The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin

THE END TB STRATEGY

World Health Organization

The use of molecular line probe assays
for the detection of resistance
to isoniazid and rifampicin

Policy update

World Health
Organization

WHO Library Cataloguing-in-Publication Data

The use of molecular line probe assay for the detection of resistance to isoniazid and rifampicin: policy update.
Contents: web annex 1: GRADE summary of findings tables – web annex 2: Evidence to recommendations tables – web annex 3: references to studies included and excluded from the review
1. Tuberculosis, Multidrug-resistant. 2. Antitubercular Agents. 3. Rifampin - pharmacology. 4. Isoniazid – pharmacology. 5. Drug Resistance. 6. Molecular Probes. 7. Guideline.

I. World Health Organization.

ISBN 978 92 4 151126 1 (NLM classification: WF 310)
Subject headings are available from WHO institutional repository

Designed by GPS Publishing

Printed in Spain

WHO/HTM/TB/2016.12

Contents

Acknowledgements

This document was prepared by Christopher Gilpin and Alexei Korobitsyn with input from Karin Weyer (all at WHO's Global TB Programme) on the basis of consensus agreed at a Guideline Development Group meeting convened by WHO in Montreux, Switzerland, on 2 March 2016.

WHO Steering Group

Christopher Gilpin, Karin Weyer, Alexei Korobitsyn, Wayne van Gemert and Fuad Mirzayev (all at WHO's Global TB Programme)

Chair of the WHO Guideline Development Group

Holger Schünemann (McMaster University, Canada)

GRADE methodologist of the WHO Guideline Development Group

Holger Schünemann (McMaster University, Canada)

Members of the WHO Guideline Development Group

Gavin Churchyard (The Aurum Institute, South Africa), Daniela Maria Cirillo (HSR San Raffaele Scientific Institute/TB Supranational Reference Laboratory, Italy), Chris Coulter (Queensland Mycobacterium Reference Laboratory, Australia), Greg Fox (University of Sydney, Australia), Moses Joloba (National Reference Laboratory of the National TB and Leprosy Programme, Uganda), James Posey (Centers for Disease Control and Prevention, United States of America), Michael Rich (Partners in Health, United States of America), Leen Rigouts (Institute of Tropical Medicine, Belgium), Thomas M. Shinnick (independent consultant, United States of America), Rebecca Tadokera (Human Sciences Research Council, South Africa) (unable to attend), Marie Alice Telles (independent tuberculosis laboratory consultant for the Pan American Health Organization, Brazil), Francis Varaine (Médecins Sans Frontières, France)

Systematic review authors

Ruvandhi R. Nathavitharana (Beth Israel Deaconess Medical Center, United States), Patrick Cudahy (Yale Medical Center, United States), Samuel G. Schumacher (FIND [Foundation for Innovative New Diagnostics], Switzerland), Karen Steingart (Liverpool School of Tropical Medicine, England), Madhukar Pai (McGill University, Canada), Claudia M. Denkinger (FIND, Switzerland)

Observers

Sevim Ahmedov (United States Agency for International Development, United States), Emmanuelle Cambau (Groupe Hospitalier Lariboisière-Fernand Widal, France), David Dolinger (FIND), Miranda Langendam (University of Amsterdam, The Netherlands), Thomas Schön (Kalmar County Hospital and Linköping University, Sweden), Belay Tessema (FIND, Switzerland)

External Review Group

Heather Alexander (Centers for Disease Control and Prevention, United States), Martina Casenghi (Médecins Sans Frontières Access Campaign, Switzerland), Kathleen England (KNCV Tuberculosis Foundation, The Netherlands), Rumina Hasan (TB Supranational Reference Laboratory Pakistan, Aga Khan University, Pakistan), Nazir Ismail (TB Supranational Reference Laboratory, National Institute of Communicable Diseases, South Africa), Beatrice Lopez (Buenos Aires TB Supranational Reference Laboratory, Argentina), Richard Lumb (Adelaide TB Supranational Reference Laboratory, Australia), Satoshi Mitarai (Japan Anti-Tuberculosis Association, Japan), Alaine Umubyeyi Nyaruhirira (Management Sciences for Health, South Africa), Rohit Sarin (LRS Institute of TB and Respiratory Diseases, India), Alena Shrahina (National TB Programme, Belarus),

Acknowledgement of financial support

Funding from the Bill and Melinda Gates Foundation as well as the United States Agency for International Development through United States Agency for International Development—WHO Consolidated Grant No. GHA-G-00-09-00003/US 2014-741 is gratefully acknowledged.

Declaration and management of conflicts of interest

All contributors completed a WHO Declaration of Interests form. All declarations were evaluated by members of the Steering Group to determine whether a possible financial conflict of interest might warrant exclusion from membership of the Guideline Development Group or the External Review Group, or from the discussion part of the guideline development process. Contributors with intellectual conflicts of interest were not excluded from membership of the Guideline Development Group, as broader expertise on drug-susceptibility testing was considered to be part of the criteria for selection. In addition, the diversity and representation in the groups was large enough to balance and overcome any potential intellectual conflicts of interest. During the guideline development process and the meeting, the emergence of intellectual conflicts of interest was monitored by the Chair and any perceived intellectual conflict of interest was discussed with members of the Guideline Development Group.

The following interests were declared.

None declared

Holger Schünemann (Chair), Moses Joloba, Miranda Langendam, James Posey, Thomas Schön, Karen Steingart, Belay Tessema, Grant Theron, Francis Varaine, Ruvandhi R. Nathavitharana, Patrick Cudahy, Samuel Schumacher, Karen Steingart, Claudia M. Denkinger, Belay Tessema declared no conflicts of interest.

Declared, determined to be insignificant

Sevim Ahmedov declared that his participation in the meeting was covered by the United States Agency for International Development.

Emmanuelle Cambau was reimbursed by ESCMID for participating in the European Congress on Clinical Microbiology and Infectious Diseases from 2012 to 2015.

Gavin Churchyard received a research grant to evaluate the national roll-out of the GeneXpert MTB/RIF assay in South Africa (the Bill and Melinda Gates Foundation provided US$ 11 million to the Aurum Institute, South Africa).

Daniela Maria Cirillo declared that she had received research grants from FIND and the Italian government (€17 000) to evaluate a new TB test.

Chris Coulter declared taking part in short-term consultancies for WHO (less than 5 000 Australian dollars), receiving a research grant to study TB transmission in Australia, taking part in a whole-genome sequencing study (National Health and Medical Research Council research collaboration grant of less than 18 000 Australian dollars), and providing laboratory support services to Papua New Guinea (240 000 Australian dollars for funding provided by the Australian government to the TB Supranational Reference Laboratory).

David Dolinger is employed by a commercial entity and receives US$ 190 000 per year; he is also working with FIND to assess new TB diagnostics.

Gregory Fox received the Otsuka/Union Young Innovators' Award at the 2015 Union World Conference on Lung Health (US$ 10 000 in airfare, accommodation and per diem for the meeting).

Michael Rich is employed by Partners in Health to work on clinical care guidelines and on the programmatic management of multidrug-resistant tuberculosis; he has also consulted on behalf of WHO; he is conducting research on bedaquiline and delamanid as a recipient of UNITAID's endTB grant.

Leen Rigouts has been involved in evaluating Nipro line probe assays for pyrazinamide and second-line agents.

Thomas M. Shinnick declared that he was a former employee of the United States Centers for Disease Control and Prevention. The Centers for Disease Control and Prevention has supported his travel and research related to his work on the laboratory services needed to control tuberculosis. He declared that he had represented the centers' positions on the laboratory services needed for tuberculosis diagnosis, treatment and control, and served on the Data and Safety Monitoring Board organized by Otsuka Pharmaceutical for the clinical trial of delamanid. He declared that no remuneration had been received.

Maria Alice Telles declared that she had worked for FIND as a consultant providing training on the GeneXpert MTB/RIF assay (US$ 4 000) and participated in a meeting on the BACTEC mycobacterial growth indicator tube (known as MGIT; Becton Dickinson, Franklin Lakes, NJ, United States), with Beckton Dickinson funding travel and per diem expenses.

Declared, determined to be significant

None

Date of review: 2020 or earlier if significant additional evidence becomes available.

Abbreviations

CI	confidence interval
DST	drug-susceptibility testing
FIND	Foundation for Innovative New Diagnostics
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GDG	Guideline Development Group
HIV	human immunodeficiency virus
MDR-TB	multidrug-resistant tuberculosis
MGIT	mycobacterial growth indicator tube
MIC	minimum inhibitory concentration
MTBC	*Mycobacterium tuberculosis* complex
PICO	Population, Intervention, Comparator, Outcome
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
TB	tuberculosis
WHO	World Health Organization

Executive summary

Background

Drug-resistant tuberculosis (TB) continues to threaten global TB control and remains a major public health concern in many countries. Globally, an estimated 3.3% of new cases and 20% of previously treated cases have multidrug-resistant TB (MDR-TB). In 2014, there were an estimated 480 000 new cases of MDR-TB and approximately 190 000 deaths from MDR-TB. The World Health Organization's (WHO's) End TB Strategy calls for the early diagnosis of TB and universal drug-susceptibility testing (DST), highlighting the critical role of laboratories in the post-2015 era in rapidly and accurately detecting TB and drug resistance.[1]

Molecular methods based on nucleic acid amplification have considerable advantages for the scale-up of programmatic management and the surveillance of drug-resistant TB, offering quicker diagnosis, standardized testing and the potential for high throughput. Molecular tests for detecting drug resistance to rifampicin alone or in combination with resistance to isoniazid have been recommended for use by WHO since 2008. These tests include the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, United States) and commercial line probe assays (LPAs), such as the GenoType MTBDR*plus assay* (Hain Lifescience, Nehren, Germany), which detect the presence of mutations associated with drug resistance to rifampicin.

In 2008, WHO approved the use of commercial LPAs for detecting *Mycobacterium tuberculosis* complex (MTBC) and rifampicin resistance in sputum smear-positive specimens (direct testing) and in cultured isolates of MTBC (indirect testing). A systematic review at that time, evaluating the diagnostic accuracy of two commercially available LPAs – the INNO-LiPA Rif.TB assay (Innogenetics, Ghent, Belgium) and the GenoType MTBDR*plus* (version 1) (subsequently referred to as Hain version 1) – provided evidence for WHO's endorsement.[2, 3]

Although excellent accuracy was reported for both tests in detecting rifampicin resistance, their diagnostic accuracy for isoniazid resistance had lower sensitivity, despite excellent specificity. Because there were inadequate data to allow stratification by smear status, WHO's recommendation for using LPAs was limited to culture isolates or smear-positive sputum specimens. Further data have since been published on the use of LPAs; newer versions of LPA technology have since been developed, such as the Hain GenoType MTBDR*plus* version 2 (subsequently referred to as Hain version 2); and other manufacturers have entered the market, including Nipro (Tokyo, Japan), which developed the Nipro NTM+MDRTB detection kit 2 (subsequently referred to as Nipro).

In 2015, FIND (the Foundation for Innovative New Diagnostics) evaluated the Nipro and the Hain version 2 LPAs and compared them with Hain version 1. The study demonstrated equivalence among the three commercially available LPAs for detecting TB and resistance to rifampicin and isoniazid.[4]

---

1 Global tuberculosis report 2015. Geneva: World Health Organization; 2015 (WHO/HTM/TB/2015.22; http://www.who.int/tb/publications/global_report/gtbr15_main_text.pdf, accessed 4 August 2016).

2 Ling DI, Zwerling AA, Pai M. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. Eur Respir J. 2008;32:1165–74.

3 Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB): policy statement. Geneva: World Health Organization; 2008 (http://www.who.int/tb/features_archive/policy_statement.pdf, accessed 4 August 2016).

4 Report for WHO: non-inferiority evaluation of Nipro NTM+MDRTB and Hain GenoType MTBDRplus V2 line probe assays. Geneva: FIND; 2015 (http://www.finddx.org/wp-content/uploads/2016/04/LPA-report_noninferiority-study_oct2015.pdf, accessed 4 September 2016).

