The GenoType® MTBDR*sl* test for resistance to second-line anti-tuberculosis drugs (Protocol)

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[Diagnostic Test Accuracy Protocol]

The GenoType® MTBDR*sl* test for resistance to second-line anti-tuberculosis drugs

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ABSTRACT

This is the protocol for a review and there is no abstract. The objectives are as follows:

To obtain summary estimates of the diagnostic accuracy of GenoType® MTBDR*sl* for detection of resistance to the fluoroquinolones in patient specimens or culture isolates confirmed as TB positive.

To obtain summary estimates of the diagnostic accuracy of GenoType® MTBDR*sl* for detection of resistance to second-line injectable drugs in patient specimens or culture isolates confirmed as TB positive.

To obtain summary estimates of the diagnostic accuracy of GenoType® MTBDR*sl* for detection of XDR-TB in patient specimens or culture isolates confirmed as TB positive.

Purpose of index test: GenoType® MTBDR*sl* used as an initial test replacing phenotypic culture-based DST as the initial test.

We plan to investigate heterogeneity in relation to the reference tests (genetic sequencing, culture-based DST, and culture-based DST followed by genetic sequencing), as well as by type of testing (indirect or direct). We also plan to investigate heterogeneity in relation to HIV status, conditions of the specimens (fresh or frozen, volume of specimen) and patient population (patients suspected of having MDR-TB or XDR-TB).

BACKGROUND

Tuberculosis (TB) is an infectious airborne disease caused by *Mycobacterium tuberculosis* 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 finding viable TB bacilli in fluid or tissue. The symptoms of active pulmonary TB include a persistent cough (for at least two weeks), fever, night sweats, weight loss, chills, haemoptysis, and fatigue. In 2011, an estimated 8.7

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million people developed TB and 1.4 million people died from TB. TB that is drug sensitive (also referred to as drug-susceptible TB) may be effectively treated with a standardized regimen of first-line anti-TB drugs, along with supervision and support (WHO 2012). However, TB bacilli may become drug resistant, meaning that first-line anti-TB drugs no longer kill TB bacilli. Drug-resistance usually develops because of inappropriate or incorrect use of first-line drugs.

The global emergence of drug-resistant TB (DR-TB) threatens to destabilise global TB control. In 2010, approximately 5% of the 8.8 million new cases of TB were drug resistant (WHO 2009; WHO 2012). Therapy for DR-TB requires treatment for more than 12 months, is toxic, and exceptionally expensive. In South Africa, treatment of approximately 6000 cases of DR-TB consumes approximately 60% of the country's annual TB drug budget). 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 fluoroquinolone drugs and second-line injectable drugs. Fluoroquinolone drugs include ofloxacin and moxifloxacin. Second-line injectable drugs 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 fluoroquinolone and at least one of the three injectable second-line drugs (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 XDR-TB in China (Zhao 2012). Modelling studies (Basu 2007; Dowdy 2008; Basu 2009) 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). Early treatment initiation may therefore help curtail the spread of DR-TB through the disruption of person-to-person transmission. There is thus an urgent need for rapid tests that allow for 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 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 tests, 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 commercial tests for drug resistance, which are frequently 'genotypic' in nature and detect the presence of specific mutations known to be associated with drug resistance, have offered considerable promise for the diagnosis of DR-TB.

One of the challenges in this review is the choice of the reference standard used to determine the presence or absence of the target conditions (described below). The best reference standard for testing for the presence of drug resistance is generally considered to be genetic sequencing. However, because of the technical aspects, costs, and time associated with this method, it is rarely feasible to perform sequencing on all samples suspected of DR-TB. The most widely used reference standard for drug resistance testing, phenotypic culture-based DST, is considered substantially imperfect, being likely to assign a false-positive result in situations where the index test result is positive and the reference standard result is negative. Recognizing that phenotypic DST is imperfect, researchers may perform genetic sequencing for selected samples where index test and phenotypic DST reference standard results do not agree (usually index test positive/phenotypic DST reference standard negative). In this scenario, selected TB samples (not all) with discordant results will receive a second reference standard test (namely genetic sequencing) to resolve the discordant results. This type of analysis is referred to as discrepant analysis. In the review, we plan to look at the different reference standards as a potential methodological source of heterogeneity.

Target condition being diagnosed

We will consider the following three target conditions: resistance of *M. tuberculosis* to fluoroquinolones; resistance of *M. tuberculosis* to second-line injectable drugs; and XDR-TB.

