susceptible to the SLIDs
Tessema 2012a	Technical. No information on resistance to FQs, SLIDs or XDR-TB
Tessema 2012b	Technical. No information on resistance to FQs, SLIDs or XDR-TB
Totten 2011	Conference abstract.
Zhang 2011	Conference abstract.

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DATA

Presented below are all the data for all of the tests entered into the review.

Tests. Data tables by test

Test	No. of studies	No. of participants
1 Indirect, FQ, culture	16	1766
2 Indirect, Ofl, culture	11	1544
3 Indirect, Mx, culture	4	222
4 Indirect, SLID, culture	14	1637
5 Indirect, Ak, culture	9	1017
6 Indirect, Kn, culture	9	1342
7 Indirect, Cm, culture	10	1406
8 Indirect, XDR, culture	8	880
9 Indirect, FQ, sequencing	7	974
10 Indirect, SLID, sequencing	6	873
11 Indirect, XDR, sequencing	3	541
12 Indirect, FQ, sequencing and culture	7	1211
13 Indirect, SLID, sequencing and culture	7	1491
14 Indirect, XDR, sequencing and culture	2	435
15 Indirect, FQ, culture followed by sequencing of discrepants	3	427
16 Indirect, SLID, culture followed by sequencing of discrepants	3	619
17 Direct, FQ, culture	7	1033
18 Direct, Ofl, culture	3	622
19 Direct, SLID, culture	6	947
20 Direct, Ak, culture	4	803
21 Direct, Kn, culture	4	418
22 Direct, Cm, culture	4	425
23 Direct, XDR, culture	3	644
24 Direct, FQ, culture followed by sequencing of discrepants	2	685
25 Direct, SLID, culture followed by sequencing of discrepants	2	666
26 Direct, XDR, culture followed by sequencing of discrepants	1	516

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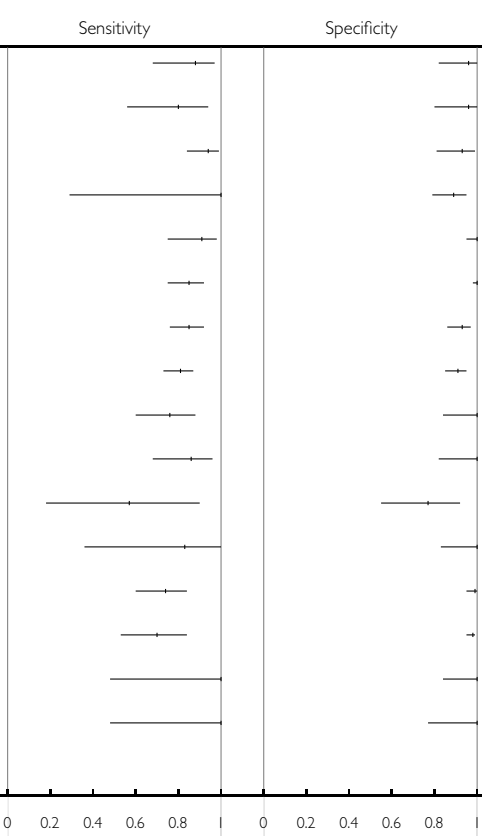
Test 1. Indirect, FQ, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 1 Indirect, FQ, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brossier 2010	21	1	3	27	0.88 [0.68, 0.97]	0.96 [0.82, 1.00]
Chikamatsu 2012	16	1	4	25	0.80 [0.56, 0.94]	0.96 [0.80, 1.00]
Fan 2011	49	3	3	39	0.94 [0.84, 0.99]	0.93 [0.81, 0.99]
Ferro 2013	3	8	0	62	1.00 [0.29, 1.00]	0.89 [0.79, 0.95]
Hillemann 2009	29	0	3	74	0.91 [0.75, 0.98]	1.00 [0.95, 1.00]
Huang 2011	63	0	11	160	0.85 [0.75, 0.92]	1.00 [0.98, 1.00]
Ignatyeva 2012	69	7	12	92	0.85 [0.76, 0.92]	0.93 [0.86, 0.97]
Jin 2013	111	11	26	113	0.81 [0.73, 0.87]	0.91 [0.85, 0.95]
Kiet 2010	31	0	10	21	0.76 [0.60, 0.88]	1.00 [0.84, 1.00]
Kontsevaya 2011	25	0	4	19	0.86 [0.68, 0.96]	1.00 [0.82, 1.00]
Lacoma 2012	4	5	3	17	0.57 [0.18, 0.90]	0.77 [0.55, 0.92]
Lopez-Roa 2012	5	0	1	20	0.83 [0.36, 1.00]	1.00 [0.83, 1.00]
Miotto 2012	42	1	15	116	0.74 [0.60, 0.84]	0.99 [0.95, 1.00]
Said 2012	26	7	11	292	0.70 [0.53, 0.84]	0.98 [0.95, 0.99]
van Ingen 2010	5	0	0	21	1.00 [0.48, 1.00]	1.00 [0.84, 1.00]
Zivanovic 2012	5	0	0	14	1.00 [0.48, 1.00]	1.00 [0.77, 1.00]



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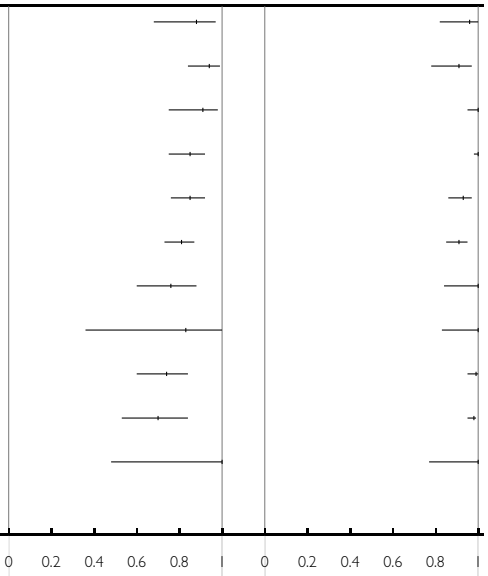
Test 2. Indirect, Ofl, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 2 Indirect, Ofl, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brossier 2010	21	1	3	27	0.88 [0.68, 0.97]	0.96 [0.82, 1.00]
Fan 2011	48	4	3	39	0.94 [0.84, 0.99]	0.91 [0.78, 0.97]
Hillemann 2009	29	0	3	74	0.91 [0.75, 0.98]	1.00 [0.95, 1.00]
Huang 2011	63	0	11	160	0.85 [0.75, 0.92]	1.00 [0.98, 1.00]
Ignatyeva 2012	69	7	12	92	0.85 [0.76, 0.92]	0.93 [0.86, 0.97]
Jin 2013	111	11	26	113	0.81 [0.73, 0.87]	0.91 [0.85, 0.95]
Kiet 2010	31	0	10	21	0.76 [0.60, 0.88]	1.00 [0.84, 1.00]
Lopez-Roa 2012	5	0	1	20	0.83 [0.36, 1.00]	1.00 [0.83, 1.00]
Miotto 2012	42	1	15	116	0.74 [0.60, 0.84]	0.99 [0.95, 1.00]
Said 2012	26	7	11	292	0.70 [0.53, 0.84]	0.98 [0.95, 0.99]
Zivanovic 2012	5	0	0	14	1.00 [0.48, 1.00]	1.00 [0.77, 1.00]

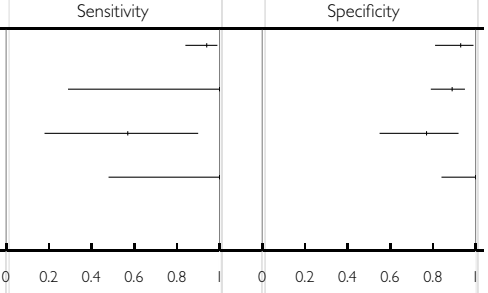


Test 3. Indirect, Mx, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 3 Indirect, Mx, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Fan 2011	49	3	3	39	0.94 [0.84, 0.99]	0.93 [0.81, 0.99]
Ferro 2013	3	8	0	62	1.00 [0.29, 1.00]	0.89 [0.79, 0.95]
Lacoma 2012	4	5	3	17	0.57 [0.18, 0.90]	0.77 [0.55, 0.92]
van Ingen 2010	5	0	0	21	1.00 [0.48, 1.00]	1.00 [0.84, 1.00]



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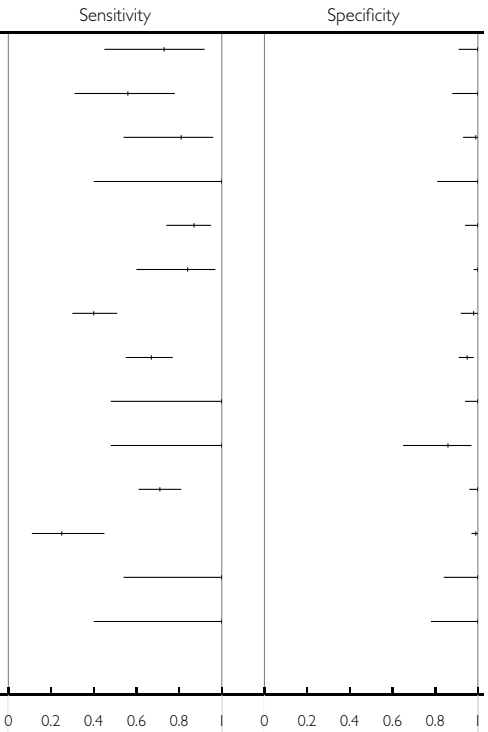
Test 4. Indirect, SLID, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 4 Indirect, SLID, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brossier 2010	11	0	4	37	0.73 [0.45, 0.92]	1.00 [0.91, 1.00]
Chikamatsu 2012	10	0	8	28	0.56 [0.31, 0.78]	1.00 [0.88, 1.00]
Fan 2011	13	1	3	77	0.81 [0.54, 0.96]	0.99 [0.93, 1.00]
Ferro 2013	4	0	0	18	1.00 [0.40, 1.00]	1.00 [0.81, 1.00]
Hillemann 2009	40	0	6	60	0.87 [0.74, 0.95]	1.00 [0.94, 1.00]
Huang 2011	16	0	3	215	0.84 [0.60, 0.97]	1.00 [0.98, 1.00]
Ignatyeva 2012	37	2	55	87	0.40 [0.30, 0.51]	0.98 [0.92, 1.00]
Jin 2013	48	9	24	180	0.67 [0.55, 0.77]	0.95 [0.91, 0.98]
Kiet 2010	5	0	0	57	1.00 [0.48, 1.00]	1.00 [0.94, 1.00]
Lacoma 2012	5	3	0	19	1.00 [0.48, 1.00]	0.86 [0.65, 0.97]
Miotto 2012	60	0	24	90	0.71 [0.61, 0.81]	1.00 [0.96, 1.00]
Said 2012	7	4	21	300	0.25 [0.11, 0.45]	0.99 [0.97, 1.00]
van Ingen 2010	6	0	0	21	1.00 [0.54, 1.00]	1.00 [0.84, 1.00]
Zivanovic 2012	4	0	0	15	1.00 [0.40, 1.00]	1.00 [0.78, 1.00]