Objectives, rationale and methods used to develop the guidance

This document updates existing WHO policy on the use of molecular LPAs for detecting MTBC and resistance to isoniazid and rifampicin directly from sputum specimens and by the indirect testing of MTBC culture isolates.

The objectives of this policy guidance are to:

- evaluate the diagnostic accuracy of molecular LPAs for detecting MTBC and rifampicin resistance directly from smear-positive sputum specimens and indirectly from isolates of MTBC;
- evaluate the diagnostic accuracy of molecular LPAs for detecting MTBC and isoniazid resistance directly from smear-positive sputum specimens and indirectly from isolates of MTBC.

WHO's policy recommendations developed from the evidence synthesis process by the Guideline Development Group are summarized below.

WHO's policy recommendations

For persons with a sputum smear-positive specimen or a cultured isolate of MTBC, commercial molecular LPAs may be used as the initial test instead of phenotypic culture-based DST to detect resistance to rifampicin and isoniazid (conditional recommendation, moderate certainty in the evidence for the test's accuracy).

Remarks

a. These recommendations apply to the use of LPAs for testing sputum smear-positive specimens (direct testing) and cultured isolates of MTBC (indirect testing) from both pulmonary and extrapulmonary sites.

b. LPAs are not recommended for the direct testing of sputum smear-negative specimens.

c. These recommendations apply to the detection of MTBC and the diagnosis of MDR-TB but acknowledge that the accuracy of detecting resistance to rifampicin and isoniazid differs and, hence, the accuracy of a diagnosis of MDR-TB is reduced overall.

d. These recommendations do not eliminate the need for conventional culture-based DST, which will be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance.

e. Conventional culture-based DST for isoniazid may still be used to evaluate patients when the LPA result does not detect isoniazid resistance. This is particularly important for populations with a high pre-test probability of resistance to isoniazid.

f. These recommendations apply to the use of LPA in children based on the generalization of data from adults.

1. Background

Tuberculosis (TB) remains a large-scale public health problem. Key global priorities for TB care and control include improving case-detection and detecting cases earlier, including cases of smear-negative disease. In 2014, only 63% (6 million) of an estimated 9.6 million people who developed TB were reported to the World Health Organization (WHO), meaning that globally 37% of the estimated cases of TB are undetected. WHO has identified the development and evaluation of new diagnostic tools as an essential part of future TB control efforts.[1]

Conventional methods for mycobacteriological culture and drug-susceptibility testing (DST) are slow and cumbersome, requiring sequential procedures for isolating mycobacteria from clinical specimens, identifying *Mycobacterium tuberculosis* complex (MTBC), and performing in vitro testing of strain susceptibility to anti-TB agents. During this time, patients may be inappropriately treated, drug-resistant strains may continue to spread, and resistance may be amplified. Compared with culture-based DST, genotypic (molecular) methods, such as line probe assays (LPAs), offer quicker diagnosis, a standardized and safer procedure when performed directly on sputum specimens, the potential for high throughput, and they are suitable for supporting the programmatic management and surveillance of drug-resistant TB.

The GenoType MTBDR*plus* LPA (Hain Lifescience, Nehren, Germany), subsequently referred to as Hain version 1, was the first commercial LPA recommended for use by WHO in 2008.[2] It remains the most widely studied LPA. Further data have been published on the use of LPAs, and newer versions of LPA technology have since been developed including (1) the Hain GenoType MTBDR*plus* version 2 (subsequently referred to as Hain version 2) and (2) the Nipro NTM+MDRTB detection kit 2 (Nipro, Tokyo, Japan), subsequently referred to as Nipro. These newer LPAs aim to improve the sensitivity of MTBC detection and to simultaneously detect resistance to rifampicin and isoniazid.

In 2015, FIND (the Foundation for Innovative New Diagnostics) evaluated the Nipro and the Hain version 2 LPAs and compared them with Hain version 1. The study demonstrated equivalence among the three commercially available LPAs for detecting TB and resistance to rifampicin and isoniazid.[3]

Also in 2015, WHO commissioned an updated systematic review of the accuracy of commercial LPAs for detecting MTBC and resistance to rifampicin and isoniazid. A total of 74 studies were identified, comprising 94 unique datasets (see Annex 1). Of these, 83 datasets evaluated Hain version 1, 5 evaluated Hain version 2, and 6 evaluated the Nipro assay.

In accordance with WHO's standards for assessing evidence when formulating policy recommendations, the GRADE approach (Grading of Recommendations Assessment, Development and Evaluation, see http://www.gradeworkinggroup.org/) was used. GRADE provides a structured framework for evaluating the accuracy of diagnostic tests and their impact on patients and public health. The systematic review assessed the accuracy of the Hain version 1, Hain version 2 and Nipro assays in the direct

[1] Global tuberculosis report 2015. Geneva: World Health Organization; 2015 (WHO/HTM/TB/2015.22; http://www.who.int/tb/publications/global_report/gtbr15_main_text.pdf, accessed 15 April 2016).

[2] Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB): policy statement. Geneva: World Health Organization; 2008 (http://www.who.int/tb/features_archive/policy_statement.pdf, accessed 15 April 2016).

[3] Report for WHO: non-inferiority evaluation of Nipro NTM+MDRTB and Hain GenoType MTBDRplus V2 line probe assays. Geneva: FIND; 2015 (http://www.finddx.org/wp-content/uploads/2016/04/LPA-report_noninferiority-study_oct2015.pdf, accessed 15 April 2016).

testing of sputum samples from patients with signs and symptoms of TB, as well as in the indirect testing of cultures of MTBC.

The evidence reviewed and this policy guidance apply to the use of only these commercial assays. Other assays for detecting MTBC and resistance to rifampicin and isoniazid were not evaluated. Any new or generic assay intended to detect the presence of MTBC and mutations associated with drug resistance to rifampicin and isoniazid should be adequately evaluated and validated in the settings where it is intended to be used, as per WHO's policy.[4]

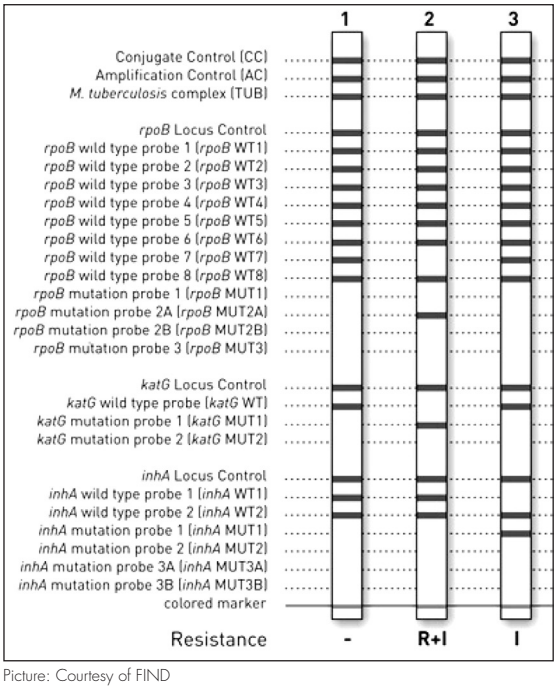
1.1 Index tests

The Hain version 1 and version 2 assays include *rpo*B probes to detect rifampicin resistance, *kat*G probes to detect mutations associated with high-level isoniazid resistance, and *inh*A probes to detect mutations usually associated with low-level isoniazid resistance. The probes used to detect wild-type and specific mutations are the same for both versions of the Hain LPA (Fig. 1a). The Nipro assay underwent Japanese registration in 2012 and allows for the identification of MTBC and resistance to rifampicin and isoniazid. The Nipro assay also differentiates *M. avium*, *M. intracellulare* and *M. kansasii* from other non-tuberculous mycobacteria (Fig. 1b).

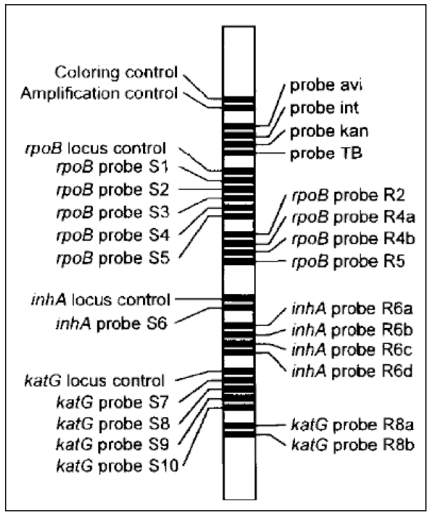
The *rpo*B, *kat*G and *inh*A mutation probes are the same for the three assays with the exception of the *kat*G S315N mutation, which is included in the Nipro assay but not in Hain version 1 or version 2. There are some minor variations in the codon regions covered for the wild type among Hain version 1 and version 2 and the Nipro.

Figure 1. Examples of different line probe assay strip readouts: (a) Hain GenoType MTBDR*plus* version 1 and version 2 (Hain Lifescience, Nehren, Germany) and (b) Nipro NTM+MDRTB Detection Kit 2 (Nipro, Tokyo, Japan)

(a)



(b)



Picture: Courtesy of FIND

[4] Implementing tuberculosis diagnostics: policy framework. Geneva: World Health Organization; 2015 (WHO/HTM/TB/2015.11; http://apps.who.int/iris/bitstream/10665/162712/1/9789241508612_eng.pdf, accessed 18 April 2016).

2. Methods

2.1 Evidence synthesis

Following the 2015 systematic review, WHO's Global TB Programme convened a Guideline Development Group (GDG) in March 2016 to assess the data and update the 2008 policy recommendations on using commercial LPAs to detect MTBC and resistance to isoniazid and rifampicin. The evaluation used the GRADE system to determine the quality of the evidence and provide information on the strength of the recommendations using PICO questions agreed by the GDG. PICO refers to the following four elements that should be included in questions that govern a systematic search of the evidence: the Population targeted by the action or intervention (in the case of systematic reviews of the accuracy of a diagnostic test, P is the population of interest); the Intervention (I is the index test); the Comparator (C is the comparator test or tests); and the Outcome (O is usually sensitivity and specificity). The PICO questions for the review are given below.

Overarching question

Should LPA results be used to guide clinical decisions to use rifampicin and isoniazid in patients diagnosed with TB?

PICO questions addressed by the Guideline Development Group

1. Should LPAs be used to guide clinical decisions to use rifampicin in the direct testing of specimens and the indirect testing of culture isolates from patients with signs and symptoms consistent with TB?
2. Should LPAs be used to guide clinical decisions to use isoniazid in the direct testing of specimens and the indirect testing of culture isolates from patients with signs and symptoms consistent with TB?
3. Should LPAs be used to diagnose multidrug-resistant TB (MDR-TB) in patients with signs and symptoms consistent with TB?
4. Should LPAs be used to diagnose TB in patients with signs and symptoms consistent with TB but for whom sputum-smear results are negative?

A comprehensive search was performed of the following databases for relevant citations: PubMed, Embase, BIOSIS, Web of Science, LILACS and the Cochrane Collaboration. The search was restricted from January 2004 to August 2015 because the first-generation Hain assay was introduced in October 2004. In addition, laboratory experts and the tests' manufacturers (Hain Lifescience and Nipro) were contacted for lists of additional published studies. Reference lists from included studies were also searched.

No language restriction was applied but at the full-text review stage studies were restricted to English, French and Spanish. Abstracts or conference proceedings were not included in the review as these usually do not include methodological details, and data are often subject to change.

In an effort to maximize the data, all studies that determined the diagnostic accuracy of the index test in comparison with a defined reference standard were included along with studies that used case–control designs. Included studies were those from which data could be extracted for true positives, false positives, false negatives and true negatives to calculate sensitivity and specificity estimates and 95% confidence intervals (CIs) for individual studies. The results from individual studies were graphed by plotting the estimates of sensitivity and specificity (and their 95% CIs) in forest plots.