Index test(s)

The GenoType® MTBDR*sl* assay (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

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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 fluoroquinolones (including ofloxacin and levofloxacin), second-line injectable drugs (including kanamycin, amikacin, and capreomycin), and ethambutol, respectively. Since ethambutol is a first-line drug, we will not determine the accuracy of GenoType® MTBDR*sl* assay for ethambutol resistance in the review.

The assay can be performed either on a patient specimen (direct testing) or on a culture grown from the patient specimen (indirect testing). This is dependent on the quantity of TB in the patient specimen. The manufacturer recommends that if the specimen contains bacilli that can be seen using a light microscope and an acid-fast stain (smear-positive), the assay is performed directly on the specimen (Figure 1). 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 lastly 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 6.6 Middlebrook slant is inoculated prior to picking the colonies from the agar after incubation for a period of time.

Figure 1. Clinical pathway diagram showing how molecular drug susceptibility testing (DST), such as testing with 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), the 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 (e.g. the MTBDRplus assay) is performed first and, only if resistance to the first-line drugs is indicated, is tested further for resistance to the second-line drugs performed using the MTBDRsl assay. Where molecular testing is not available, phenotypic testing for drug resistance is typically performed on culture-positive isolates. This phenotypic testing is being replaced by molecular-based methods as indicated. However, as represented by the dashed lines, it is still usually performed in research studies seeking to measure the accuracy of the molecular test. Furthermore, some research studies also perform gene sequencing on any specimens with discordant results.