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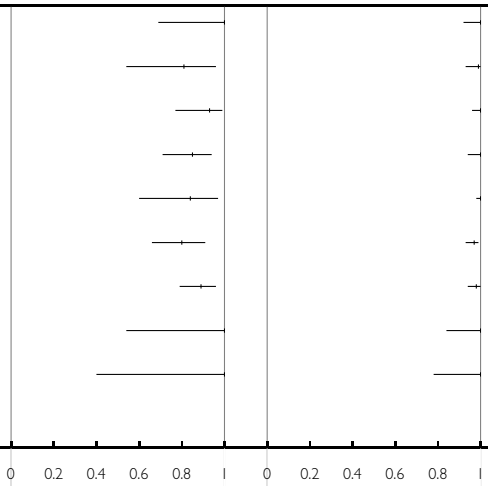
Test 5. Indirect, Ak, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 5 Indirect, Ak, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brossier 2010	10	0	0	42	1.00 [0.69, 1.00]	1.00 [0.92, 1.00]
Fan 2011	13	1	3	77	0.81 [0.54, 0.96]	0.99 [0.93, 1.00]
Ferro 2013	27	0	2	102	0.93 [0.77, 0.99]	1.00 [0.96, 1.00]
Hillemann 2009	39	0	7	60	0.85 [0.71, 0.94]	1.00 [0.94, 1.00]
Huang 2011	16	0	3	215	0.84 [0.60, 0.97]	1.00 [0.98, 1.00]
Ignatyeva 2012	37	4	9	130	0.80 [0.66, 0.91]	0.97 [0.93, 0.99]
Miotto 2012	58	2	7	107	0.89 [0.79, 0.96]	0.98 [0.94, 1.00]
van Ingen 2010	6	0	0	21	1.00 [0.54, 1.00]	1.00 [0.84, 1.00]
Zivanovic 2012	4	0	0	15	1.00 [0.40, 1.00]	1.00 [0.78, 1.00]



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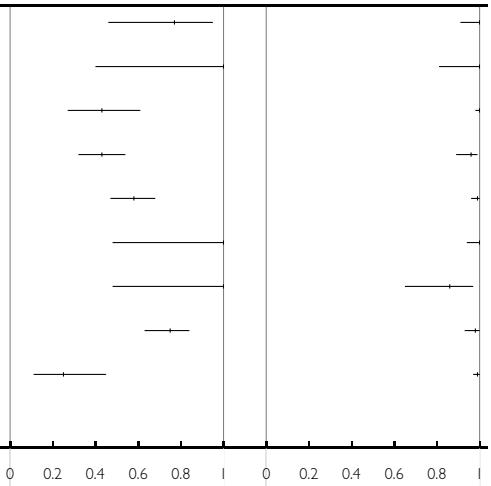
Test 6. Indirect, Kn, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 6 Indirect, Kn, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brossier 2010	10	0	3	39	0.77 [0.46, 0.95]	1.00 [0.91, 1.00]
Ferro 2013	4	0	0	18	1.00 [0.40, 1.00]	1.00 [0.81, 1.00]
Huang 2011	16	0	21	197	0.43 [0.27, 0.61]	1.00 [0.98, 1.00]
Ignatyeva 2012	37	4	50	89	0.43 [0.32, 0.54]	0.96 [0.89, 0.99]
Jin 2013	55	2	40	164	0.58 [0.47, 0.68]	0.99 [0.96, 1.00]
Kiet 2010	5	0	0	57	1.00 [0.48, 1.00]	1.00 [0.94, 1.00]
Lacoma 2012	5	3	0	19	1.00 [0.48, 1.00]	0.86 [0.65, 0.97]
Miotto 2012	56	2	19	95	0.75 [0.63, 0.84]	0.98 [0.93, 1.00]
Said 2012	7	4	21	300	0.25 [0.11, 0.45]	0.99 [0.97, 1.00]



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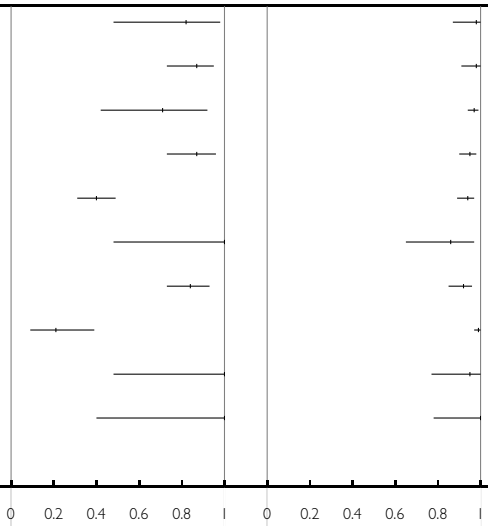
Test 7. Indirect, Cm, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 7 Indirect, Cm, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brossier 2010	9	1	2	40	0.82 [0.48, 0.98]	0.98 [0.87, 1.00]
Hillemann 2009	39	1	6	60	0.87 [0.73, 0.95]	0.98 [0.91, 1.00]
Huang 2011	10	6	4	214	0.71 [0.42, 0.92]	0.97 [0.94, 0.99]
Ignatyeva 2012	34	7	5	134	0.87 [0.73, 0.96]	0.95 [0.90, 0.98]
Jin 2013	49	8	75	129	0.40 [0.31, 0.49]	0.94 [0.89, 0.97]
Lacoma 2012	5	3	0	19	1.00 [0.48, 1.00]	0.86 [0.65, 0.97]
Miotto 2012	49	9	9	101	0.84 [0.73, 0.93]	0.92 [0.85, 0.96]
Said 2012	7	4	26	295	0.21 [0.09, 0.39]	0.99 [0.97, 1.00]
van Ingen 2010	5	1	0	21	1.00 [0.48, 1.00]	0.95 [0.77, 1.00]
Zivanovic 2012	4	0	0	15	1.00 [0.40, 1.00]	1.00 [0.78, 1.00]



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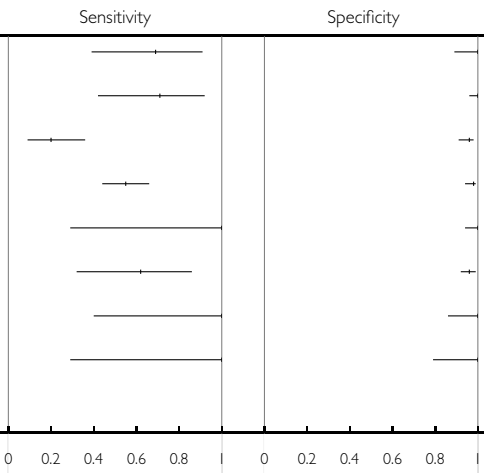
Test 8. Indirect, XDR, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 8 Indirect, XDR, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Chikamatsu 2012	9	0	4	33	0.69 [0.39, 0.91]	1.00 [0.89, 1.00]
Hillemann 2009	10	0	4	92	0.71 [0.42, 0.92]	1.00 [0.96, 1.00]
Ignatyeva 2012	8	6	32	134	0.20 [0.09, 0.36]	0.96 [0.91, 0.98]
Jin 2013	46	4	37	174	0.55 [0.44, 0.66]	0.98 [0.94, 0.99]
Kiet 2010	3	0	0	62	1.00 [0.29, 1.00]	1.00 [0.94, 1.00]
Miotto 2012	8	6	5	155	0.62 [0.32, 0.86]	0.96 [0.92, 0.99]
van Ingen 2010	4	0	0	25	1.00 [0.40, 1.00]	1.00 [0.86, 1.00]
Zivanovic 2012	3	0	0	16	1.00 [0.29, 1.00]	1.00 [0.79, 1.00]

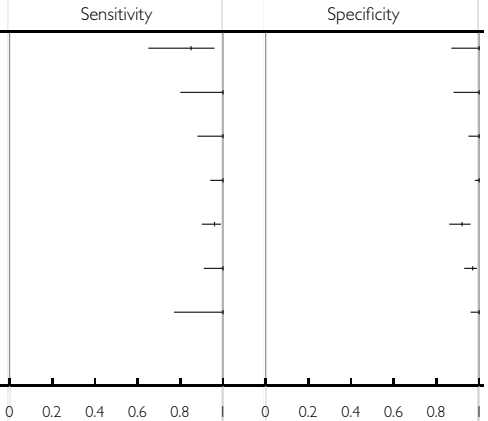


Test 9. Indirect, FQ, sequencing.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 9 Indirect, FQ, sequencing

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brossier 2010	22	0	4	26	0.85 [0.65, 0.96]	1.00 [0.87, 1.00]
Chikamatsu 2012	17	0	0	29	1.00 [0.80, 1.00]	1.00 [0.88, 1.00]
Hillemann 2009	29	0	0	77	1.00 [0.88, 1.00]	1.00 [0.95, 1.00]
Huang 2011	63	0	0	171	1.00 [0.94, 1.00]	1.00 [0.98, 1.00]
Jin 2013	110	12	5	134	0.96 [0.90, 0.99]	0.92 [0.86, 0.96]
Miotto 2012	39	4	0	131	1.00 [0.91, 1.00]	0.97 [0.93, 0.99]
Surcouf 2011	14	0	0	87	1.00 [0.77, 1.00]	1.00 [0.96, 1.00]