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

- The reference standard for the detection of MTBC was a positive culture using either solid or liquid media.

- The reference standard for detecting rifampicin and isoniazid resistance was phenotypic culture-based DST using either solid or liquid culture and incorporating the anti-TB agent of interest.
- A composite reference standard included both culture-based phenotypic DST and sequencing of the same specimens. Results were classified as follows:
  - if a specimen was resistant according to culture-based DST or had a mutation conferring resistance that was associated with a particular anti-TB agent, the specimen was classified as being resistant to a particular drug;
  - if both culture-based DST and sequencing indicated susceptibility, the specimen was classified as being susceptible to a particular agent;
  - if results were discrepant between culture-based DST and sequencing, the final determination was based on whether the sequencing mutations detected were thought to be clinically significant (that is, associated with resistance) using the TB Drug Resistance Mutation (TB DReaM) database and the relational sequencing TB data platform (ReSeq TB) as references;
  - if conventional DST showed susceptibility but sequencing identified mutations recognized to be associated with resistance, the composite reference standard was considered resistant;
  - if conventional DST showed resistance but sequencing did not identify mutations associated with resistance, the composite reference standard was considered resistant (as mutations were assumed to be outside of the region sequenced).

A composite reference standard was preferred because there is evidence that strains with disputed mutations that may not be detected as resistant by phenotypic DST are clinically important because they are associated with worse treatment outcomes.

Sequencing is considered a reliable method for detecting mutations known to be associated with phenotypic drug resistance; however, not all resistance-determining mechanisms for rifampicin and isoniazid are known. As a consequence, targeted sequencing may not detect all strains with phenotypic resistance because mutations that confer resistance may occur outside the area of a particular gene that is targeted for sequencing.

Using the GRADE framework, calculations of test sensitivity and specificity were used as proxy measures for patient outcomes; these outcomes were based on the relative importance or impact of false-positive and false-negative results. Poor sensitivity would result in false-negative results so that patients with rifampicin-resistant TB or MDR-TB would not be placed on an effective treatment regimen. This would have negative consequences in terms of the time to initiation of an effective regimen, the development of additional drug resistance, as well as morbidity, mortality and further transmission of disease. Poor specificity would result in false-positive results so that patients without a diagnosis of TB or drug resistance would be prescribed unnecessary treatment, which could have serious adverse effects.

Rates for true positives, true negatives, false positives and false negatives were calculated using pre-test probabilities based on the results of routine surveillance of TB drug resistance that have been overseen by WHO since 1994.[5] Prevalences of 5% and 15% were used to cover the overall levels of resistance to rifampicin. The 5% level was used to represent the upper level of rifampicin resistance among new TB cases and 15% to represent the lower limit of rifampicin resistance among previously treated persons.[6]

[5] Sandgren A, Strong M, Muthukrishnan P, Weiner BK, Church GM, Murray MB. Tuberculosis Drug Resistance Mutation database. PLOS Med. 2009:6:e1000002. doi:10.1371/journal.pmed.1000002.

[6] Global tuberculosis report 2015. Geneva: World Health Organization; 2015 (WHO/HTM/TB/2015.22; http://www.who.int/tb/publications/global_report/gtbr15_main_text.pdf, accessed 18 April 2016).

Prevalences of 5%, 15% and 90% were used for isoniazid. The 5% level was used to represent a population with a low level of isoniazid monoresistance; the 15% level represented a population with a high level of isoniazid monoresistance; and 90% represented the overall prevalence of isoniazid resistance associated with rifampicin resistance. Prevalences of 1%, 5% and 10% were used to cover resistance associated with MDR-TB. These thresholds were chosen based on the findings from global drug-resistance surveillance among TB patients in 217 countries.

The evaluation of the impact on patients was based on a balance among the following values:

- *true positives* – the benefit to patients from rapid diagnosis and treatment;
- *true negatives* – the benefit to patients who would be spared unnecessary treatment (the benefits of reassurance and alternative diagnosis);
- *false positives* – the likelihood of anxiety and morbidity caused by additional testing, unnecessary treatment and possible adverse effects; the possible stigma associated with a diagnosis of TB; and the chance that a false positive might halt further diagnostic evaluation;
- *false negatives* – the increased risk of morbidity and mortality, delayed initiation of treatment and the continued risk of TB transmission.

2.2 Guideline Development Group meeting

The WHO Steering Group was responsible for scoping the guideline, drafting the PICO questions and overseeing evidence retrieval and analyses. The Steering Group was also responsible for selecting the members of the GDG and the External Review Group, for managing Declarations of Interests and for organizing the GDG meeting. A brief biography of each of the GDG members was made available for public scrutiny on the website of the WHO Global TB Programme (http://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/) 2 weeks prior to the GDG meeting.

PICO questions were drafted by the WHO Steering Group and were presented to the GDG for discussion and modification. The Steering Group also prepared an initial list of relevant outcomes, including desirable effects and undesirable effects, and requested the GDG to identify any other important outcomes.

On 1 February 2016, a webinar was conducted with members of the GDG prior to the meeting to review the preliminary findings from the systematic reviews, refine and finalize the proposed patient outcomes and to rate their relative importance. The following outcomes for each PICO question were determined, and the ratings of their importance were unanimously agreed:

- critical outcomes – diagnostic accuracy as reflected by true-positive, true-negative, false-positive and false-negative results;
- important outcomes – impact on the time to diagnosis, ease of use and acceptability of the test, and cost.

The format for the tables tables showing the decisions from evidence to recommendations was discussed and agreed upon by the GDG members during the webinar. The format includes the following sections: description of the problem, accuracy of the diagnostic test, patients' values and preferences, the certainty of the evidence of a test's accuracy, the benefits and harms of using the test, the resources required, equity, the acceptability of the test, the feasibility of implementing the test, and how to use neutral language to formulate the recommendations.

The tables showing the decisions made from evidence to recommendations were developed for each of the PICO questions to guide the process of developing the recommendations. These tables were completed during the meeting.

The meeting was chaired by a guideline methodologist with expertise in guideline development processes and methods. The methodologist participated in the initial planning, scoping and in developing the key questions for the GDG meeting. During the meeting, the methodologist helped the GDG formulate recommendations based on the evidence presented. Decisions were based on consensus, which was defined as unanimous agreement among all GDG members. Consensus was achieved for both of the tables showing the decisions made from evidence to recommendations for direct tests (see Online annex 3, Tables 12 and 14). The remaining tables showing the decisions made from evidence to recommendations for indirect testing were compiled by the WHO Steering Group and circulated to the GDG for their agreement following the meeting (see Online annex 3, Tables 13 and 15). All tables were subsequently agreed to by consensus among the GDG members.

2.3 External Review Group

The findings and recommendations from the GDG meeting were sent to an External Review Group of international experts in the field of TB laboratory diagnostics, which included representatives from the WHO TB Supranational Reference Laboratory Network, WHO's Strategic and Technical Advisory Group for TB and members of the core group of the Global Laboratory Initiative Working Group of the Stop TB Partnership. The External Review Group did not identify any major errors or missing data in the policy guidance. Members of the External Review Group confirmed that they had no concerns regarding any of the recommendations or any other setting-specific issues, nor were there any implications for implementation that were not addressed.

3. Scope

This document provides a pragmatic summary of the evidence and recommendations on using commercial LPAs to detect resistance to rifampicin and isoniazid in adults with signs and symptoms consistent with TB and who are at risk for MDR-TB. It should be read in conjunction with the *Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis*[7] and the 2015 WHO framework for implementing TB diagnostics.[8]

3.1 Target audience

This guidance is intended to be used by clinicians treating patients with TB and drug-resistant TB, and managers and laboratory directors working in TB programmes in coordination with external laboratory consultants, donor agencies, technical advisers, laboratory technicians, procurement officers for laboratory equipment, service providers in the private sector, relevant government sectors, and implementation partners that are involved in country-level strengthening of MDR-TB diagnostic and treatment services. Individuals responsible for programme planning, budgeting, mobilizing resources and implementing training activities for the programmatic management of drug-resistant TB may also benefit from this document.

[7] Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis. Geneva: World Health Organization; 2014 (WHO/HTM/TB/2011.6; http://apps.who.int/iris/bitstream/10665/130918/1/9789241548809_eng.pdf?ua=1&ua=1, accessed 19 April 2016).

[8] Implementing tuberculosis diagnostics: policy framework. Geneva: World Health Organization; 2015 (WHO/HTM/TB/2015.11; http://apps.who.int/iris/bitstream/10665/162712/1/9789241508612_eng.pdf, accessed 19 April 2016).

4. Evidence base for policy formulation

The literature search identified 1 650 citations and, of these, 218 full-text articles were reviewed (Fig. 2). A total of 74 studies were identified for inclusion in the systematic review. References to the included studies are provided in Annex 1 (excluded studies are provided in Online annex 4). Sixteen of these studies contributed data to more than one analysis, resulting in a total of 94 datasets. Thirteen studies used different populations of patients or specimens to perform indirect and direct testing and, thus, were included as two separate datasets. Only one of these studies performed head-to-head testing of all three target LPAs on directly tested clinical specimens and indirectly tested isolates and these data were included as six separate datasets. One study performed indirect testing on two different populations with two different phenotypic reference standards and these data were included as two separate datasets. Two studies examined two different populations of TB patients and were included as four separate datasets. No studies performed LPA testing on specimens and culture isolates from the same patients, precluding direct within-study comparisons.

LPAs were compared with a phenotypic culture-based DST reference standard and a composite reference standard that combined the results from genetic sequencing with results from phenotypic culture-based DST. Phenotypic DST was the primary reference standard applied to all participants for all analyses. These analyses were stratified, first, by susceptibility or resistance to rifampicin or isoniazid, or both, and, second, by type of LPA testing (indirect testing or direct testing). Within each stratum, estimates of the studies' observed sensitivities and specificities were plotted in forest plots with 95% confidence intervals. In cases in which adequate data were available, data were combined for meta-analysis by fitting the bivariate random-effects model.[9, 10]