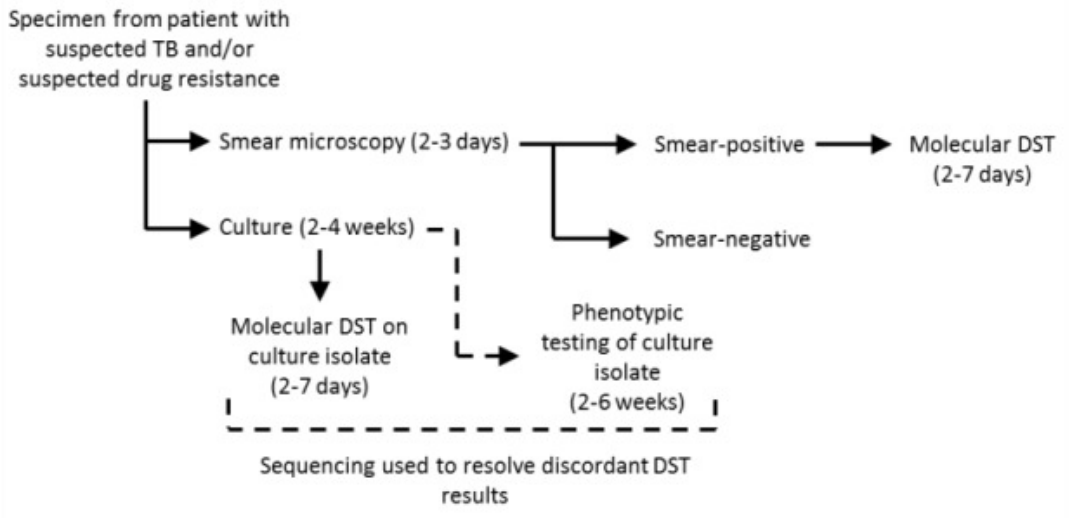

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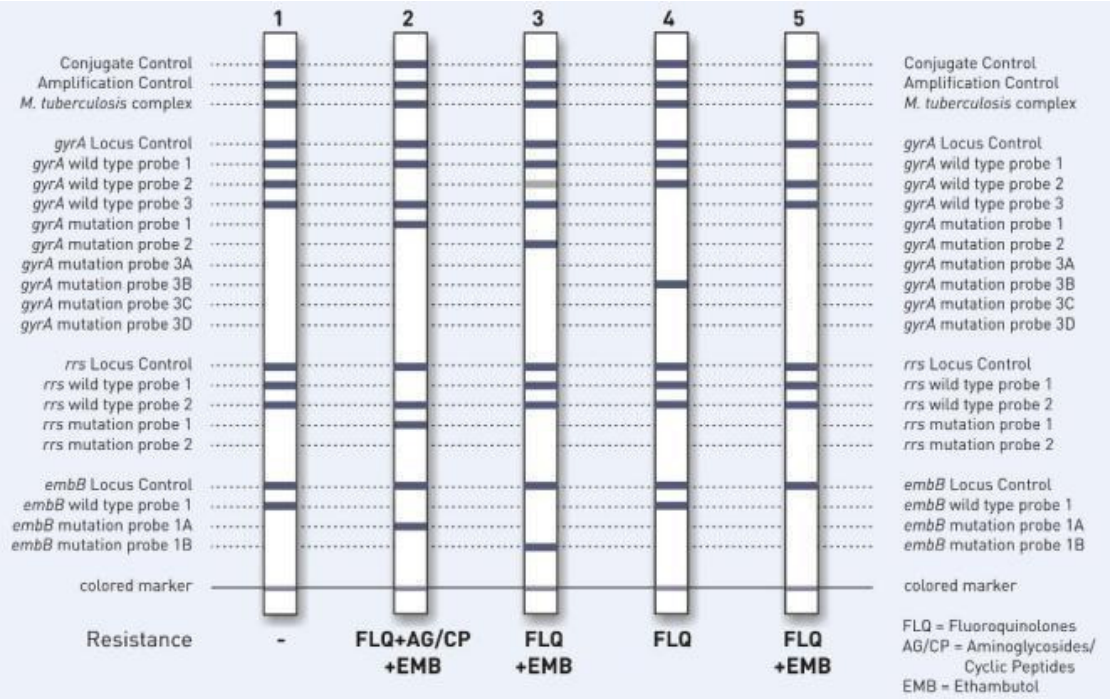
Figure 2. Examples of different GenoType® MTBDRsl strip readouts.



A template is supplied by the manufacturer to help read the strips (Figure 3) where the banding patterns are scored by eye, transcribed, and manually fed into the Laboratory Information System (LIS). In high-volume settings, an automated reader, the GenoScan®, can be incorporated to interpret the banding patterns automatically and give a suggested interpretation (an example output of the machine is shown in Figure 4). 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 GenoType® MTBDR*sl* can also be used to confirm a diagnosis of XDR-TB.

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Figure 3. An example of the manufacturer-supplied result template.