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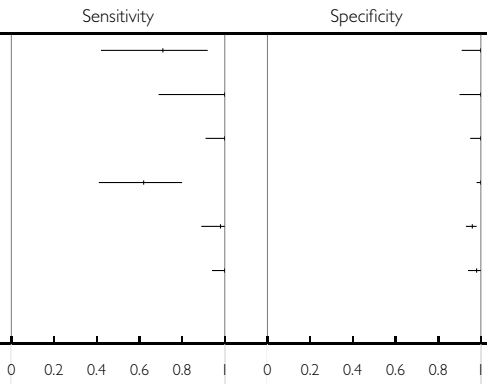
Test 10. Indirect, SLID, sequencing.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 10 Indirect, SLID, sequencing

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brossier 2010	10	0	4	38	0.71 [0.42, 0.92]	1.00 [0.91, 1.00]
Chikamatsu 2012	10	0	0	36	1.00 [0.69, 1.00]	1.00 [0.90, 1.00]
Hillemann 2009	40	0	0	66	1.00 [0.91, 1.00]	1.00 [0.95, 1.00]
Huang 2011	16	0	10	208	0.62 [0.41, 0.80]	1.00 [0.98, 1.00]
Jin 2013	49	8	1	203	0.98 [0.89, 1.00]	0.96 [0.93, 0.98]
Miotto 2012	58	2	0	114	1.00 [0.94, 1.00]	0.98 [0.94, 1.00]

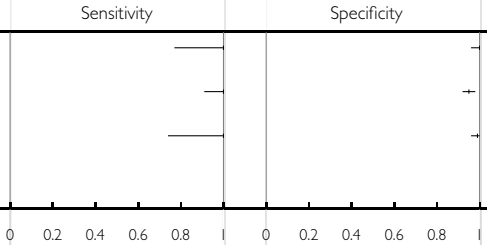


Test 11. Indirect, XDR, sequencing.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 11 Indirect, XDR, sequencing

Study	TP	FP	FN	TN	Sensitivity	Specificity
Hillemann 2009	14	0	0	92	1.00 [0.77, 1.00]	1.00 [0.96, 1.00]
Jin 2013	40	10	0	211	1.00 [0.91, 1.00]	0.95 [0.92, 0.98]
Miotto 2012	12	2	0	160	1.00 [0.74, 1.00]	0.99 [0.96, 1.00]



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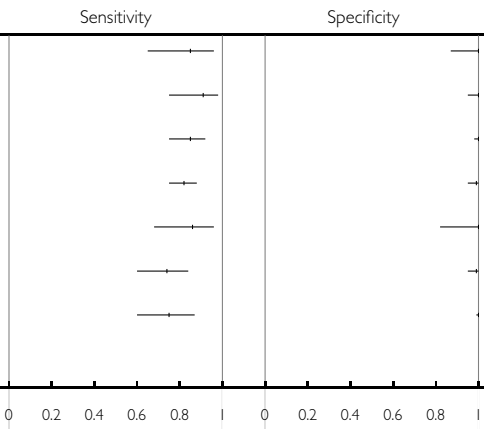
Test 12. Indirect, FQ, sequencing and culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 12 Indirect, FQ, sequencing and culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brossier 2010	22	0	4	26	0.85 [0.65, 0.96]	1.00 [0.87, 1.00]
Hillemann 2009	29	0	3	74	0.91 [0.75, 0.98]	1.00 [0.95, 1.00]
Huang 2011	63	0	11	160	0.85 [0.75, 0.92]	1.00 [0.98, 1.00]
Jin 2013	121	1	26	113	0.82 [0.75, 0.88]	0.99 [0.95, 1.00]
Kontsevaya 2011	25	0	4	19	0.86 [0.68, 0.96]	1.00 [0.82, 1.00]
Miotto 2012	42	1	15	116	0.74 [0.60, 0.84]	0.99 [0.95, 1.00]
Said 2012	33	0	11	292	0.75 [0.60, 0.87]	1.00 [0.99, 1.00]

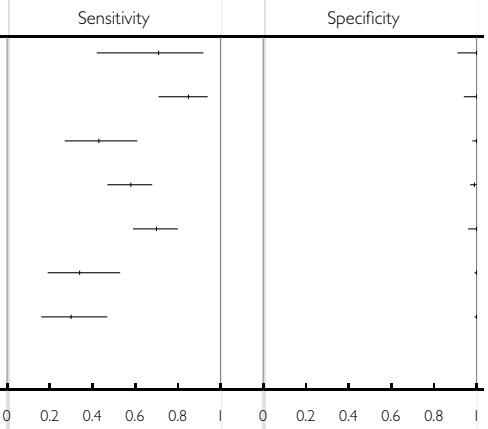


Test 13. Indirect, SLID, sequencing and culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 13 Indirect, SLID, sequencing and culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brossier 2010	10	0	4	38	0.71 [0.42, 0.92]	1.00 [0.91, 1.00]
Hillemann 2009	39	0	7	60	0.85 [0.71, 0.94]	1.00 [0.94, 1.00]
Huang 2011	16	0	21	197	0.43 [0.27, 0.61]	1.00 [0.98, 1.00]
Jin 2013	56	1	41	163	0.58 [0.47, 0.68]	0.99 [0.97, 1.00]
Miotto 2012	59	0	25	90	0.70 [0.59, 0.80]	1.00 [0.96, 1.00]
Said 2012	11	0	21	300	0.34 [0.19, 0.53]	1.00 [0.99, 1.00]
Surcouf 2011	11	0	26	295	0.30 [0.16, 0.47]	1.00 [0.99, 1.00]



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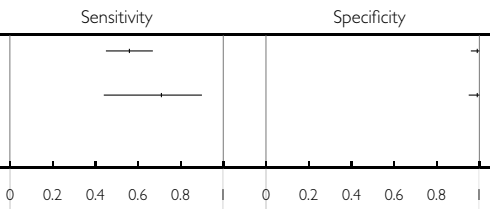
Test 14. Indirect, XDR, sequencing and culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 14 Indirect, XDR, sequencing and culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Jin 2013	48	2	37	174	0.56 [0.45, 0.67]	0.99 [0.96, 1.00]
Miotto 2012	12	2	5	155	0.71 [0.44, 0.90]	0.99 [0.95, 1.00]

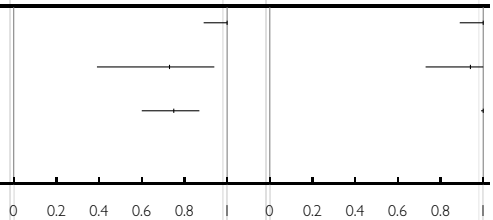


Test 15. Indirect, FQ, culture followed by sequencing of discrepants.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 15 Indirect, FQ, culture followed by sequencing of discrepants

Study	TP	FP	FN	TN	Sensitivity	Specificity
Kiet 2010	31	0	0	31	1.00 [0.89, 1.00]	1.00 [0.89, 1.00]
Lacoma 2012	8	1	3	17	0.73 [0.39, 0.94]	0.94 [0.73, 1.00]
Lopez-Roa 2012	33	0	11	292	0.75 [0.60, 0.87]	1.00 [0.99, 1.00]



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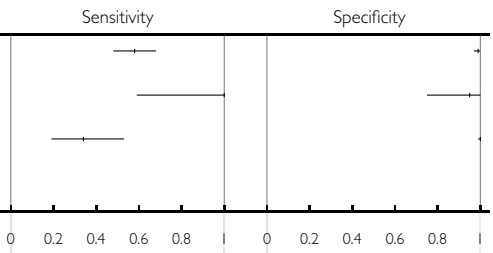
Test 16. Indirect, SLID, culture followed by sequencing of discrepants.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 16 Indirect, SLID, culture followed by sequencing of discrepants

Study	TP	FP	FN	TN	Sensitivity	Specificity
Jin 2013	56	1	40	163	0.58 [0.48, 0.68]	0.99 [0.97, 1.00]
Lacoma 2012	7	1	0	19	1.00 [0.59, 1.00]	0.95 [0.75, 1.00]
Said 2012	11	0	21	300	0.34 [0.19, 0.53]	1.00 [0.99, 1.00]

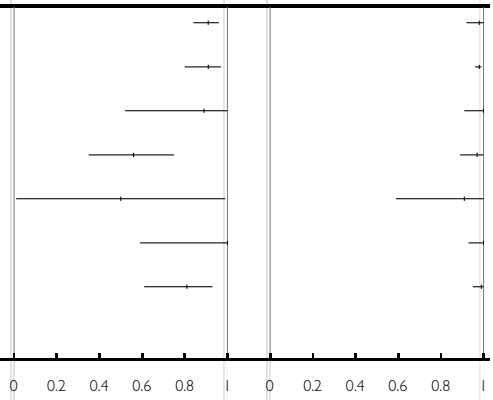


Test 17. Direct, FQ, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 17 Direct, FQ, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Ajbani 2012	96	1	9	64	0.91 [0.84, 0.96]	0.98 [0.92, 1.00]
Barnard 2012	49	9	5	453	0.91 [0.80, 0.97]	0.98 [0.96, 0.99]
Hillemann 2009	8	0	1	41	0.89 [0.52, 1.00]	1.00 [0.91, 1.00]
Kontsevaya 2013	15	2	12	61	0.56 [0.35, 0.75]	0.97 [0.89, 1.00]
Lacoma 2012	1	1	1	10	0.50 [0.01, 0.99]	0.91 [0.59, 1.00]
Miotto 2012	7	0	0	49	1.00 [0.59, 1.00]	1.00 [0.93, 1.00]
Tukvadze 2014	21	1	5	111	0.81 [0.61, 0.93]	0.99 [0.95, 1.00]



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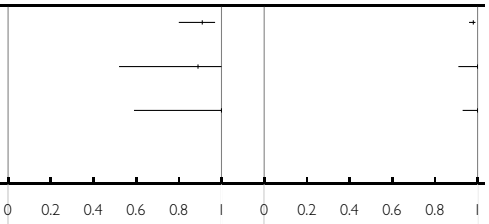
Test 18. Direct, Ofl, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 18 Direct, Ofl, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Barnard 2012	49	9	5	453	0.91 [0.80, 0.97]	0.98 [0.96, 0.99]
Hillemann 2009	8	0	1	41	0.89 [0.52, 1.00]	1.00 [0.91, 1.00]
Miotto 2012	7	0	0	49	1.00 [0.59, 1.00]	1.00 [0.93, 1.00]