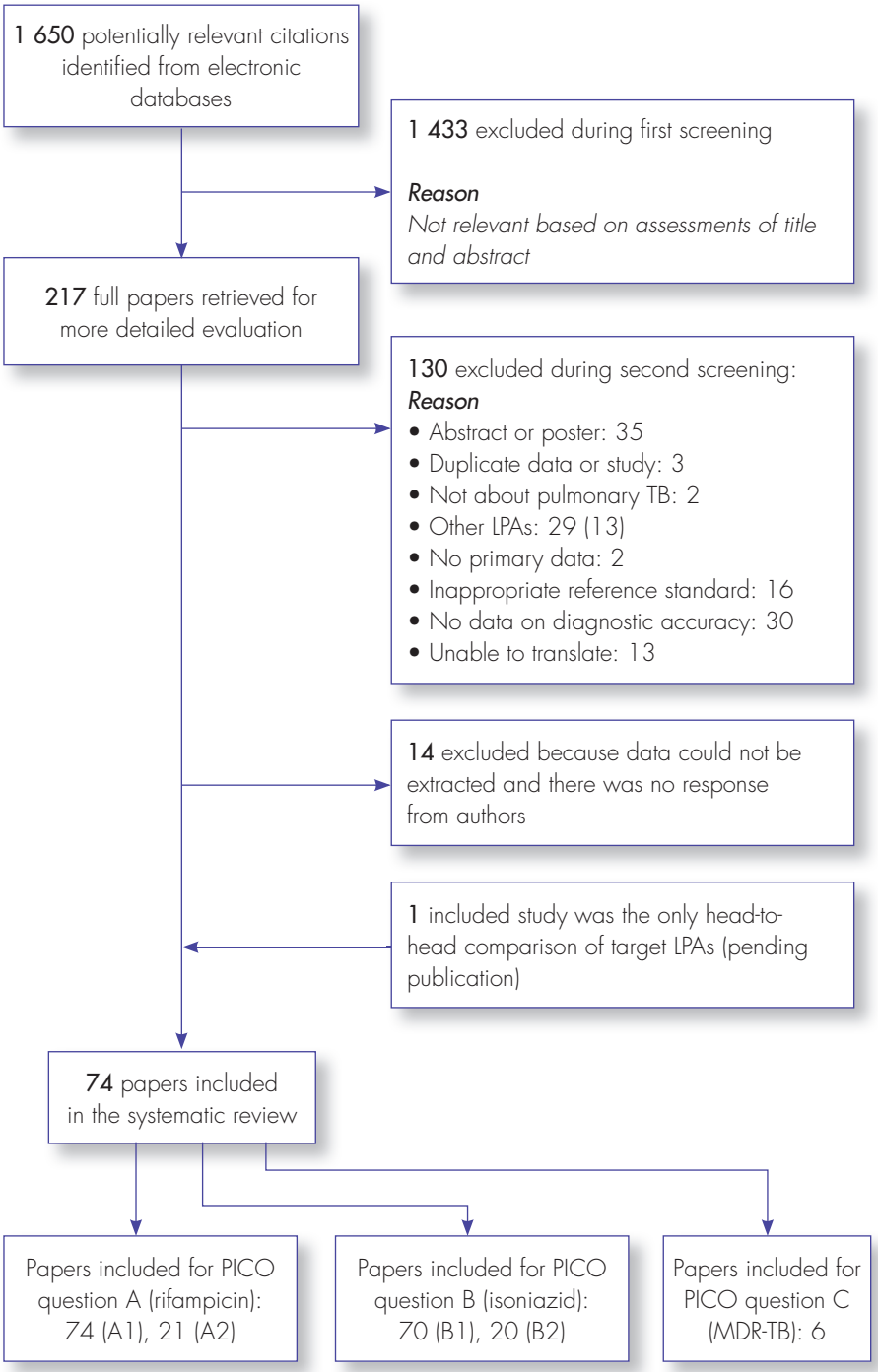
Several studies did not contribute to both sensitivity (no true positives and no false negatives) and specificity (no true negatives and no false positives) but to only one of the two. For these studies, a univariate, random effects meta-analysis of the estimates of sensitivity or specificity was performed separately to make optimal use of the data. The results from the univariate analysis (using all studies) were compared with the results from the bivariate analysis of the subset of studies that contributed to estimates of both sensitivity and specificity.

If there were at least four studies for index tests with data that contributed only to sensitivity or specificity, a univariate, random effects meta-analysis was performed to assess one summary estimate, assuming no correlation between sensitivity and specificity. In cases in which there were fewer than four studies or if substantial heterogeneity was evident on forest plots that precluded a meta-analysis, a descriptive analysis was performed for these index tests. Forest plots were visually assessed for heterogeneity among the studies within each index test and in the summary plots for variability in estimates and the width of the prediction region, with a wider prediction region suggesting more heterogeneity.

[9] Chu H, Cole SR. Bivariate meta-analysis of sensitivity and specificity with sparse data: a generalized linear mixed model approach. J Clin Epidemiol. 2006;59:1331–32; author reply 1332–33. doi:10.1016/j.jclinepi.2006.06.011.

[10] Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol. 2005;58:982–90. doi:10.1016/j.jclinepi.2005.02.022.

Figure 2. Selection of studies evaluating the accuracy of line probe assays (LPAs) for detecting resistance to rifampicin and isoniazid



PICO questions

1. Should LPAs be used to guide clinical decisions to use rifampicin in the direct testing of specimens (A1) and the indirect testing of culture isolates (A2) from patients with signs and symptoms consistent with TB?
2. Should LPAs be used to guide clinical decisions to use isoniazid in the direct testing of specimens (B1) and the indirect testing of culture isolates (B2) from patients with signs and symptoms consistent with TB?
3. Should LPAs be used to diagnose multidrug-resistant TB (MDR-TB) in patients with signs and symptoms consistent with TB?

4.1 Quality of included studies

The quality of the included studies was appraised with the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool.[11] QUADAS-2 consists of four domains: patient selection, index test, reference standard, and flow and timing. All domains were assessed for the potential for risk of bias, and the first three domains for concerns regarding applicability (Fig. 3–5).

4.1.1 Risk of bias for detecting rifampicin resistance

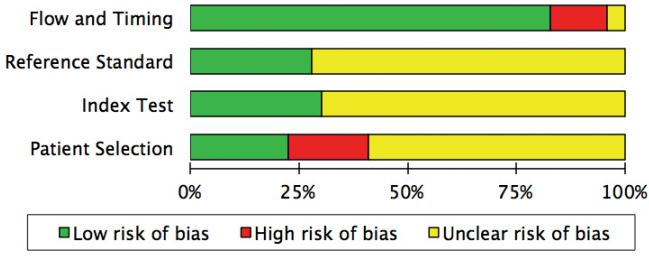
Flow and timing: In the flow and timing domain related to rifampicin resistance, 78 of the 94 datasets were judged to have a low risk of bias. Low risk in this domain indicates that the index and reference tests were performed at the same point in time on paired specimens from the same patient; the same reference standard was applied to all specimens; and all patients or specimens were included in the analysis.

Reference standard: The risk of bias was unclear for 68 of the 94 datasets because the studies did not state whether the person performing the reference test had been blinded to the results of the index test. Applicability was judged to be of low concern in all datasets.

Index test: The risk of bias was unclear for 66 of the 94 datasets because the studies did not state whether the person performing the index test had been blinded to the results of the reference standard. Applicability was judged to be of low concern in 86 of the 94 datasets. Eight datasets that reported variations in test processing that did not follow the manufacturer's recommendations were judged to have a high risk of bias.

Patient selection: In the patient selection domain related to rifampicin resistance, 17 of the 94 datasets were judged to have a high risk of bias. A total of 21 datasets were judged to have a low risk of bias. In 56 datasets, the risk of bias was unclear. In 54 of these 56 datasets, the method of sampling patients or specimens was not specified; 1 dataset had an unclear design; and in 1 dataset it was unclear whether there had been inappropriate exclusions. Applicability was judged to be a low risk in 75 of the 94 datasets and an unclear risk in 19 datasets that did not specify the type of patients tested or the type of laboratory setting.

Figure 3. Risk of bias for detecting rifampicin resistance: review authors' judgements about each domain across studies



[11] Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529–36. doi:10.7326/0003-4819-155-8-201110180-00009.

4.1.2 Risk of bias for detecting isoniazid resistance

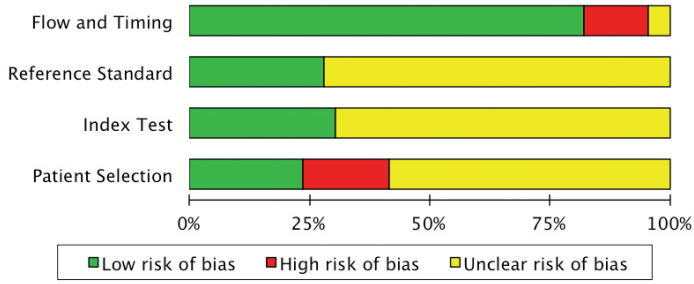
Flow and timing: In the flow and timing domain related to isoniazid resistance, 74 of the 90 datasets were judged to have a low risk of bias.

Reference standard: The risk of bias was unclear for 65 of the 90 datasets because the studies did not state whether the person performing the index test had been blinded to the results of the reference standard. Applicability was judged to be of low concern in all datasets.

Index test: The risk of bias was unclear for 63 of the 90 datasets because the studies did not state whether the person performing the index test had been blinded to the results of the reference standard. Applicability was judged to be of low concern in 82 of the 90 datasets. Eight datasets that reported variations in test processing that did not follow the manufacturer's recommendations were judged to have a high risk for bias.

Patient selection: Overall, in the patient selection domain related to isoniazid resistance, there was an unclear risk of bias for 53 of the 90 datasets. This was predominantly because the method of sampling patients was not defined. Applicability was judged to be a low risk in 71 of the 90 datasets and an unclear risk in 19 datasets that did not specify the type of patients tested or the type of laboratory setting.

Figure 4. Risk of bias for detecting isoniazid resistance: review authors' judgements about each domain across studies



4.1.3 Risk of bias for detecting Mycobacterium tuberculosis complex

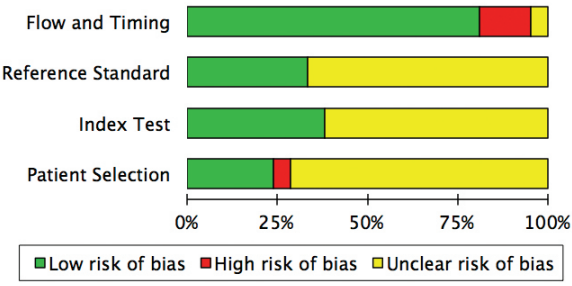
Flow and timing: In the flow and timing domain, all six datasets were judged to have a low risk of bias for detecting MTBC.

Reference standard: In the reference standard domain, there was an unclear risk of bias for three of the six datasets because the studies did not specify whether the person performing the reference test had been blinded to the results of the index test.

Index test: In the index test domain, there was an unclear risk of bias for two of the six datasets because the studies did not specify whether the person performing the index test had been blinded to the results of the reference standard.

Patient selection: In the patient selection domain, there was an unclear risk of bias for five of the six datasets that evaluated the accuracy of detecting MTBC. This was mainly because the method of sampling patients or specimens was not specified. Applicability was judged to be a low risk in five of the six included datasets and an unclear risk in one dataset that did not specify the laboratory setting.

Figure 5. Risk of bias for detecting Mycobacterium tuberculosis complex: review authors' judgements about each domain across studies



The certainty of the evidence (also called the quality of the evidence or confidence in effect estimates) was assessed using the GRADE approach[12, 13] and GRADEpro guideline development tool software.[14] In the context of a systematic review, the ratings of the certainty of the evidence reflect the extent of the confidence that the estimates of the effect are correct (including test accuracy and associations). The certainty of the evidence was rated as high (no points subtracted), moderate (one point subtracted), low (two points subtracted) or very low (more than two points subtracted) based on five domains: risk of bias, indirectness, inconsistency, imprecision and publication bias. One point was subtracted when a serious issue was identified and two points were subtracted when a very serious issue was identified in any of the domains used to judge the certainty of the evidence.

4.2 Accuracy for detecting rifampicin resistance

Altogether, 91 studies were included in the bivariate analysis, with a total of 21 225 samples that included 6 789 confirmed rifampicin-resistant TB cases (32%). Meta-analysis of the studies that reported data on sensitivity and specificity independently of the type of LPA or the type of testing performed (direct or indirect) or the type of phenotypic reference standard used revealed a pooled sensitivity of 96.7% (95% CI: 95.6–97.5) and a pooled specificity of 98.8% (95% CI: 98.2–99.2).

The pooled sensitivity estimate from a univariate analysis that included three additional datasets, with a total of 22 078 samples, contributing data only for sensitivity was 96.5% (95% CI: 95.6–97.3). Because it was anticipated that studies included in this systematic review would be diverse with respect to the sample types tested and assays used, the results were analysed separately for direct testing of sputum specimens and indirect testing of culture isolates. Pooled analysis stratified by LPA demonstrated a slightly lower sensitivity for Hain version 2 and Nipro (95.0% and 94.3%, respectively) compared with Hain version 1 (97.1%), although confidence intervals overlapped and specificity was similar (respectively, 98.3%, 98.1% and 98.9%).