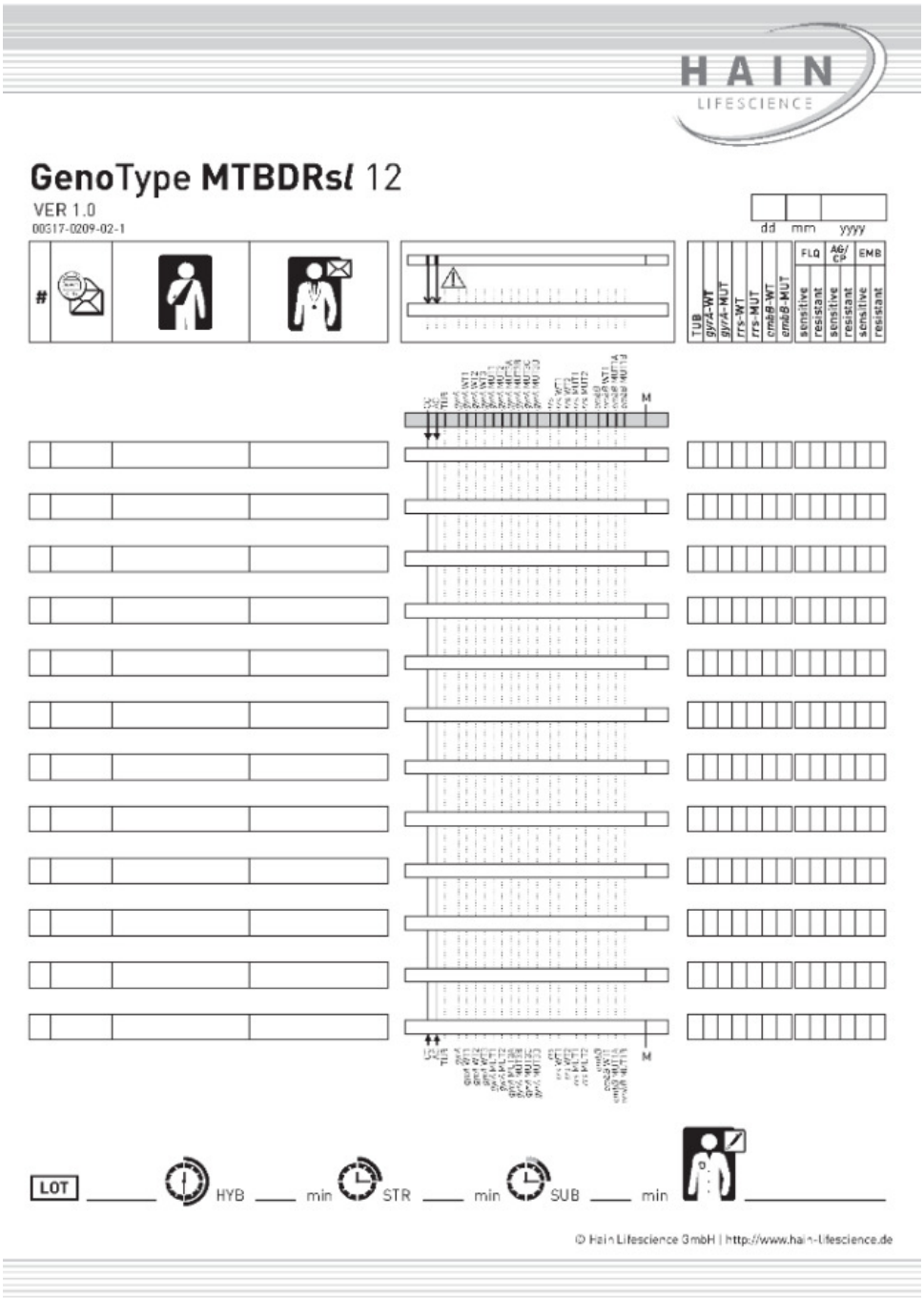

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Figure 4. Example of a readout from an automated strip reader. The results are generated automatically and validated manually by a technician.

An example of different GenoType® MTBDR*sl* results is shown in Figure 2. 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 and/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 (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

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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/AS kit fail to produce a positive identification for any of the 17 species covered by the GenoType® CM/AS kit. The manufacturer also recommends that if resistance to the fluoroquinolones or any of the second-line injectables is detected, but resistance to ethambutol is not, additional phenotypic testing should be performed in order to exclude ethambutol resistance.

Clinical pathway

The clinical pathway is shown in (Figure 1). 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 has been failing therapy). 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 expect research studies evaluating the accuracy of molecular DSTs, such as the MTBDR*sl* test, to almost always include phenotypic DST as a reference standard. Furthermore, we also expect some studies to use genetic sequencing to resolve any discordant index test-reference standard results.

Prior test(s)

As detailed in Figure 4 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)

Diagnosis of resistance to the fluoroquinolone drugs and the second-line injectable drugs, and the diagnosis of XDR-TB

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 fluoroquinolones alone; resistance to second-line injectable drugs alone; or resistance to both fluoroquinolones and second-line injectable drugs (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 is 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 GenoType® MTBDR*sl* assay, present a promising potential solution to this problem. To date, we are aware of approximately 15 studies examining the diagnostic accuracy of this assay for resistance to second-line drugs. Some of these studies have been performed by direct testing on patient specimens and other studies have been performed by indirect testing on pure culture isolates grown from patient specimens.

OBJECTIVES

To obtain summary estimates of the diagnostic accuracy of GenoType® MTBDR*sl* for detection of resistance to the fluoroquinolones in patient specimens or culture isolates confirmed as TB positive.

To obtain summary estimates of the diagnostic accuracy of GenoType® MTBDR*sl* for detection of resistance to second-line injectable drugs in patient specimens or culture isolates confirmed as TB positive.

To obtain summary estimates of the diagnostic accuracy of GenoType® MTBDR*sl* for detection of XDR-TB in patient specimens or culture isolates confirmed as TB positive.

Purpose of index test: GenoType® MTBDR*sl* used as an initial test replacing phenotypic culture-based DST as the initial test.

Secondary objectives

We plan to investigate heterogeneity in relation to the reference tests (genetic sequencing, culture-based DST, and culture-based DST followed by genetic sequencing), as well as by type of testing (indirect or direct). We also plan to investigate heterogeneity in relation to HIV status, conditions of the specimens (fresh or frozen, volume of specimen) and patient population (patients suspected of having MDR-TB or XDR-TB).