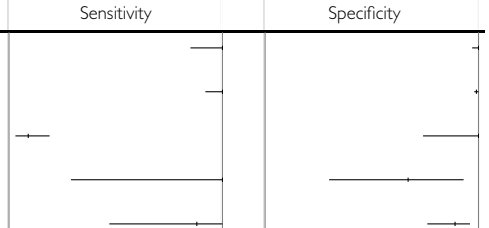


Test 19. Direct, SLID, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 19 Direct, SLID, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Ajbani 2012	22	0	0	128	1.00 [0.85, 1.00]	1.00 [0.97, 1.00]
Barnard 2012	43	3	0	470	1.00 [0.92, 1.00]	0.99 [0.98, 1.00]
Kontsevaya 2013	6	0	59	12	0.09 [0.03, 0.19]	1.00 [0.74, 1.00]
Lacoma 2012	3	3	0	6	1.00 [0.29, 1.00]	0.67 [0.30, 0.93]
Miotto 2012	7	5	1	41	0.88 [0.47, 1.00]	0.89 [0.76, 0.96]
Tukvadze 2014	19	1	47	71	0.29 [0.18, 0.41]	0.99 [0.93, 1.00]



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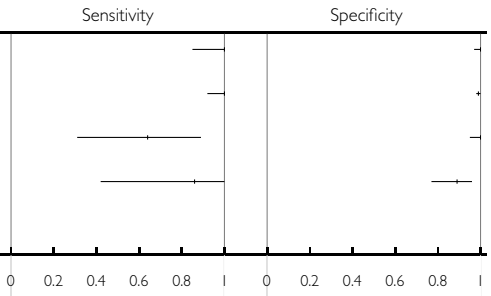
Test 20. Direct, Ak, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 20 Direct, Ak, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Ajbani 2012	22	0	0	128	1.00 [0.85, 1.00]	1.00 [0.97, 1.00]
Barnard 2012	43	3	0	470	1.00 [0.92, 1.00]	0.99 [0.98, 1.00]
Kontsevaya 2013	7	0	4	72	0.64 [0.31, 0.89]	1.00 [0.95, 1.00]
Miotto 2012	6	5	1	42	0.86 [0.42, 1.00]	0.89 [0.77, 0.96]

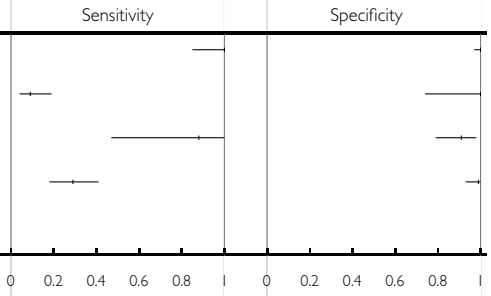


Test 21. Direct, Kn, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 21 Direct, Kn, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Ajbani 2012	22	0	0	128	1.00 [0.85, 1.00]	1.00 [0.97, 1.00]
Kontsevaya 2013	6	0	58	12	0.09 [0.04, 0.19]	1.00 [0.74, 1.00]
Miotto 2012	7	4	1	42	0.88 [0.47, 1.00]	0.91 [0.79, 0.98]
Tukvadze 2014	19	1	47	71	0.29 [0.18, 0.41]	0.99 [0.93, 1.00]



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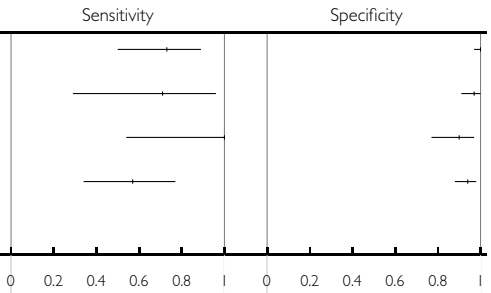
Test 22. Direct, Cm, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 22 Direct, Cm, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Ajbani 2012	16	0	6	128	0.73 [0.50, 0.89]	1.00 [0.97, 1.00]
Kontsevaya 2013	5	2	2	74	0.71 [0.29, 0.96]	0.97 [0.91, 1.00]
Miotto 2012	6	5	0	43	1.00 [0.54, 1.00]	0.90 [0.77, 0.97]
Tukvadze 2014	13	7	10	108	0.57 [0.34, 0.77]	0.94 [0.88, 0.98]

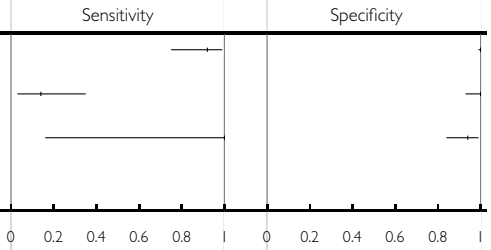


Test 23. Direct, XDR, culture.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 23 Direct, XDR, culture

Study	TP	FP	FN	TN	Sensitivity	Specificity
Barnard 2012	24	2	2	488	0.92 [0.75, 0.99]	1.00 [0.99, 1.00]
Kontsevaya 2013	3	0	19	52	0.14 [0.03, 0.35]	1.00 [0.93, 1.00]
Miotto 2012	2	3	0	49	1.00 [0.16, 1.00]	0.94 [0.84, 0.99]



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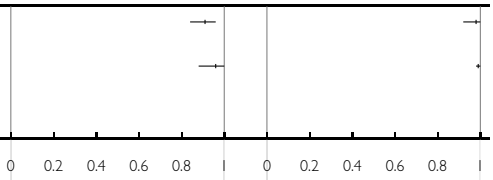
Test 24. Direct, FQ, culture followed by sequencing of discrepants.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 24 Direct, FQ, culture followed by sequencing of discrepants

Study	TP	FP	FN	TN	Sensitivity	Specificity
Ajbani 2012	96	1	9	64	0.91 [0.84, 0.96]	0.98 [0.92, 1.00]
Barnard 2012	54	4	2	455	0.96 [0.88, 1.00]	0.99 [0.98, 1.00]

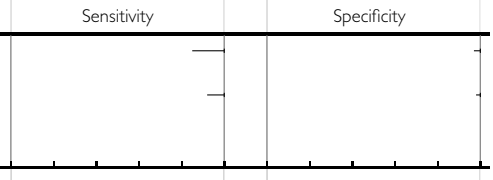


Test 25. Direct, SLID, culture followed by sequencing of discrepants.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 25 Direct, SLID, culture followed by sequencing of discrepants

Study	TP	FP	FN	TN	Sensitivity	Specificity
Ajbani 2012	22	0	0	128	1.00 [0.85, 1.00]	1.00 [0.97, 1.00]
Barnard 2012	44	2	0	470	1.00 [0.92, 1.00]	1.00 [0.98, 1.00]



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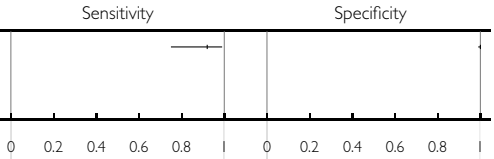
Test 26. Direct, XDR, culture followed by sequencing of discrepants.

Review: The diagnostic accuracy of the GenoType MTBDR*sl* assay for the detection of resistance to second-line anti-tuberculosis drugs

Test: 26 Direct, XDR, culture followed by sequencing of discrepants

Study	TP	FP	FN	TN	Sensitivity	Specificity
Miotto 2012	24	2	2	488	0.92 [0.75, 0.99]	1.00 [0.99, 1.00]



ADDITIONAL TABLES

Table 1. Map of review showing the number of eligible studies, according to reference standard and target condition, which performed indirect testing

Target condition, drug resistance to...	Reference standard			
	Culture, n/N (%)	Genetic testing, n/N (%)	Genetic and culture testing, n/N (%)	Culture followed by genetic testing of discordant results, n/N (%)
Fluoroquinolones	16/16 (100)†	7/16 (44)	7/16 (44)	3/16 (19)
Ofloxacin	11/16 (69)	0	0	0
Moxifloxacin	4/16 (25)	0	0	0
Levofloxacin	1/16 (6)	0	0	0
Second-line injectable drugs	14/14 (100)†	6/14 (43)	7/14 (50)	3/14 (21)
Amikacin	9/14 (64)	0	0	0
Kanamycin	8/14 (57)	0	0	0
Capreomycin	10/14 (71)	0	0	0
XDR-TB	8/8 (100)	3/8 (38)	2/8 (25)	0

†A total of 16 and 14 studies were included that evaluated MTBDR*sl* against a fluoroquinolone and a second-line injectable drug, culture reference standard. These form the denominators to generate percentages of these studies that included a particular additional reference standard.

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Table 2. Map of review showing the number of eligible studies, according to reference standard and target condition, which performed direct testing

Target condition, drug resistance to...	Reference standard			
	Culture, n/N (%)	Genetic testing, n/N (%)	Genetic and culture testing, n/N (%)	Culture followed by genetic testing of discordant results, n/N (%)
Fluoroquinolones	7/7 (100)[†]	0	0	2/7 (29)
Ofloxacin	3/7 (43)	0	0	1/7 (14)
Second-line injectable drugs	6/6 (100)[†]	0	0	2/5 (40)
Amikacin	4/6 (67)	0	0	1/6 (17)
Kanamycin	4/6 (67)	0	0	0
Capreomycin	6/6 (100)	0	0	0
XDR-TB	3/3 (100)	0	0	1/3 (34)

[†]A total of six and five studies were included that evaluated MTBDR*sl* against a fluoroquinolone and a second-line injectable drug, culture reference standard. These form the denominators to generate percentages of these studies that included a particular additional reference standard.