4.2.1 Direct testing for detecting rifampicin resistance

Altogether, 48 datasets were available for LPAs used to detect rifampicin resistance by

[12] Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, et al . GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. 2011;64(4):401–6. doi:10.1016/j.jclinepi.2010.07.015.
[13] Schünemann H, Brożek J, Guyatt G, Oxman A, editors. Handbook for grading the quality of evidence and the strength of recommendations using the GRADE approach. Hamilton, Ontario: McMaster University, Evidence Prime; 2013 (http://gdt.guidelinedevelopment.org/app/handbook/handbook.html, accessed 20.04.16).
[14] GRADEpro GDT: GRADEpro guideline development tool [software]. Hamilton, Ontario: McMaster University, Evidence Prime; 2015 (https://gradepro.org, accessed 20.04.16).

direct testing of 10 560 sputum specimens that included 2 876 specimens from confirmed rifampicin-resistant TB cases (27%). Compared with a culture-based DST reference standard, the pooled sensitivity across studies was 96.3% (95% CI: 94.6–97.5) and the pooled specificity was 98.2% (95% CI: 97.2–98.8).

A total of 41 datasets assessed the Hain version 1 assay (Fig. 6); 4 datasets (Bablishvili et al. 2015, Catanzaro et al. 2015, Crudu et al. 2012, Nathavitharana et al. 2016) evaluated Hain version 2 on 1 872 specimens that included 827 rifampicin-resistant TB cases (44%). Bivariate meta-analysis revealed that the pooled sensitivity across the four studies evaluating Hain version 2 was 95.8% (95% CI: 92.6–97.6) and specificity was 98.4% (95% CI: 96.9–99.2). Because there were only three datasets that evaluated Nipro (Mitarai et al. 2012, Nathavitharana et al. 2016, Rienthong et al. 2015), a meta-analysis could not be performed separately for this assay. The three datasets evaluating Nipro included a total of 657 specimens with 182 rifampicin-resistant TB cases (28%): sensitivity estimates ranged from 75% to 100%, and specificity estimates ranged from 96.5% to 100%. The pooled sensitivity and specificity across studies for Hain version 1 did not differ substantially from the overall pooled estimates across all three LPAs reported above (for sensitivity 96.8%, 95% CI: 94.7–98.1; and for specificity 98.1%, 95% CI: 96.9–98.8). The only study (Nathavitharana et al. 2016) that compared all three LPAs side by side, directly on specimens, showed similar performance across all three tests (Table 1).

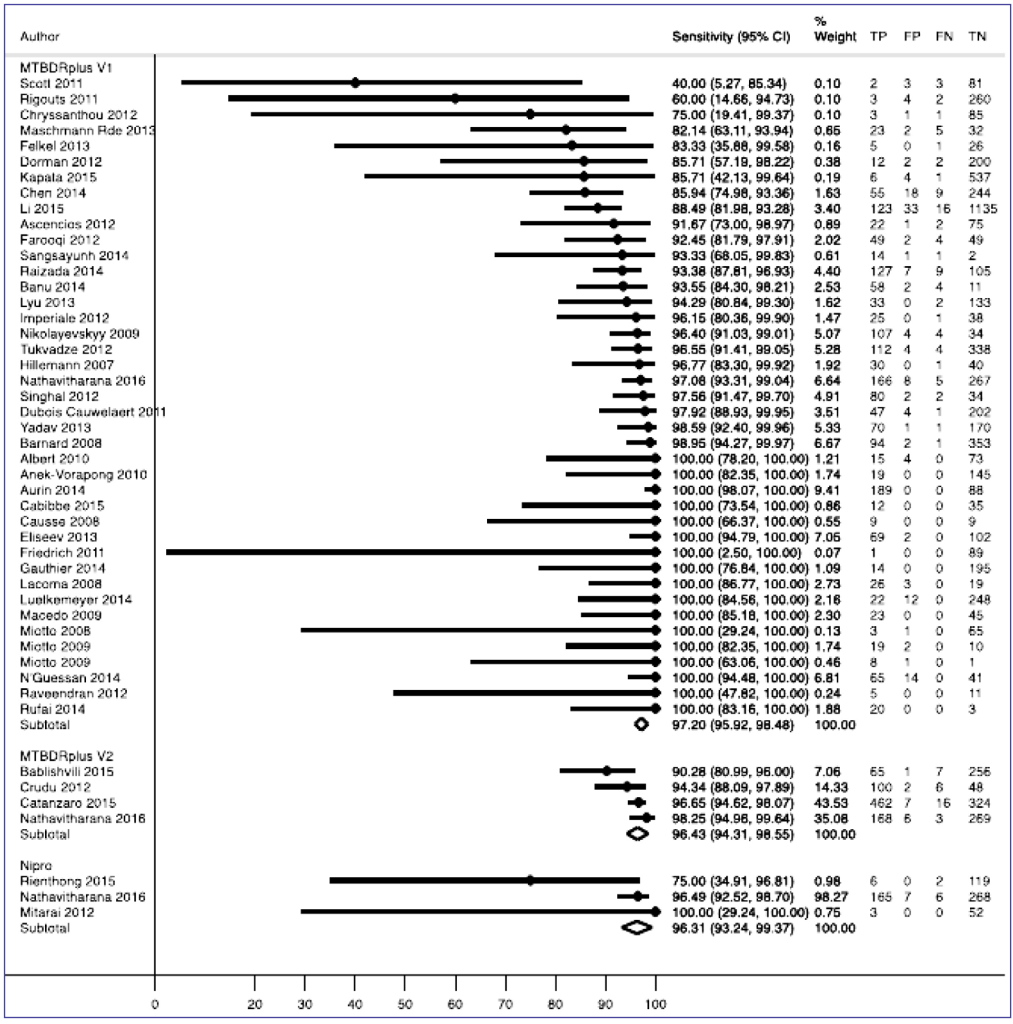
Table 1. Head- to-head comparison of three line probe assays used to detect rifampicin resistance in the direct testing of sputum specimens compared with a culture-based reference standard for drug-susceptibility testing

Line probe assay	Sensitivity[a/b]	Specificity[a/b]
Hain version 1	97.1 (93.3–99.0) (166/171)	97.1 (94.3–98.7) (267/275)
Hain version 2	98.2 (95.0–99.6) (168/171)	97.8 (95.3–99.2) (269/275)
Nipro	96.5 (92.5–98.7) (165/171)	97.5 (94.8–99.0) (268/275)

[a] Values are percentage (95% confidence interval)
[b] Absolute numbers

For individual studies, the point estimates for sensitivity ranged from 40% to 100%, and the point estimates for specificity ranged from 50% to 100% (Fig. 6, 7). Outliers with lower sensitivity were predominantly datasets with limited numbers of resistant specimens (fewer than 10) and, thus, were accompanied by wide confidence intervals. Heterogeneity also appeared to be limited for specificity, aside from a few outliers that were predominantly datasets with fewer than 10 rifampicin-susceptible specimens. One large outlier study (N'Guessan et al. 2014) demonstrated a low specificity of 74.5%. The patient population included in this study was unclear.

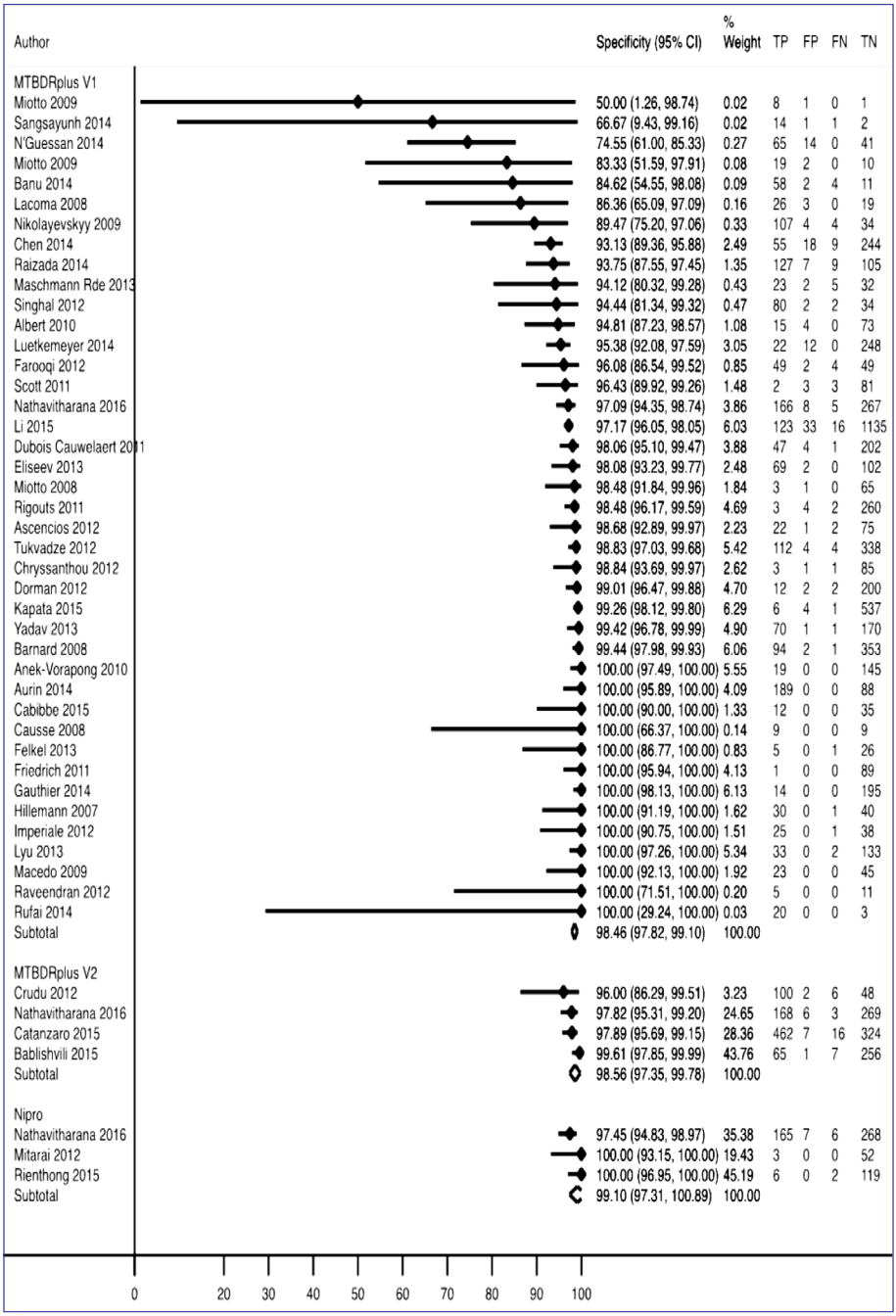
Figure 6. Forest plot for the sensitivity of all line probe assays evaluated for diagnosing rifampicin resistance in sputum specimens that were tested directly[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are stratified by the version of the line probe assay and ordered by decreasing sensitivity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

Figure 7. Forest plot for the specificity of all line probe assays evaluated for diagnosing rifampicin resistance in sputum specimens that were tested directly[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are stratified by the version of the line probe assay and ordered by decreasing specificity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

4.2.2 Indirect testing for detecting rifampicin resistance

In 43 datasets, LPA was used to detect rifampicin resistance by indirect testing of 10 696 culture isolates of MTBC that included 3 913 cultures with confirmed rifampicin-resistant TB (37%). Compared with a phenotypic culture-based DST reference standard, the pooled sensitivity across studies was 96.9% (95% CI: 95.5–98.0) and the pooled specificity was 99.3% (95% CI: 98.6–99.6).

Altogether, 39 datasets assessed the Hain version 1 assay. One dataset (Nathavitharana et al. 2016) evaluated Hain version 2 on 376 culture isolates that included 172 rifampicin-resistant TB strains (46%) and found a sensitivity of 91.3% and specificity of 98.0% compared with a culture-based DST reference standard. Because there only three datasets evaluated Nipro (Mitarai et al. 2012, Nathavitharana et al. 2016, Rienthong et al. 2015), a meta-analysis could not be performed separately for this assay. The three datasets evaluating Nipro included a total of 952 culture isolates with 357 rifampicin-resistant TB strains (38%): sensitivity estimates ranged from 92.8% to 98.9%, and specificity estimates ranged from 97.3% to 98.2%. The pooled sensitivity and specificity across studies for Hain version 1 did not differ substantially from the overall pooled estimates across all three LPAs reported above (for sensitivity 97.3%, 95% CI: 95.7–98.3; and for specificity 99.5%, 95% CI: 98.8–98.8%). The only study (Nathavitharana et al. 2016) that compared all three LPAs side by side on culture isolates showed similar performance across all three tests (Table 2).