METHODS

Criteria for considering studies for this review

Types of studies

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We will include all studies that determine the diagnostic accuracy of the index test in comparison with a defined reference standard. Such studies are typically cross-sectional in nature. However, we will include all types of study designs, including case-control designs, in which cases and controls are sampled from the same patient population if we do not have sufficient cross-sectional studies. For multi-site studies that tested the same panel of TB isolates, we will select one site based on the experience of the laboratory technicians (some experience, but not extensive experience with the assay) and results that fell in the middle range (neither the best nor the worst results and represenative of the other sites). We will only include studies from which data can be extracted for true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN).

Participants

We will include patients/specimens of any age who are suspected of having resistance to any of the second-line TB drugs, as well as patients/specimens with confirmed MDR-TB, from all settings.

Index tests

We will include studies that evaluate the GenoType® MTBDR*sl* assay.

Target conditions

We will consider three target conditions:

1. Resistance to any of the fluoroquinolones. The fluoroquinolones include ofloxacin, levofloxacin, and moxifloxacin.
2. Resistance to any of the second-line injectable drugs. The second-line injectable drugs include two aminoglycosides, kanamycin and amikacin, and one cyclic peptide, capreomycin.
3. XDR-TB

For the fluoroquinolones, 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 fluoroquinolones: ofloxacin, levofloxacin, and moxifloxacin (Sirgel 2012b). The same holds true for the *rrs* gene and the two aminoglycosides, kanamycin and amikacin (Sirgel 2012a). In reference to capreomycin, the 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 second-line injectables drugs together, and thus including careomycin with the aminoglycosides, may be a limitation. However, the index test results are reported in this manner. We will discuss concerns about detection of capreomycin resistance in the review.

Reference standards

The following reference standards will be used to define the target conditions:

1. Genetic sequencing of the *gyrA* and *rrs* genes
2. 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.
3. 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. Phenotypic culture-based DST is the conventional reference standard, but it is considered to be less than 100% accurate in verifying the target conditions. Genetic sequencing is considered to be more accurate than phenotypic culture-based DST; however, genetic sequencing is usually applied only to culture isolates when results for index test/phenotypic culture-based DST do not agree. In this latter situation, there is potential for risk of bias (verification bias) because the same reference standard is not being used to verify all index test results. Another limitation of genetic sequencing is that this method may not target all of the resistance-determining regions in the TB genome.

We will carry out separate analyses for the three different reference standards. In our primary analysis we will use genetic sequencing as the reference standard, though we anticipate few studies will use this reference standard for all samples. We will investigate the potential contribution of the different reference standards as sources of heterogeneity by performing two secondary analyses using the following reference standards: phenotypic culture-based DST (we expect all or nearly all studies included in the review to report results using this reference standard) and two reference standards used sequentially, ie phenotypic culture-based DST followed by selective use of genetic sequencing for discordant results.

Search methods for identification of studies

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

Electronic searches

To identify all relevant studies, we will search the following databases using the search terms and strategy described in Appendix 1: Cochrane Infectious Diseases Group Specialized Register; MEDLINE; EMBASE; ISI Web of Knowledge; MEDION; LILACS; BIOSIS; and SCOPUS. We will also search the metaRegister of Controlled Trials (mRCT) and the search portal of the

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World Health Organization (WHO) International Clinical Trials Registry Platform (www.who.int/trialsearch), to identify ongoing trials. We will also search ProQuest Dissertations & Theses A&I and the National ETD Portal (South African theses and dissertations) to identify relevant dissertations.

Searching other resources

We will review reference lists of included articles and any relevant review articles identified through the above methods. We will contact the assay manufacturer (Hain Life Sciences) to identify unpublished studies. We will contact 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 independent review authors (GT and JP) will first look at titles and abstracts identified by electronic literature searching to identify potentially eligible studies. We will select all citations identified as suitable by the two review authors during this screen (screen 1) for full-text review. Two independent review authors (GT and JP) will then review full-text papers (screen 2) for study eligibility using the predefined inclusion and exclusion criteria. During screen 2, we will resolve any discrepancies by discussion between the two review authors (GT and JP), or if they are unable to resolve, by the decision of a third review author (KRS). We will maintain a list of excluded studies and their reasons for exclusion.