Table 3. Accuracy of MTBDR*sl* for detection of resistance to fluoroquinolones and second-line injectable drugs, by reference standard and type of testing, indirect comparisons

Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Pooled sensitivity P value[1]	Pooled specificity P value[1]
Fluoroquinolone, culture, indirect testing (16 studies, 1766 participants)		**Fluoroquinolone, culture, direct testing** (7 studies, 1033 participants)			
83.1% (78.7 to 86.7)	97.7% (94.3 to 99.1)	85.1% (71.9 to 92.7)	98.2% (96.8 to 99.0)	0.670	0.293
Fluoroquinolone, culture, indirect testing (16 studies, 1766 participants)		**Fluoroquinolone, genetic sequencing, indirect testing** (7 studies, 974 participants)			
83.1% (78.7 to 86.7)	97.7 (94.3 to 99.1)	99.3% (85.9 to 100)	99.7% (92.0 to 100)	**< 0.001**	0.663

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Table 3. Accuracy of MTBDR*sl* for detection of resistance to fluoroquinolones and second-line injectable drugs, by reference standard and type of testing, indirect comparisons (*Continued*)

Fluoroquinolone, culture, indirect testing (16 studies, 1766 participants)		**Fluoroquinolone, genetic sequencing and culture, indirect testing** (7 studies, 1211 participants)			
83.1% (78.7 to 86.7)	97.7% (94.3 to 99.1)	82.0 (77.7 to 85.6)	99.8 (98.5 to 100)	0.983	**< 0.001**
Ofloxacin, culture, indirect testing (11 studies, 1544 participants)		**Moxifloxacin, culture, indirect testing** (4 studies, 222 participants)			
82.9% (79.5, 85.9)	98.2% (96.1, 99.1)	91.4% (64.7 to 98.4)	90.6% (79.3 to 96.1)	0.239	0.061
Second-line injectable drugs, culture, indirect testing (14 studies, 1637 participants)		**Second-line injectable drugs, culture, direct testing** (6 studies, 947 participants)			
76.9% (61.1 to 87.6)	99.5% (97.1 to 99.9)	94.4% (25.2 to 99.9)	98.2% (88.9 to 99.7)	0.451	**0.005**
Second-line injectable drugs, culture, indirect testing (14 studies, 1637 participants)		**Second-line injectable drugs, indirect, genetic sequencing** (6 studies, 873 participants)			
76.9% (61.1 to 87.6)	99.5% (97.1 to 99.9)	97.0% (77.0 to 99.7)	99.5% (94.5 to 100)	**0.047***	0.935*
Second-line injectable drugs, culture, indirect testing (14 studies, 1637 participants)		**Second-line injectable drugs, genetic sequencing and culture, indirect testing** (7 studies, 1491 participants)			
76.9% (61.1 to 87.6)	99.5% (97.1 to 100)	56.7% (40.8 to 71.3)	99.9% (99.2 to 100)	0.340	**0.003**
Amikacin, indirect, culture (9 studies, 1017 participants)		***Kanamycin, culture, indirect testing*** (9 studies, 1342 participants)			
87.9% (82.1 to 92.0)	99.5% (97.5 to 99.9)	66.9% (44.1 to 83.8)	98.6% (96.1 to 99.5)	**0.006**	0.262
Amikacin, culture, indirect testing (9 studies, 1017 participants)		***Capreomycin, culture, indirect testing*** (10 studies, 1406 participants)			
87.9% (82.1 to 92.0)	99.5% (0.975 to 0.999)	79.5% (58.3 to 91.4)	95.8% (93.4 to 97.3)	0.309*	**0.003***
Kanamycin, culture, indirect testing (9 studies, 1342 participants)		***Capreomycin, culture, indirect testing*** (10 studies, 1406 participants)			

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Table 3. Accuracy of MTBDR*sl* for detection of resistance to fluoroquinolones and second-line injectable drugs, by reference standard and type of testing, indirect comparisons (*Continued*)

66.9% (44.1 to 83.8)	98.6% (96.1 to 99.5)	79.5% (58.3 to 91.4)	95.8% (93.4 to 97.3)	0.437	**0.043**
Amikacin, culture, indirect testing (9 studies, 1017 participants)		***Amikacin, culture, direct testing*** (4 studies, 803 participants)			
87.9% (82.1 to 92.0)	99.5% (0.975 to 0.999)	97.3% (55.1 to 99.9)	99.3% (92.3 to 99.9)	0.739	**0.035**

An indirect comparison uses all studies. Indirect statistical comparisons for the purpose of determining pooled accuracy estimates are not to be confused with indirect MTBDR*sl* testing which involves testing of the culture isolate.

*Indicates the model allowed the variances of the random effects to be associated with the covariate.

[1] Likelihood ratio test for evidence of a significant difference between accuracy estimates.

Table 4. Accuracy of MTBDR*sl* for detection of resistance to fluoroquinolones and second-line injectable drugs, by reference standard and type of testing, direct comparisons

Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Pooled sensitivity P value[1]	Pooled specificity P value[1]
Fluoroquinolone, culture, indirect testing (6 studies, 873 participants)		**Fluoroquinolone, genetic sequencing, indirect testing** (6 studies, 873 participants)			
82.4% (77.6 to 86.3)	98.8% (94.3 to 99.8)	99.3% (81.2 to 100)	99.3% (90.8 to 100)	**< 0.001**	0.971
Fluoroquinolone, culture, indirect testing (7 studies, 1211 participants)		**Fluoroquinolone, genetic sequencing and culture, indirect testing** (7 studies, 1211 participants)			
81.8% (77.2 to 85.7)	99.0% (95.0 to 99.8)	82.0% (77.7 to 85.6)	99.8% (98.5 to 100)	0.795	**< 0.001**
Second-line injectable drugs, culture, indirect testing (6 studies, 873 participants)		**Second-line injectable drugs, genetic sequencing, indirect testing** (6 studies, 873 participants)			
74.6% (66.2 to 81.5)	99.9% (71.8 to 100)	97.0% (77.0 to 99.7)	99.5% (94.5 to 100)	0.053*	0.349*
Second-line injectable drugs, culture, indirect testing (6 studies, 1159 participants)		**Second-line injectable drugs, genetic sequencing and culture, indirect testing** (6 studies, 1159 participants)			

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Table 4. Accuracy of MTBDR*sl* for detection of resistance to fluoroquinolones and second-line injectable drugs, by reference standard and type of testing, direct comparisons (*Continued*)

70.5% (52.0 to 84.1)	99.8% (93.8 to 100)	61.3% (45.8 to 74.8%)	99.9% (99.0 to 100)	0.729	**0.015**
Amikacin, culture, indirect testing (6 studies, 618 participants)		**Capreomycin, culture, indirect testing** (6 studies, 618 participants)			
87.1% (77.0 to 93.1)	99.9% (80.8 to 100)	85.6% (78.0 to 90.9)	96.8% (94.8 to 98.0)	0.989	**0.029**
Kanamycin, culture, indirect testing (6 studies, 1086 participants)		**Capreomycin, culture, indirect testing** (6 studies, 1086 participants)			
54.3% (34.5 to 72.8)	98.3% (94.8 to 99.5)	69.7% (38.0 to 89.6)	96.1% (93.3 to 97.8)	0.594	0.188

A direct comparison uses only studies that directly compared the two evaluations. Direct statistical comparisons for the purpose of determining pooled accuracy estimates are not to be confused with direct testing that pertains to the method for testing with MTBDR*sl*.

*Indicates the model allowed the variances of the random effects to be associated with the covariate.

[1]Likelihood ratio test for evidence of a significant difference between accuracy estimates.

Table 5. Accuracy of MTBDR*sl* for detection of extensively drug-resistant TB, by reference standard and type of testing, direct comparisons

Type of testing	Number of participants (studies)	Number of resistant cases (TPs + FNs)	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)
Culture testing				
Indirect	880 (8 studies)	173	70.9% (42.9 to 88.8)	98.8% (96.1, 99.6)
Direct	644 (3 studies)	50	*	*
Genetic testing				
Indirect	541 (3 studies)	66	100.0% (94.6 to 100.0)‡	97.5% (95.6 to 98.7)‡
Direct	0 (0 studies)	0	Not applicable	Not applicable
Genetic and culture testing				
Indirect	435 (2 studies)	102	58.8% (49.1 to 67.9)	98.8% (96.8 to 99.5)
Direct	0 (0 studies)	0	Not applicable	Not applicable
Culture followed by genetic testing of discordant results				

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Table 5. Accuracy of MTBDR*sl* for detection of extensively drug-resistant TB, by reference standard and type of testing, direct comparisons (*Continued*)

Indirect	0 (0 studies)	0	Not applicable	Not applicable
Direct	516 (1 study)	26	92.3% (74.9 to 99.1)	99.6% (98.5 to 100.0)

*We observed considerable heterogeneity and did not pool results.
‡We observed little heterogeneity and determined summary estimates of sensitivity and specificity separately using a fixed-effect model.
TP = true positive; FN = false negative.

Table 6. Sensitivity analyses for the fluoroquinolones

Culture, indirect testing			Culture, direct testing		
Number of participants (studies)	**Pooled sensitivity (95% CI)**	**Pooled specificity (95% CI)**	**Number of participants (studies)**	**Pooled sensitivity (95% CI)**	**Pooled specificity (95% CI)**
All studies of fluoroquinolones					
1766 (16 studies)	83.1% (78.7 to 86.7)	97.7% (94.3 to 99.1)	1033 (7 studies)	85.1% (71.9 to 92.7)	98.2% (96.8 to 99.0)
Consecutive or random sampling					
1493 (10 studies)	81.5% (77.8 to 84.8)	98.5% (94.0 to 99.6)	504 (5 studies)	85.6% (68.1 to 94.3)	99.0% (96.4 to 99.8)
Cross-sectional studies					
1166 (10 studies)	83.3% (77.1 to 88.1)	96.8% (91.0 to 98.9)	927 (5 studies)	82.0% (65.7 to 91.6)	98.0% (96.7 to 98.8)
Index test results blinded to reference standard results					
1307 (9 studies)	81.2% (77.0 to 84.8)	98.1% (92.1 to 99.6)	845 (5 studies)	85.1% (64.2 to 94.8)	97.8% (95.8 to 98.9)
Reference standard results blinded to index test results					
1104 (8 studies)	78.6% (73.3 to 83.1)	96.9% (93.4 to 98.6)	742 (3 studies)	91.6 (86.3 to 94.9)	98.3 (96.8 to 99.1)

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Table 7. Sensitivity analyses for the second-line injectable drugs

Culture, indirect testing			Culture, direct testing		
Number of participants (studies)	**Pooled sensitivity (95% CI)**	**Pooled specificity (95% CI)**	**Number of participants (studies)**	**Pooled sensitivity (95% CI)**	**Pooled specificity (95% CI)**
All studies of second-line injectable drugs					
1637 (14 studies)	76.9% (61.1 to 87.6)	99.5% (97.1 to 99.9)	947 (6 studies)	94.4% (25.2 to 99.9)	98.2% (88.9 to 99.7)
Consecutive or random sampling					
1391 (9 studies)	76.5% (53.8 to 90.1)	99.7% (95.9 to 100.0)	419 (4 studies)	71.9% (9.6 to 98.4)	98.9% (87.4 to 99.9)
Cross-sectional studies					
1035 (8 studies)	77.0% (51.5 to 91.3)	98.7% (94.7 to 99.7)	893 (5 studies)	99.2% (0.5 to 100.0)	99.1% (90.7 to 99.9)
Index test results blinded to reference standard results					
1255 (8 studies)	74.3 (46.9 to 90.4)	98.9 (94.6 to 99.8)	809 (5 studies)	98.6% (12.7 to 100.0)	98.1% (81.7 to 99.8)
Reference standard results blinded to index test results					
1102 (8 studies)	70.4% (42.7 to 88.4)	99.2% (94.7 to 99.9)	720 (3 studies)	99.0% (79.7 to 100.0)	98.9% (89.6 to 100.0)

APPENDICES

Appendix 1. The manufacturer-supplied result template

Figure 15 is an example of the manufacturer supplied result template.