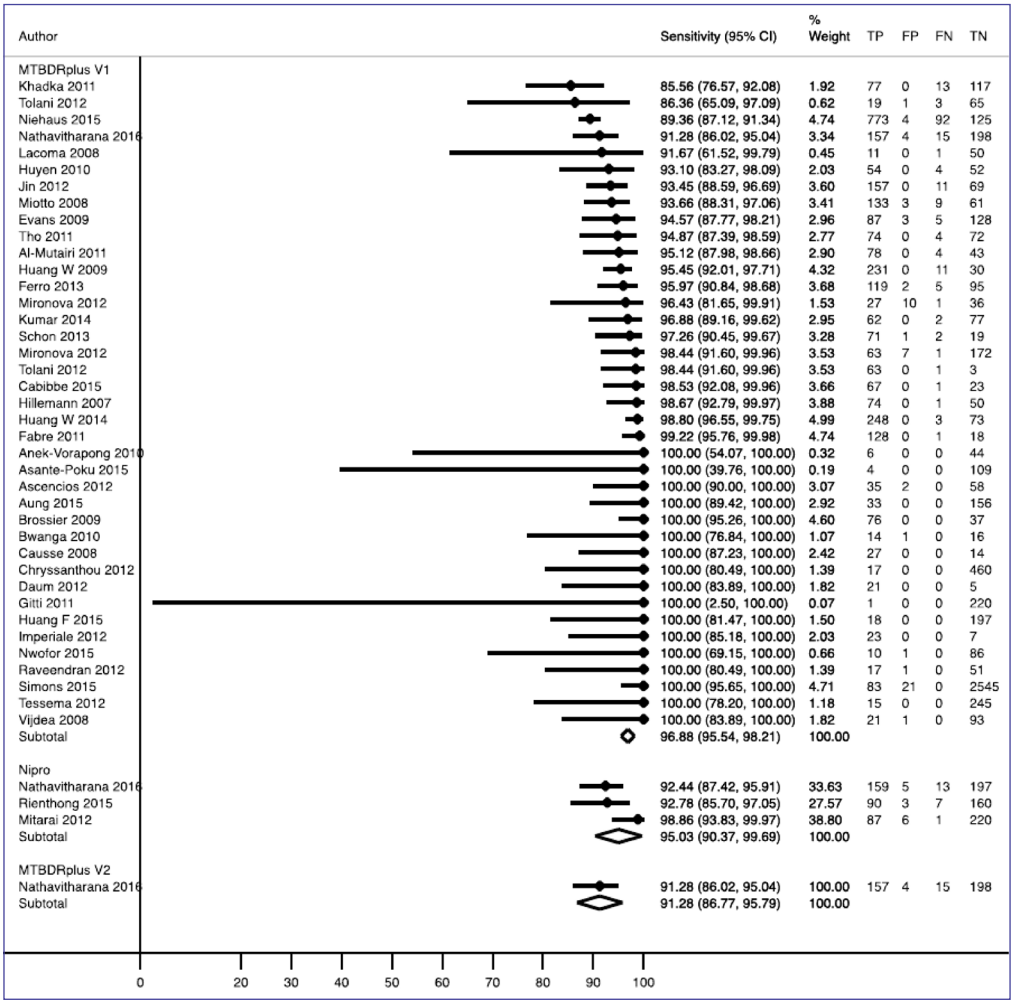
Table 2. Head- to-head comparison of three line probe assays used to detect rifampicin resistance in the indirect testing of cultures of *Mycobacterium tuberculosis* complex compared with a culture-based reference standard for drug-susceptibility testing

Line probe assay	Sensitivity[a/b]	Specificity[a/b]
Hain version 1	91.3 (86.0–95.0) (157/172)	97.1 (94.3–98.7) (267/275)
Hain version 2	91.3 (86.0–95.0) (157/172)	97.1 (94.3–98.7) (267/275)
Nipro	92.4 (87.4–95.9) (159/172)	97.5 (94.3–99.2) (197/202)

[a] Values are percentage (95% confidence interval)
[b] Absolute numbers

For individual studies, the point estimates for sensitivity ranged from 85.6% to 100%, and specificity ranged from 78.3% to 100% (Fig. 8, 9). Results for both sensitivity and specificity were more homogeneous than those for direct testing. A study of TB patients undergoing retreatment (Khadka et al. 2011) demonstrated a sensitivity of 85.6%. Another study (Tolani , D'Souza, & Mistry 2012) demonstrated a sensitivity of 86.3% on a group of persons presumed to have TB but without risk factors for MDR-TB. A third study (Niehaus et al. 2015) of 1 000 routine LPA results reported a sensitivity of 89.3% for detecting rifampicin resistance, although the reason for the lower sensitivity was unclear. There was one outlier study (Mironova et al. 2012) with a specificity of 78.3%. The outlier study used both the BACTEC mycobacterial growth indicator tube (MGIT) liquid media DST (Becton Dickinson, Franklin Lakes, NJ, United States) and the Löwenstein–Jensen proportion method as the phenotypic reference standards (in separate study populations); the lower specificity was noted for the isolates that were compared with the Löwenstein–Jensen proportion method.

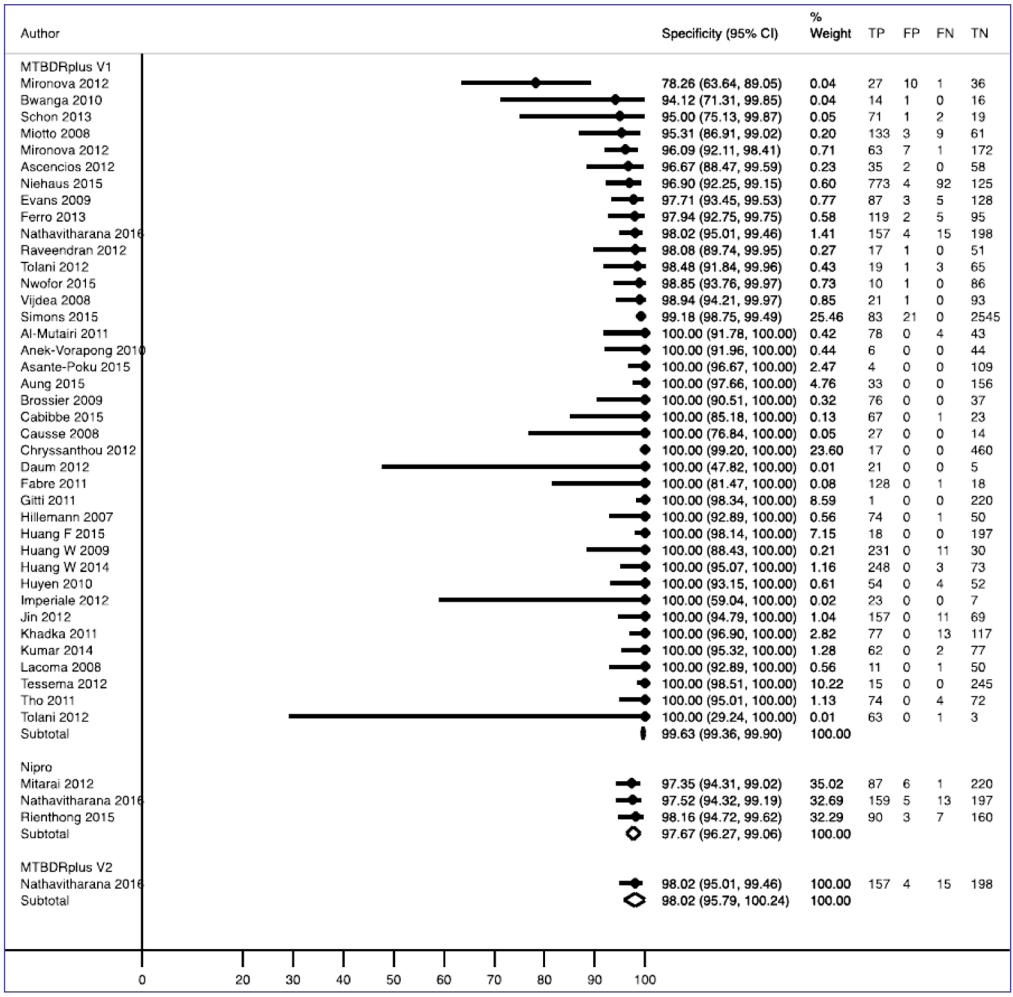
Figure 8. Forest plot for the sensitivity of all line probe assays evaluated for detecting rifampicin resistance by indirect testing of Mycobacterium tuberculosis complex culture isolates[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are stratified by the version of the line probe assay and ordered by decreasing sensitivity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

Figure 9. Forest plot for the specificity of all line probe assays evaluated for detecting rifampicin resistance by indirect testing of *Mycobacterium tuberculosis* complex culture isolates[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are stratified by the version of the line probe assay and ordered by decreasing sensitivity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

4.2.3 Comparison of diagnostic accuracy: direct versus indirect testing

The pooled sensitivity estimates of LPA for detecting rifampicin resistance were almost identical for LPA performed directly on sputum specimens and indirectly on culture isolates (96.3% and 96.9%, respectively). The pooled specificity estimate was slightly increased for indirect testing (99.3% compared with 98.2%).

No studies performed LPA testing on specimens and culture isolates from the same patients, thus precluding direct within-study comparisons.

Only one study (Nathavitharana et al. 2016) performed a head-to-head comparison of all three LPAs, and it found that Hain version 2 and Nipro were equivalent to Hain version 1 for detecting rifampicin resistance in both direct and indirect testing.[15] In this study, the sensitivities for detecting rifampicin resistance for all three LPAs were lower for indirect testing of MTBC culture isolates than for the direct testing of sputum specimens (90–91% compared with 97–98%, respectively). The reduced sensitivity was due to the use of strains with resistance-conferring mutations that had been pre-selected to challenge the performance of the LPAs and were not intended to represent a typical population-based frequency of the distribution of resistance-conferring mutations. The specificities demonstrated for the two methods (direct versus indirect) did not significantly differ (97.5–98.5% compared with 97–97.5%).

4.2.4 Accuracy of detecting rifampicin resistance compared with a composite reference standard

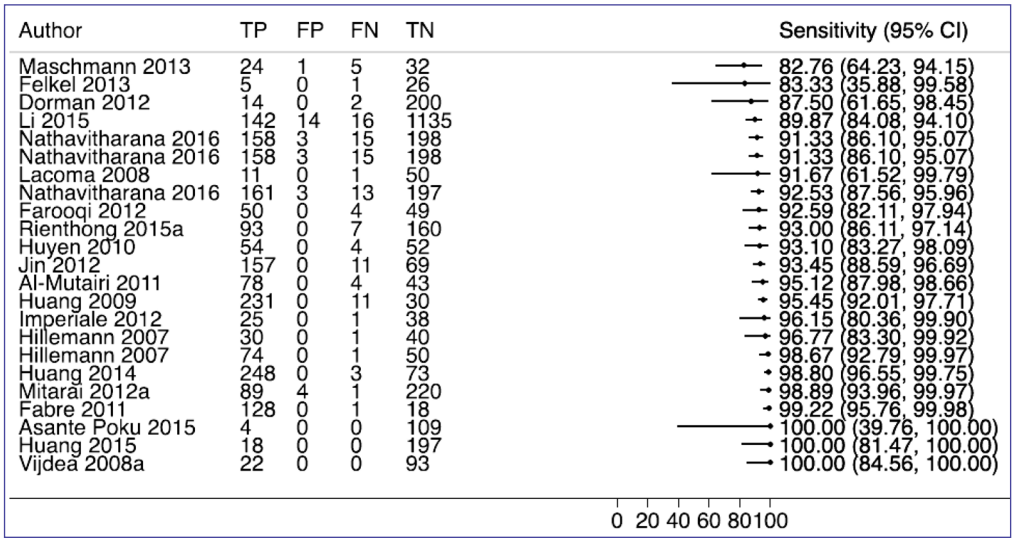
A total of 23 datasets were identified that included data on the accuracy of using LPA to detect rifampicin resistance compared with a composite reference standard that included both DNA sequencing of the rpoB gene and phenotypic culture-based DST. A total of 5 483 cultures of MTBC that included 2 091 (38%) rifampicin-resistant strains were evaluated. Bivariate meta-analysis of these studies revealed a pooled sensitivity of 95.3% (95% CI: 93.4–96.6) and a pooled specificity of 99.5% (95% CI: 98.6–99.8).