Data extraction and management

Two independent review authors (GT and JP) will extract a set of data from each study using a piloted data extraction form. Based on the pilot data extraction, the extraction form will be finalized. Two independent review authors (GT and JP) will then extract data on the following characteristics:

- Details of study: first author; publication year; case country of residence; World Bank country income status; 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 fluoroquinolones; resistance to second-line injectable drugs; XDR-TB.
- Reference standards: (name and manufacturer); type; percentage of patients whose reference standard was 'indeterminate' (contaminated, sequencing failed etc.).
- 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 true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN); number of indeterminate, missing, or unavailable 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 will contact authors of primary studies for missing data or clarifications. We will enter all data into a database manager. Whenever possible, we will extract data that use patient as the unit of analysis, ie one Genotype® MTBDR*sl* result per one specimen from one patient. However, some of the studies may provide data using 'specimen' as the unit of analysis, meaning in some situations one patient may have submitted more than one specimen. We will therefore, in sensitivity analyses for each target condition, compare pooled sensitivity and specificity for detection of drug resistance in all studies with pooled sensitivity and specificity in the subset of studies that provides one result per patient**.**

Assessment of methodological quality

We will appraise the quality of included studies with the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool (Whiting 2011). QUADAS-2 consists of four domains: patient selection, index test, reference standard, and flow and timing. We will assess all domains for the potential for risk of bias. In addition, we will assess the first three domains for concerns regarding applicability. We will used specific questions, called signalling questions, for each domain to form judgments about the risk of bias. One review author (GT) will pilot the tool with two of the included studies. We will finalize the tool based on experience we gain from the pilot testing. Two review authors will independently assess methodological quality of included studies with the finalized tool. We will not generate a summary "quality score" because of problems associated with such scores (Juni 1999; Whiting 2005). We have provided the domains of the QUADAS-2 tool and their interpretation in Appendix 2.

Statistical analysis and data synthesis

Firstly, we will provide a descriptive analysis of the results of the primary studies. We will provide results separately for each of the three target conditions. We will base the results of the index test

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on categorical assay results defined by the visual readout of the Genotype® MTBDR*sl* strip.

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

1. Sensitive to either fluoroquinolones, or second-line injectable drugs (referred to as 'aminoglycosides/cyclic peptides'), or both (conjugation and amplification bands present; TUB band present; gene locus band present; all *wt* bands for each gene present; no mutation bands present).
2. Resistant to either fluoroquinolones or second-line injectable drugs, 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 amplification control).
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).

We will consider results reported as 'indeterminate', 'no TB', or 'no result' (3, 4, and 5 above) to be indeterminate index test results.

Assignment of results to the fluoroquinolones and/or second-line injectable drugs category:

The GenoType® MTBDR*sl* assay detects the presence of mutations in genes that cause drug resistance to fluoroquinolones, or second-line injectable drugs, or both. It does not report whether there is resistance to individual drugs within these categories (ofloxacin and levofloxacin in the case of the fluoroquinolones; amikacin, kanamycin, and capreomycin in the case of second-line injectable drugs). 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 a reference standard to confirm second-line injectable drug resistance. In such a scenario, if the phenotypic DST and GenoType® MTBDR*sl* assay results were concordant and positive for resistance, we would classify this as second-line injectable drug resistance. We would adopt the same approach for the fluoroquinolones. Similarily, if the index tests reports resistance to a second-line injectable drug, and, in the case of genetic sequencing being used as a reference standard, the presence of mutations in the *rrs* gene is confirmed, we would record this as a concordant result positive for second-line injectable drugs.

We will perform descriptive analyses using Stata version 12.0 and will display key study characteristics in tables. For each study we will determine sensitivity and specificity of the assay along with the 95% confidence intervals (CIs) and generate forest plots using Review Manager (RevMan).

Where sufficient data are available, we will undertake meta-analyses to estimate the pooled sensitivity and specificity. We will use the following approach: firstly, we will group studies according to the target condition evaluated. Since the index test uses a common threshold for a positive result, we will use the bivariate random-effects regression model (Reitsma 2005; Macaskill 2010). Then, within each target condition, we will classify two groups according to the type of testing, either direct testing or indirect testing. For our primary analysis, we will use genetic sequencing as the reference standard. In our investigations of heterogeneity, we will explore the effect of using phenotypic culture-based DST as a reference standard and culture-based DST followed by genetic sequencing of discordant results. The following scheme demonstrates in part how we will present results:

I. Target condition: Resistance to fluoroquinolones

A. Direct testing

1. Reference standard is genetic sequencing
2. Reference standard is culture-based DST
3. Reference standard is culture-based DST followed by genetic sequencing

I. Target condition: Resistance to fluoroquinolones

B. Indirect testing

1. Reference standard is genetic sequencing
2. Reference standard is culture-based DST
3. Reference standard is culture-based DST followed by genetic sequencing

II. Target condition: resistance to second-line injectable drugs

A. Direct testing

1. Reference standard is genetic sequencing

Sequence repeats as in I.A.