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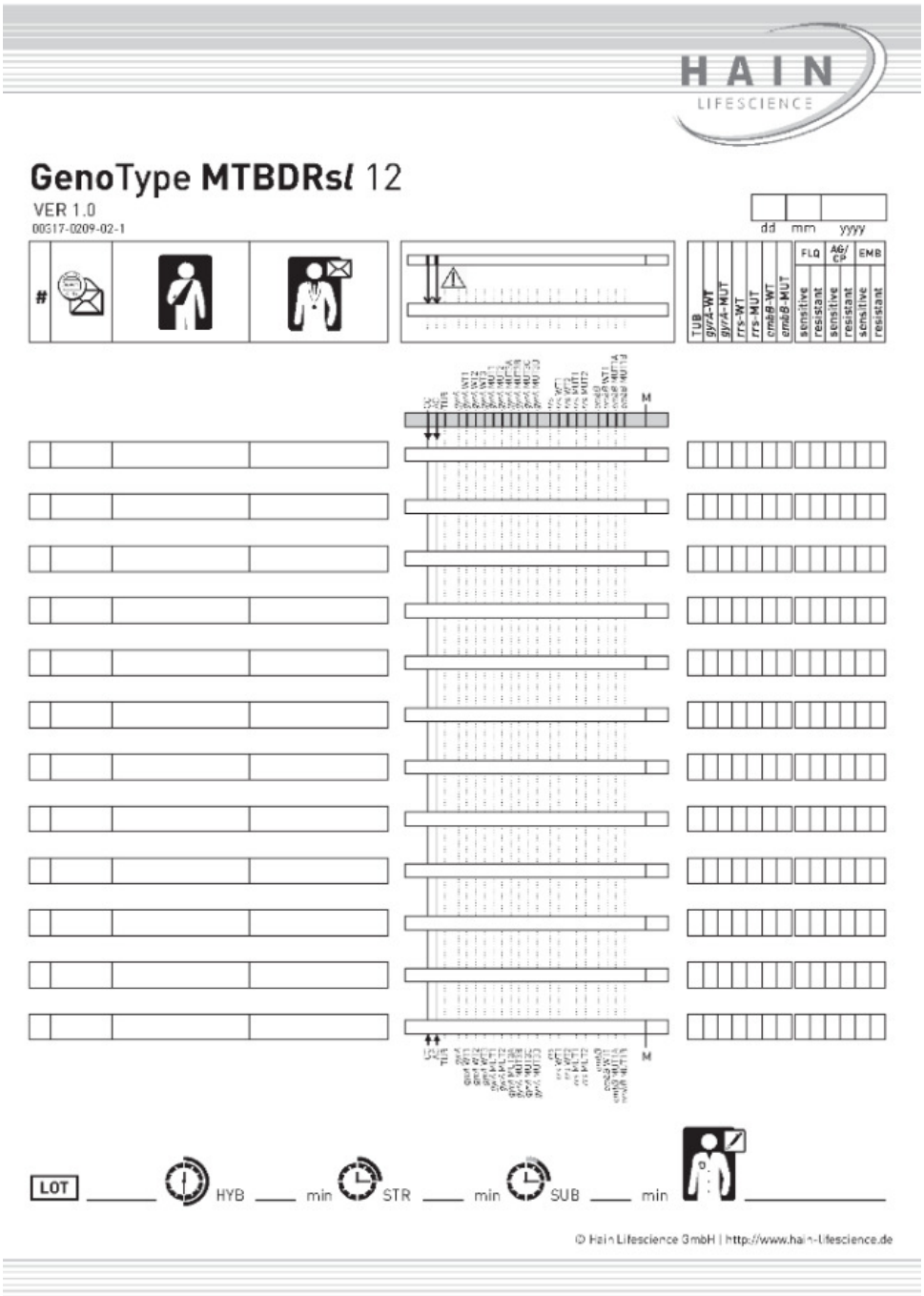
Figure 15. An example of the manufacturer-supplied result template.



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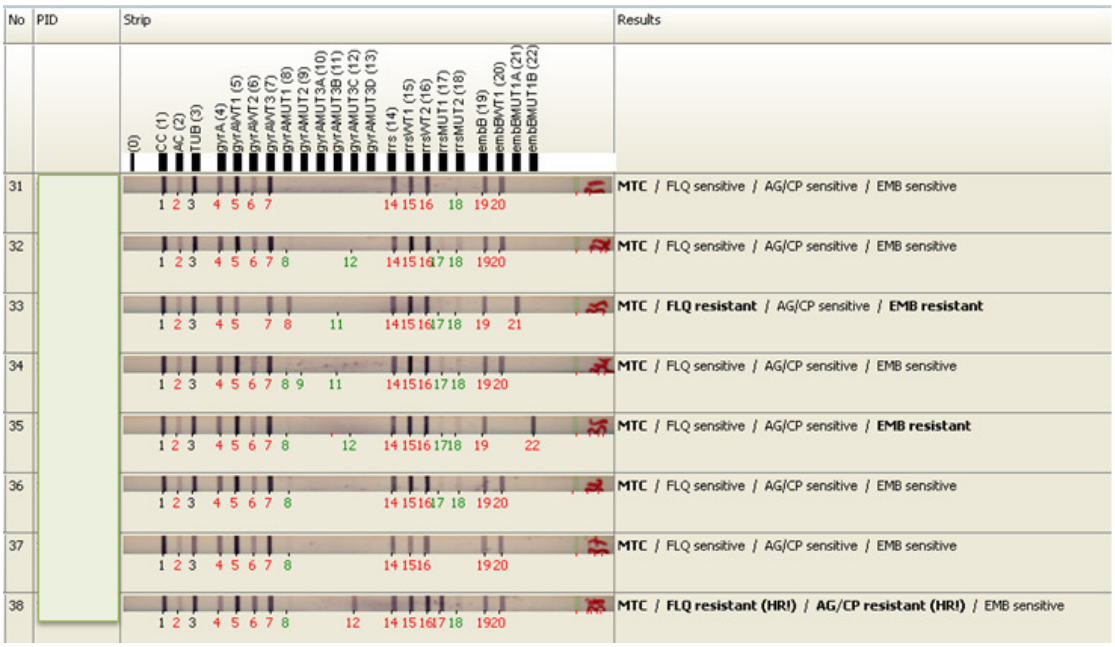
Appendix 2. Readout from an automated strip reader

Figure 16 is an example of a readout from an automated strip reader. The results are generated automatically and validated manually by a technician.

Figure 16. An example of a readout from an automated strip reader. The results are generated automatically and validated manually by a technician.



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Appendix 3. Detailed search strategy

Medline (PubMed)

1. MTBDR*.ti/ab.
2. Genotype MTBDR*.ti/ab
3. or/1-2
4. exp Tuberculosis, Pulmonary/
5. exp Tuberculosis, Multidrug-Resistant/
6. MDR-TB.ti/ab
7. XDR-TB.ti/ab
8. Mycobacterium tuberculosis/
9. TB.ti/ab
10. tuberculosis.ti/ab
11. or/4-10
12. 3 and 11

EMBASE (OVID)

1. tuberculosis.mp. or lung tuberculosis/ or Mycobacterium tuberculosis/ or multidrug resistant tuberculosis/
2. (MDR-TB or XDR-TB).mp.
3. exp Mycobacterium tuberculosis/
4. 1 or 2 or 3
5. (MTBDR* or "Genotype MTBDR*").mp
6. 4 and 5

Web of Knowledge (SCI-expanded, Conference Proceedings science) and BIOSIS previews

Topic=(MTBDR*) AND Topic=(tuberculosis OR TB OR MDR-TB OR XDR-TB)

LILACS

(tuberculosis OR TB OR mycobacterium OR MDR-TB OR XDR-TB) (Words) AND (MTBDR$) (Wor

SCOPUS

(tuberculosis OR TB OR mycobacterium OR MDR-TB OR XDR-TB) (title, abstract, keywords) AND (MTBDR*) (title, abstract, keywords)

CIDG Specialized register

(tuberculosis OR TB OR mycobacterium OR MDR-TB OR XDR-TB) AND (MTBDR*)

ProQuest Dissertations & Theses A&I search strategy

ab(tuberculosis) AND ab((diagnostic test* OR RDT* OR MTBDR*))

Medion

MTBDR* (title or abstract)

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metaRegister of Controlled Trials (mRCT)

(tuberculosis OR TB OR mycobacterium OR MDR-TB OR XDR-TB) AND (MTBDR*)

WHO International Clinical Trials Registry Platform

(tuberculosis OR TB OR mycobacterium OR MDR-TB OR XDR-TB) AND (MTBDR*)

Appendix 4. QUADAS-2 rules and interpretation

We use "patients" below with the understanding that studies in this Cochrane Review may be evaluating patient specimens.

Domain 1: Patient selection

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

Signaling question 1: Was a consecutive or random sample of patients enrolled?

We will score 'yes' if the study enrolled a consecutive or random sample of eligible patients; 'no' if the study selected patients by convenience; and 'unclear' if the study did not report the manner of patient selection or was not clearly reported.

Signaling question 2: Was a case-control design avoided?

We will score 'yes' if the study enrolled only TB patients with suspected resistance to second-line drugs, including patients with confirmed MDR-TB; 'no' if the study enrolled TB patients with confirmed resistance to second-line drugs; and 'unclear' for all other scenarios or if it was not clearly reported.

Signaling question 3: Did the study avoid inappropriate exclusions?