Sensitivity did not change when a composite reference standard was used because no false-negative LPA results were reclassified based on the results of sequencing. Even when sequencing results matched the LPA results – that is, when sequencing detected wild-type strains or silent mutations – the phenotypic culture-based DST result was considered to be correct. In these cases, it was assumed that there could be mutations outside of the hotspots targeted by the LPA and sequencing that were responsible for resistance (no studies performed whole-genome sequencing). However, specificity increased when a composite standard was used as 37 false-positive LPA results (from 11 datasets) were reclassified as true positives based on sequencing confirming a known resistance-conferring mutation.

Fig. 10 and Fig. 11 show the forest plots for the sensitivity and specificity of all LPAs evaluated for detecting rifampicin resistance by indirect testing of MTBC culture isolates compared against a composite reference standard. Results for both sensitivity and specificity were largely homogeneous. One study (Maschmann et al. 2013) demonstrated a sensitivity of 82.8%. Two of the five cultures of MTBC that had been incorrectly classified had insertions in codons 516–517, which may have caused hybridization of the corresponding wild-type (known as wt) probe (wt3 for codons 517–520), and the other three cultures were sequenced as wild-type strains, suggesting that resistance may have been due to the presence of mutations outside of the *rpo*B hotspot.

[15] Report for WHO: non-inferiority evaluation of Nipro NTM+MDRTB and Hain GenoType MTBDRplus V2 line probe assays. Geneva: FIND; 2015 (http://www.finddx.org/wp-content/uploads/2016/04/LPA-report_noninferiority-study_oct2015.pdf, accessed 15 April 2016).

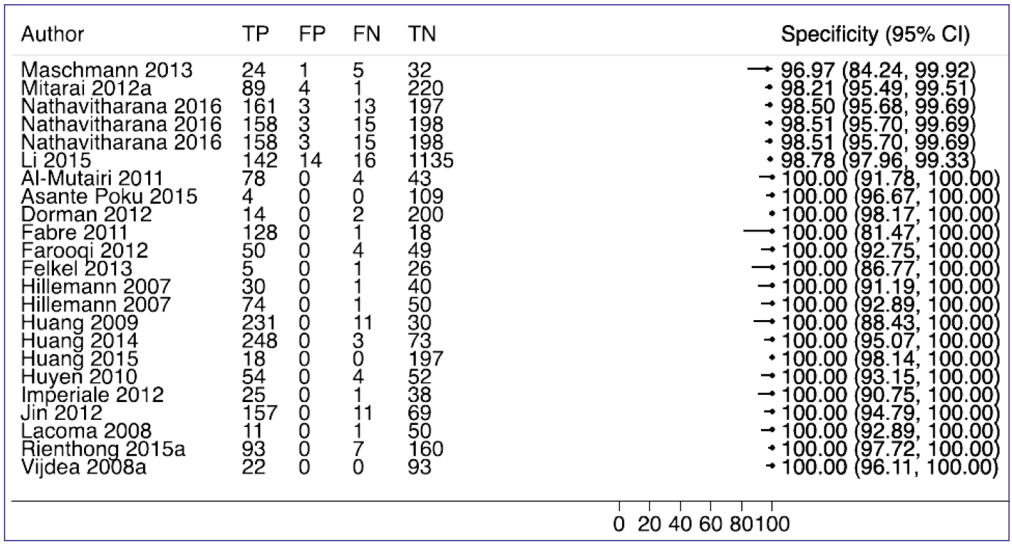
Figure 10. Forest plot for the sensitivity of all line probe assays evaluated for detecting rifampicin resistance by indirect testing of *Mycobacterium tuberculosis* complex culture isolates compared against a composite reference standard[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are ordered by decreasing sensitivity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

Figure 11. Forest plot for the specificity of all line probe assays evaluated for detecting rifampicin resistance by indirect testing of *Mycobacterium tuberculosis* complex culture isolates compared against a composite reference standard[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are ordered by decreasing sensitivity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

4.3 Accuracy for detecting isoniazid resistance

Altogether, 87 datasets were included in the bivariate analysis, with a total of 20 954 specimens that included 8 135 confirmed isoniazid-resistant TB cases (39%). Meta-analysis of the studies that reported data on sensitivity and specificity independently of the type of LPA or the type of testing performed (direct or indirect) or the type of phenotypic reference standard used revealed a pooled sensitivity of 90.2% (95% CI: 88.2–91.9) and pooled specificity of 99.2% (95% CI: 98.7–99.5%).

The pooled sensitivity estimate from a univariate analysis with three additional datasets, with a total of 21 665 samples, that contributed data only for sensitivity was largely unchanged at 89.4% (95% CI: 87.8–90.9). Pooled analysis stratified by the type of LPA demonstrated a lower sensitivity for Nipro (86.9%) and a higher sensitivity for Hain version 2 (93.6%) compared with Hain version 1 (90.2%), although specificity was similar (99.1%, 99.1% and 99.2%, respectively).

4.3.1 Direct testing for detecting isoniazid resistance

A total of 46 datasets used LPA to detect isoniazid resistance by direct testing of 10 472 sputum specimens that included 3 576 confirmed isoniazid-resistant TB cases (34%). Compared with a culture-based DST reference standard, the pooled sensitivity across studies was 89.2% (95% CI: 85.8–91.9) and the pooled specificity was 98.4% (95% CI: 97.5–98.9). One dataset contributed data only towards sensitivity but not specificity, increasing the total to 10 483 specimens. A meta-analysis including this dataset demonstrated a similar sensitivity estimate of 88.4% (95% CI: 86.0–90.8), suggesting that excluding this study did not affect the pooled estimate for sensitivity.

A total of 39 datasets assessed the Hain version 1 assay (Fig. 12, 13); 4 datasets (Bablishvili et al. 2015, Catanzaro et al. 2015, Crudu et al. 2012, Nathavitharana et al. 2016) evaluated Hain version 2 on 1 865 specimens that included 931 isoniazid-resistant TB cases (50%). Bivariate meta-analysis revealed that the pooled sensitivity across the four studies evaluating Hain version 2 was 94.5% (95% CI: 91.4–96.5) and specificity was 99.3% (95% CI: 92.6–100.0). Because there were only three datasets that evaluated Nipro (Mitarai et al. 2012, Nathavitharana et al. 2016, Rienthong et al. 2015), a meta-analysis could not be performed separately for this assay. The three datasets evaluating Nipro included a total of 653 specimens with 218 (33%) isoniazid-resistant TB cases: sensitivity estimates ranged from 50% to 94.9%, and the specificity estimates ranged from 96.5% to 97.8%. When the results for Hain version 2 and Nipro were excluded, the pooled sensitivity and specificity for Hain version 1 did not differ substantially from the overall pooled estimates across all three LPAs reported above (sensitivity 88.4%, 95% CI: 84.4–91.6; and specificity 98.3%, 95% CI: 97.4–98.9). The only study (Nathavitharana et al. 2016) that compared all three LPAs side-by-side directly on specimens showed similar performance across all three tests (Table 3).

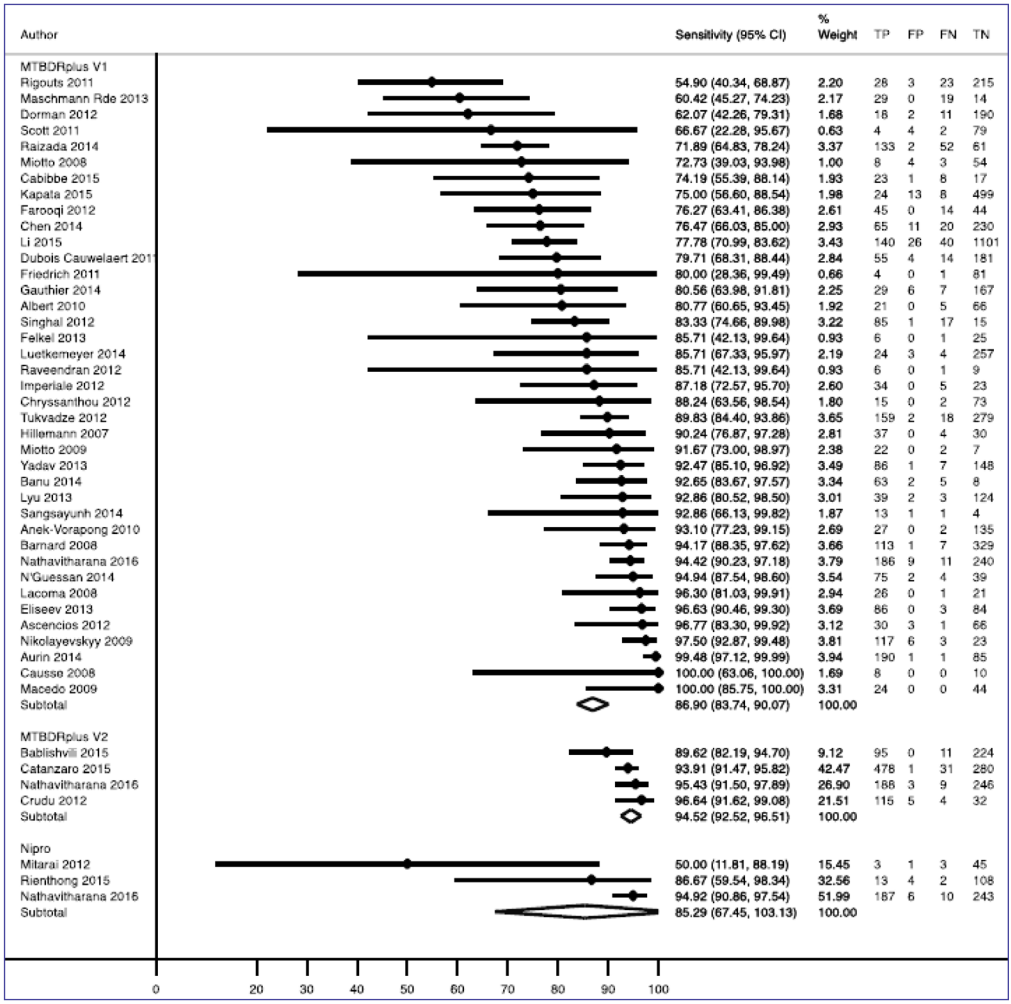
Table 3. **Head- to-head comparison of three line probe assays used to detect isoniazid resistance in the direct testing of sputum specimens compared with a culture-based reference standard for drug-susceptibility testing**

Line probe assay	Sensitivity[a/b]	Specificity[a/b]
Hain version 1	94.4 (90.2–97.2) (186/197)	96.4 (93.2–98.3) (240/249)
Hain version 2	95.4 (91.5–97.9) (188/197)	98.8 (96.5–99.8) (246/249)
Nipro	94.9 (90.9–97.5) (187/197)	97.6 (94.8–99.1) (243/249)

[a] Values are percentage (95% confidence interval)
[b] Absolute numbers

For individual studies, the point estimates for sensitivity ranged from 50% to 100%, and the point estimates for specificity ranged from 79% to 100% (Fig. 12, 13). A greater degree of heterogeneity was noted for isoniazid sensitivity compared with rifampicin sensitivity. Five datasets had a sensitivity of less than 70% for detecting isoniazid resistance. Two of these studies with lower sensitivity (Mitarai et al. 2012, Scott et al. 2011,) had limited numbers of resistant specimens (fewer than 10) and, thus, had wide confidence intervals. One study (Rigouts et al. 2011) demonstrated a sensitivity of 54% based on evaluating new smear-positive cases in the United Republic of Tanzania. Although the authors suggested that new TB cases may be more likely to have rare isoniazid resistance–conferring mutations, another dataset (Maschmann et al. 2013), which evaluated patients with treatment failure or relapse presenting to a TB reference hospital in Brazil, also demonstrated a low sensitivity (60%).