Investigations of heterogeneity

For each target condition, we will first investigate heterogeneity through visual examination of forest plots of sensitivity and specificity. Then, if sufficient studies are available, we will explore the possible influence of the reference tests (genetic sequencing, culture-based DST, or culture-based DST followed by genetic sequencing) by performing meta-analyses separately within subgroups defined by these tests. We expect several studies to report TP, FP, FN, and TN values stratified by HIV status. Therefore, we will fit the meta-analysis model separately within HIV-positive and HIV-negative subgroups to examine the effect of this covariate on the pooled sensitivity and specificity. For all subgroup analyses, we will estimate test accuracy separately by type of testing, either indirect or direct. In addition, if sufficient data are available, we plan to investigate the effect of the condition of the specimen (fresh or frozen), sample volume, and patient population (patients suspected of having either MDR-TB or XDR-TB) on summary estimates of sensitivity and specificity by adding each factor as a covariate to the bivariate model. All covariates will be at study level and dichotomous and are as follows:

- Condition of specimen: fresh; frozen.

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- Volume used for culture isolates: greater than or equal to 1 mL; less than 1 mL.
- Volume used for decontaminated sediment: greater than or equal to 500 µL; less than 500 µL.
- Patient population: patients having MDR-TB; patients suspected of having XDR-TB.

Sensitivity analyses

We will perform sensitivity analyses for three QUADAS-2 signalling questions to explore whether the results we found are robust with respect to the methodological quality of the studies. We will pose the following questions:

- Was a consecutive or random sample of patients/specimens enrolled?
- Were the reference standard results interpreted without knowledge of the results of the index test?
- Did all patients receive the same reference standard?

Assessment of reporting bias

We will 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 (Tatsioni 2005; Macaskill 2010).

Other analyses

We will summarize, if feasible, evidence on other outcomes, including time-to-diagnosis and time-to-treatment initiation. We will also summarize hands-on time for specimen processing and work-flow (including the option of using the same extracted DNA for both first-line and second-line probe assays), instrument ease-of-use, and user satisfaction. We will address these outcomes in a section of the discussion and we will present summary data in additional tables.

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* *Indicates the major publication for the study*

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APPENDICES

Appendix 1. MEDLINE search strategy

1. MTBDR*.tw .
2. Genotype MTBDR*.tw.
3. or/1-2
4. exp Tuberculosis, Pulmonary/
5. exp Tuberculosis, Multidrug-Resistant/
6. MDR-TB.tw
7. XDR-TB.tw
8. Mycobacterium tuberculosis/
9. TB.tw.
10. tuberculosis.tw.
11. or/4-10
12. 3 and 11
13. Limit 12 to ….2003-current

This is the preliminary search strategy for MEDLINE. It will be adapted for other electronic databases. All search strategies will be reported in full in the published review.

Appendix 2. QUADAS-2 rules and interpretation

We use "patients" below with the understanding that studies in this 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?

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.

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 Genotype® 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?

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Genetic sequencing (gene sequencing of loci known to be associated with drug resistance) is considered the best available reference standard. We will answer 'yes' when this reference standard is used. Phenotypic culture-based drug susceptibility testing is not 100% accurate for detection of drug resistance, in particular with respect to detection of second-line drug resistance. We will answer 'unclear' when this reference standard is used. Two reference standards used sequentially refers to culture-based drug susceptibility testing followed by genetic sequencing. We will answer 'yes' when this reference standard is used because genetic sequencing will be the arbiter of the final results.

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 (e.g. MGIT 960 drug susceptibility testing), 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.

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 this question 'yes' if the tests were paired or were separated by a few days. We will answer this question '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.

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.

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.

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. All authors edited the protocol and approved the final draft of the protocol.

DECLARATIONS OF INTEREST

KRS serves as Co-ordinator of the Evidence Synthesis and Policy Subgroup of Stop TB Partnership's New Diagnostics Working Group. The 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 protocol apart from those disclosed.

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