An inappropriate exclusion might occur if, after the laboratory technician runs the index and reference tests, he or she does not record the test results in the study. This might occur if there were resource constraints as one might find in practice, but we do not expect this to occur in the research studies included in this review. We will score 'yes' for all studies, as we do not anticipate inappropriate exclusions.

Applicability: Are there concerns that the included patients and setting do not match the review question?

We will judge 'low' concern if the selected specimens match 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 do not represent those for which the test will be used in practice, such as in individuals who are not suspected of having DR-TB. We will judge 'unclear' concern if we cannot tell.

Domain 2: Index test

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

Signaling question 1: Were the index test results interpreted without knowledge of the results of the reference standard?

We will score this question 'yes' if the reader of the assay was blinded to results of reference tests. We will score 'no' if the reader of the assay was not blinded to the results of reference tests. If the specimens were from a biobank comprised of specimens with known second-line drug resistance and the identity of these specimens was known to the assay reader, we will also answer 'no'. We will score 'unclear' if it was not stated in the paper or if the authors failed to answer this question.

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Signaling question 2: If a threshold was used, was it prespecified?

A threshold is prespecified in all versions of MTBDR*sl*. We will answer this question '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. However, we will judge these issues to be of 'low' concern for all studies in this review, as the MTBDR*sl* assay is standardized.

Domain 3: Reference standard

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

Signaling question 1: Is the reference standard likely to correctly classify the target condition?

Culture-based DST is not 100% accurate for detection of drug resistance, especially resistance to second-line drugs. However, it is the test currently endorsed by WHO when performed using WHO-recommended critical drug concentrations. Therefore, for culture-based DST, we will answer 'yes' if WHO critical concentrations were used, 'no' if they were not used and 'unclear' if the study authors do not specify.

Genetic sequencing (gene sequencing of loci known to be associated with drug resistance) is considered by researchers in this field to be the best reference standard for testing for the presence of drug resistance. Although sequencing may not be performed for all regions of the TB genome associated with resistance, we consider this to be a concern about the setting in which the test is applied, rather than a concern about risk of bias.

We will answer 'yes' when sequencing, culture and sequencing, and culture followed by discrepant sequencing are used.

Signaling question 2: Were the reference standard results interpreted without knowledge of the results of the index test?

We will score 'yes' if the reference test provided an automated result (for example, 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. We will score 'no' if the study stated that the reference standard result was interpreted with knowledge of the MTBDR*sl* assay result. We will score 'unclear' if it was not stated in the paper or if the authors failed to answer this question.

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

We judge applicability to be of 'low concern' for all studies unless studies used genetic sequencing only and did not look at known resistance determining regions outside of *gyrA* for FQ resistance and outside of *rrs* for SLID resistance, in which case we will answer 'unclear risk'.

Domain 4: Flow and timing

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

Signaling question 1: Was there an appropriate interval between the index test and reference standard?

We expect the reference standard test to be undertaken at the same time as the index test (ie each performed on a paired sample for the majority of studies). However, we expect some studies to include specimens from patients who have received a reference test on an earlier sample. The sample applies to some culture isolates, whose drug susceptibility profile might have been confirmed prior to the index test being available. We will answer 'yes' if the tests were paired or were separated by a few days. We will answer 'no' if reference and index tests were not done on paired samples and were separated by several months. As patients suspected of second-line drug resistance are often on some form of anti-TB therapy, it is possible that variation in the microbial population of specimens collected at different timepoints may occur. We will score 'unclear' if it was not stated in the paper or if the authors failed to answer this question.

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Signaling question 2: Did all patients receive the same reference standard?

We will answer 'yes' if the same reference standard was applied to all patients or a random sample of patients, 'no' if the reference standard was only applied to a selective group of patients and 'unclear' if it was not stated in the paper or if the authors failed to answer this question. We will answer 'no' when culture followed by genetic sequencing of the discrepant results was used as the reference standard because there is potential for verification bias when the same reference standard is not being used to confirm all index test results. Concerning genetic sequencing as the reference standard, the selective use of this method to resolve discordant results may be done because of the technical aspects, costs and time associated. For the reference standard 'genetic sequencing followed culture', we will answer 'no' for all studies.

Signaling 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 patients included in the two-by-two tables. We will note if the authors report the number of indeterminate assay results.

We will score '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 score 'no' if there were participants missing or excluded from the analysis and there was no explanation given. We will score 'unclear' if not enough information was given to assess whether participants were excluded from the analysis.

Appendix 5. Fluoroquinolone resistance, different reference standards

Figure 17 shows forest plots of MTBDR*sl* sensitivity and specificity for fluoroquinolone (FQ) resistance detection when performed indirectly using different reference standards. The individual studies are ordered by decreasing sensitivity. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Values between brackets are the 95% CIs of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).

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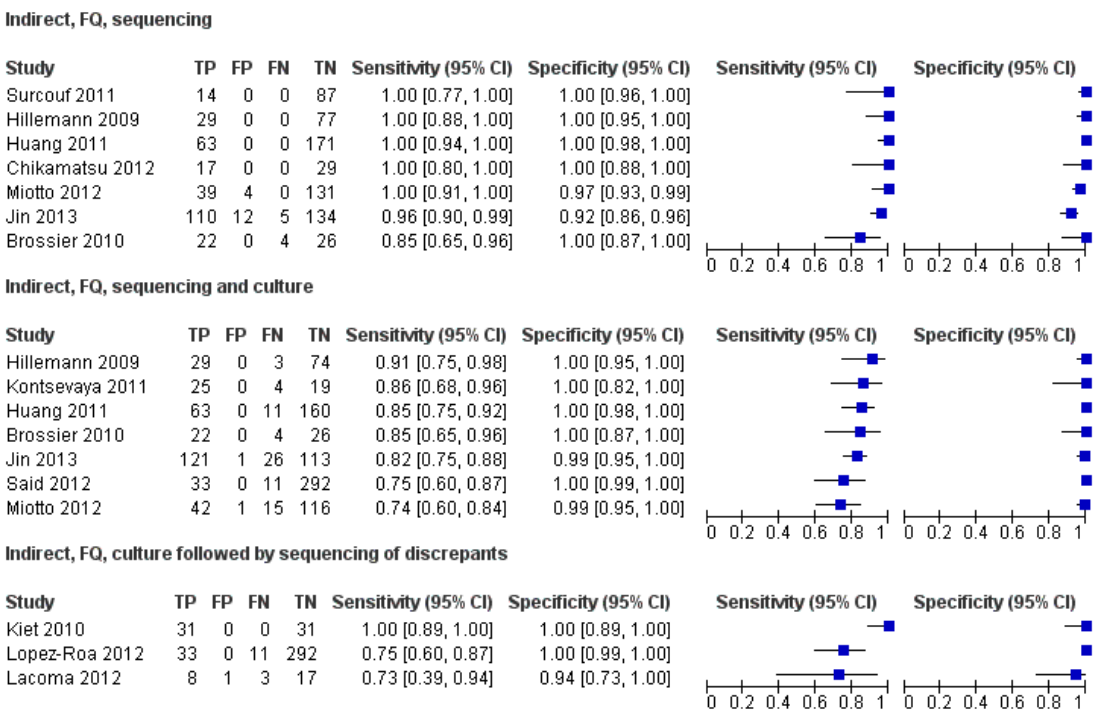
Figure 17. Forest plots of MTBDRsl sensitivity and specificity for fluoroquinolone (FQ) resistance detection when performed indirectly using different reference standards. The individual studies are ordered by decreasing sensitivity. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Values between brackets are the 95% CIs of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).



Appendix 6. Fluoroquinolone resistance, individual drugs, indirect testing

Figure 18 shows forest plots of MTBDR*sl* sensitivity and specificity for ofloxacin (Ofl) and moxifloxacin (Mx) resistance detection when performed indirectly and using phenotypic culture-based DST as a reference standard. The individual studies are ordered by decreasing sensitivity. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Values between brackets are the 95% CIs of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).

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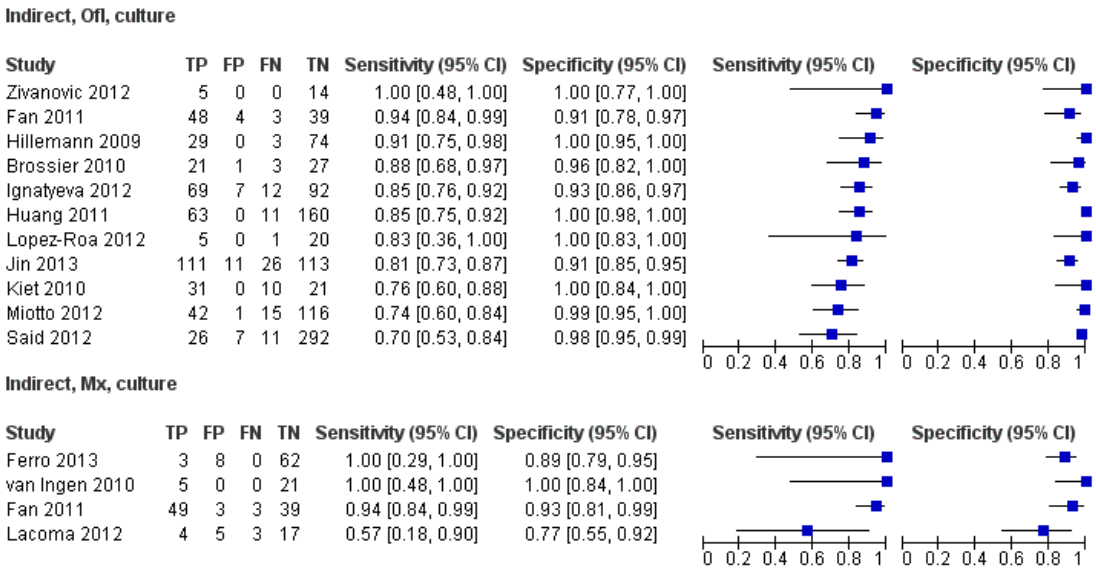
Figure 18. Forest plots of MTBDRsl sensitivity and specificity for ofloxacin (Ofl) and moxifloxacin (Mx) resistance detection when performed indirectly and using phenotypic culture-based DST as a reference standard. The individual studies are ordered by decreasing sensitivity. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Values between brackets are the 95% CIs of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).



Appendix 7. Summary plot, fluoroquinolone resistance, individual drugs

Figure 19 shows summary plots of MTBDR*sl* sensitivity and specificity comparing detection of resistance for ofloxacin (Ofl) and moxifloxacin (Mx). The solid circles correspond to the summary estimates of sensitivity and specificity and are shown with 95% confidence regions (dotted lines) and 95% prediction regions (dashed lines).

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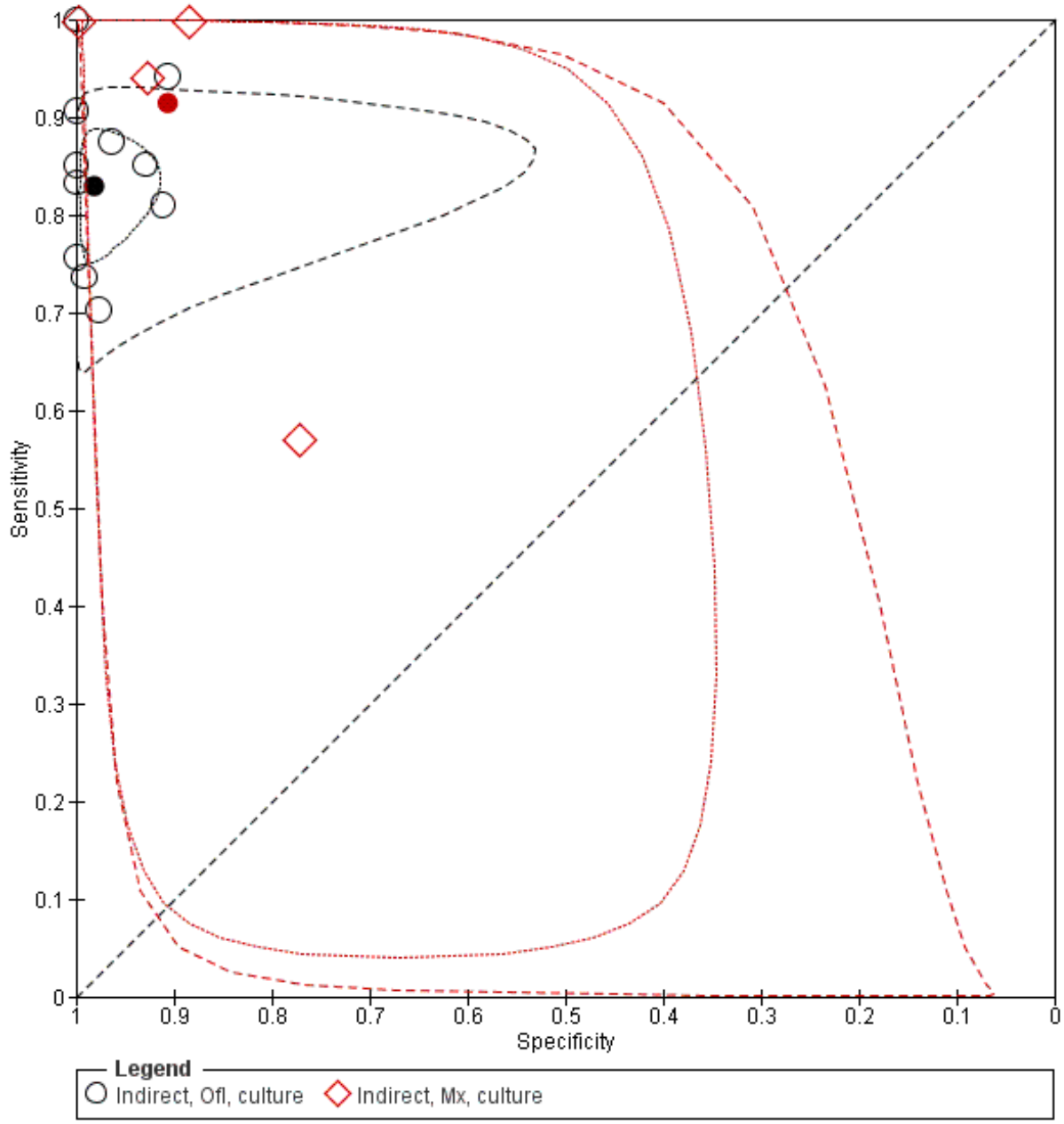
Figure 19. Summary plots of MTBDRsl sensitivity and specificity comparing direct detection of resistance for ofloxacin (Ofl) and moxifloxacin (Mx) using culture as a reference standard. The solid circles correspond to the summary estimates of sensitivity and specificity and are shown with 95% confidence regions (dotted lines) and 95% prediction regions (dashed lines).



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Appendix 8. MTBDR*sl* pooled accuracy estimates for the detection of resistance to second-line injectable drugs (when MTBDR*sl* testing was performed directly and indirectly) with Kontsevaya (2013) excluded and comparative testing using indirect statistical comparisons

Number of participants (studies)	Second-line injectable drug, culture, indirect		Second-line injectable drug, culture, direct		Sensitivity, P value[1]	Specificity, P value[1]
	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)		
2507 (19 studies)	76.9% (61.1 to 87.6)	99.5% (97.1 to 99.9)	98.0% (39.6 to 100.0)	97.8% (86.4 to 99.7)	0.202	0.003

Indirect comparisons for the purpose of determining pooled accuracy estimates are not to be confused with indirect testing that pertains to the method for testing with MTBDR*sl*..

[1]Likelihood ratio test for evidence of a significant difference between accuracy estimates.

Appendix 9. Second-line injectable drug resistance, different reference standards

Figure 20 shows forest plots of MTBDR*sl* sensitivity and specificity when performed indirectly for second-line injectable drug (SLID) resistance detection and using three different reference standards. The individual studies are ordered by decreasing sensitivity. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Values between brackets are the 95% CIs of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).

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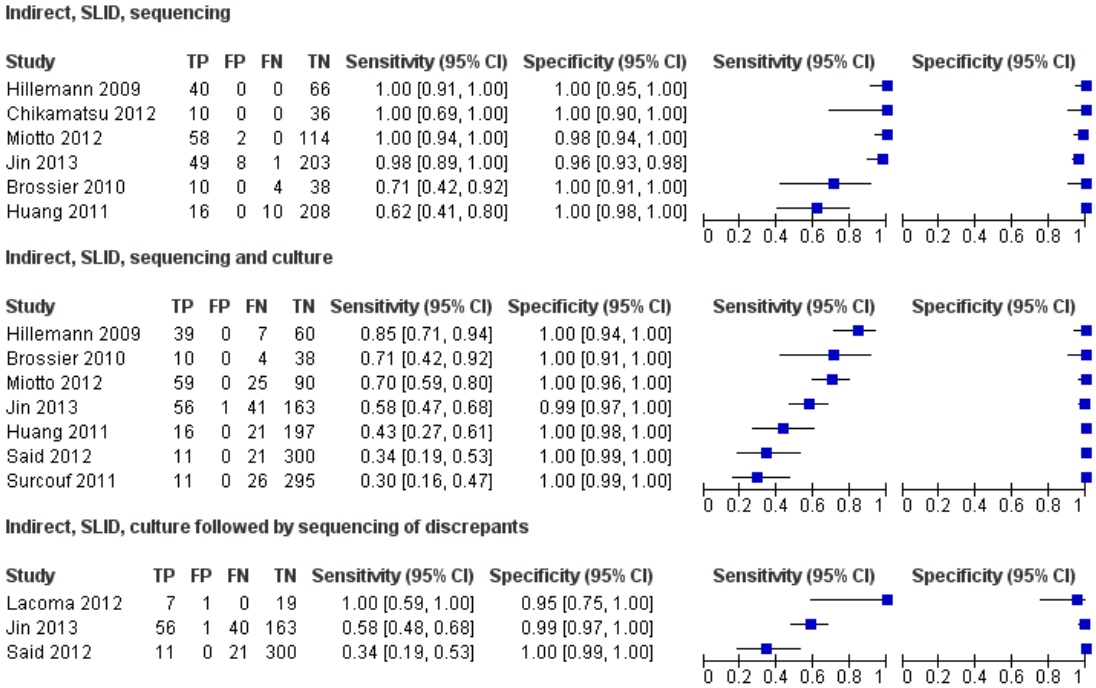
Figure 20. Forest plots of MTBDRsl sensitivity and specificity when performed indirectly for second-line injectable drug (SLID) resistance detection and using three different reference standards. The individual studies are ordered by decreasing sensitivity. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Values between brackets are the 95% CIs of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).



CONTRIBUTIONS OF AUTHORS

GT and KRS wrote the first draft of the protocol. KRS, KD and SD contributed methodological advice. MB, RW and JP gave advice on protocol content. GT and JP reviewed the studies and extracted the data. MR performed some statistical analyses. GT wrote the first draft of the review. All review authors contributed to the final manuscript.

DECLARATIONS OF INTEREST

KRS serves as Coordinator of the Evidence Synthesis and Policy Subgroup of Stop TB Partnership's New Diagnostics Working Group. The review authors have no financial involvement with any organization or entity with a financial interest in, or financial conflict with, the subject matter or materials discussed in the review apart from those disclosed.

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Grant award for support to MB

- Department for International Development (DFID), UK.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In the Cochrane Review, we added an additional reference standard defined as two reference tests used together: phenotypic culture-based DST and genetic sequencing of the same samples. We added the question, "Was a case-control design avoided?" to the sensitivity analyses. We stated in the protocol that we would perform sensitivity analyses for each target condition, using the subset of studies that provided one result per patient. However, these studies did not provide sufficient data for such analyses. After further consultation with technical experts, we changed how we assessed risk with regard to the Reference Standard and Flow and Timing Domains of QUADAS-2. Instead of answering 'unclear' to Signaling Question 1 of the Reference Standard Domain when culture was used, we decided to distinguish between studies that used culture with a WHO-recommended critical concentration in order to define resistance (answered as 'yes') and those which did not (answered as 'no') or which did not state a concentration (answered as 'unclear'). We therefore instead answered 'yes' if the recommended drug concentration was used. For the Applicability question of this domain, we answered 'unclear' if genetic sequencing was used in the reference standard and it did not examine genes (*gyrb* for the FQs and *eis* for the SLIDs) known to be associated with resistance. For Signalling Question 2 under the Flow and Timing Domain, we have now explicitly stated that we answered 'no' when culture followed by the genetic sequencing of discordant results was performed.

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