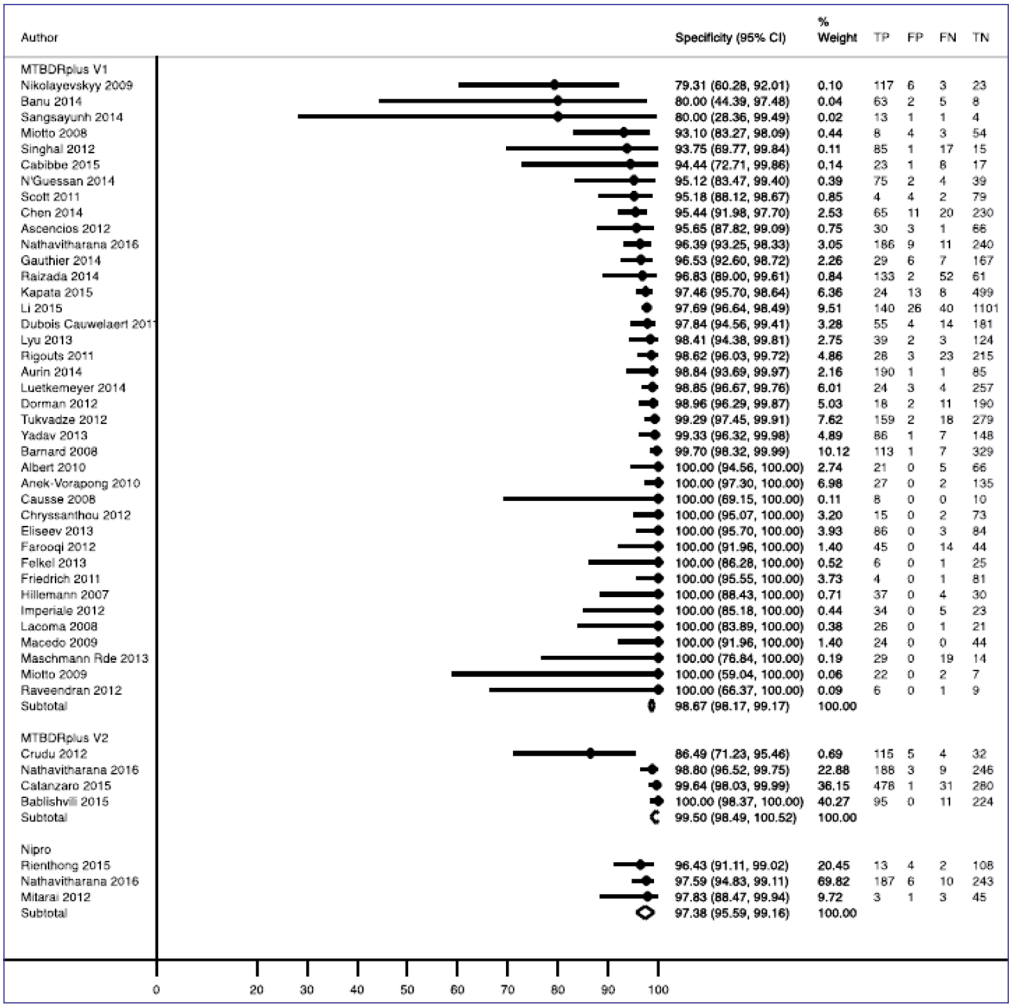
Figure 12. Forest plot for the sensitivity of all line probe assays evaluated for detecting isoniazid resistance by direct testing of sputum specimens[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are ordered by decreasing sensitivity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

Figure 13. Forest plot for the specificity of all line probe assays evaluated for detecting isoniazid resistance by direct testing of sputum specimens[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are ordered by decreasing sensitivity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

A South African study (Dorman et al. 2012) evaluating miners with signs and symptoms consistent with pulmonary TB demonstrated a sensitivity of 62% for detecting isoniazid resistance. Heterogeneity was much less pronounced for specificity estimates.

4.3.2 Indirect testing for detecting isoniazid resistance

For 40 datasets, LPA was used to detect isoniazid resistance by the indirect testing of 10 462 culture isolates of MTBC that included 4 559 cultures with confirmed isoniazid-resistant TB

(44%). Compared with a culture-based DST reference standard, the pooled sensitivity across studies was 91.0% (95% CI: 88.6–93.0) and the pooled specificity was 99.7% (95% CI: 99.3–99.9). Two datasets contributed data only towards sensitivity but not specificity, increasing the total to 11 162 samples. A meta-analysis including these studies demonstrated a sensitivity of 90.0% (95% CI: 87.8–92.1), indicating that the exclusion of these datasets did not affect the estimate of sensitivity.

A total of 36 datasets assessed the Hain version 1 assay. One dataset (Nathavitharana et al. 2016) evaluated Hain version 2 on 378 culture isolates that included 199 isoniazid-resistant TB strains (50%), and found a sensitivity of 89.4% and a specificity of 98.9% compared with a culture-based DST reference standard. Because there were only three datasets that evaluated Nipro (Mitarai et al. 2012, Nathavitharana et al. 2016, Rienthong et al. 2015), a meta-analysis could not be performed separately for this assay. The three datasets evaluating Nipro included a total of 952 culture isolates with 444 isoniazid-resistant TB strains (47%): sensitivity estimates ranged from 61.6 % to 91.6%, and the specificity estimates ranged from 99.4% to 100%. The pooled sensitivity and specificity across studies for Hain version 1 did not differ substantially from the overall pooled estimates across all three LPAs reported above (sensitivity 91.5%, 95% CI: 89.0–93.5; and specificity 99.8%, CI: 99.3–100). The only study (Nathavitharana et al. 2016) that compared all three LPAs side-by-side on culture isolates showed similar performance across all three tests (Table 4).

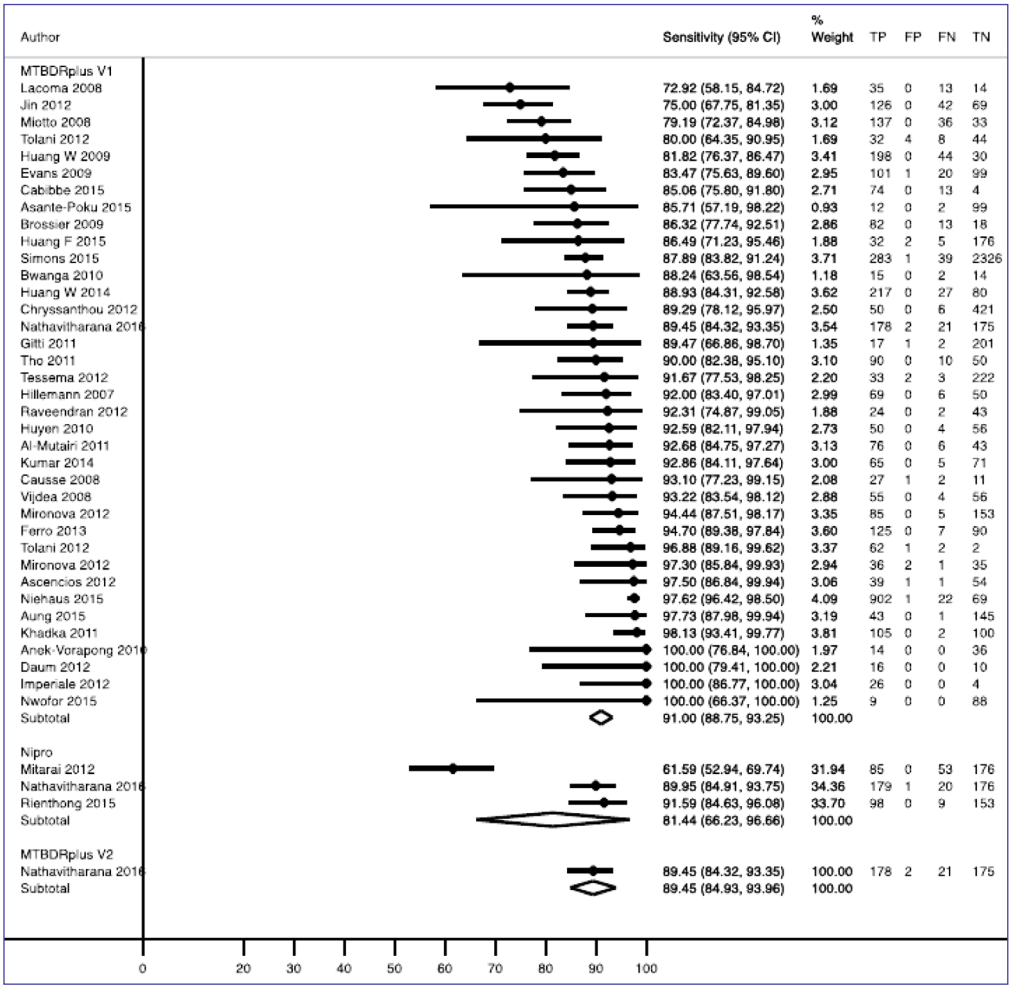
Table 4. Head- to-head comparison of three line probe assays used to detect isoniazid resistance in the indirect testing of cultures of *Mycobacterium tuberculosis* complex compared with a culture-based reference standard for drug-susceptibility testing

Line probe assay	Sensitivity[a/b]	Specificity[a/b]
Hain version 1	89.4 (84.3–93.3) (178/199)	98.9 (96.0–99.9) (175/177)
Hain version 2	89.4 (84.3–93.3) (178/199)	98.9 (96.0–99.9) (175/177)
Nipro	89.9 (84.9–93.8) (179/199)	99.4 (96.9–100) (176/177)

[a] Values are percentage (95% confidence interval)
[b] Absolute numbers

For individual studies, the point estimates for sensitivity ranged from 61.6% to 100%, and the point estimates for specificity ranged from 66.7% to 100% (Fig. 14, 15). Several studies were outliers for sensitivity, but specificity was largely homogeneous. One study in Japan (Mitarai et al. 2012), demonstrated a sensitivity of 61.6% for detecting isoniazid resistance using Nipro to test 554 culture isolates from patients with TB or infection with non-tuberculous mycobacteria. The isoniazid resistance–conferring mutations that are the most common globally occur less frequently in Japan, which could account for the lower sensitivity for detecting resistance. Another study, evaluating 62 MTBC culture isolates in Spain (Lacoma et al. 2008), found a sensitivity of 72.9%. Studies reporting higher sensitivities for detecting isoniazid resistance often have a higher proportion of strains with the S315T katG mutation, which is easier to detect by phenotypic culture-based DST because this mutation is associated with higher minimum inhibitory concentrations (MICs). In this study, 10 of the 13 strains misclassified as sensitive by LPA had low-level isoniazid resistance associated with mutations in the inhA promoter region that may be more difficult to detect with phenotypic culture-based DST. Another study (Miotto et al. 2008) found a sensitivity of 79% in an evaluation of 173 isoniazid-resistant strains, but details regarding discrepant results were not reported. One study that contained only three isoniazid-susceptible strains (Tolani, D'Souza & Mistry 2012) was an outlier, with a specificity of only 66.7% (95% CI: 9.4–99.2).

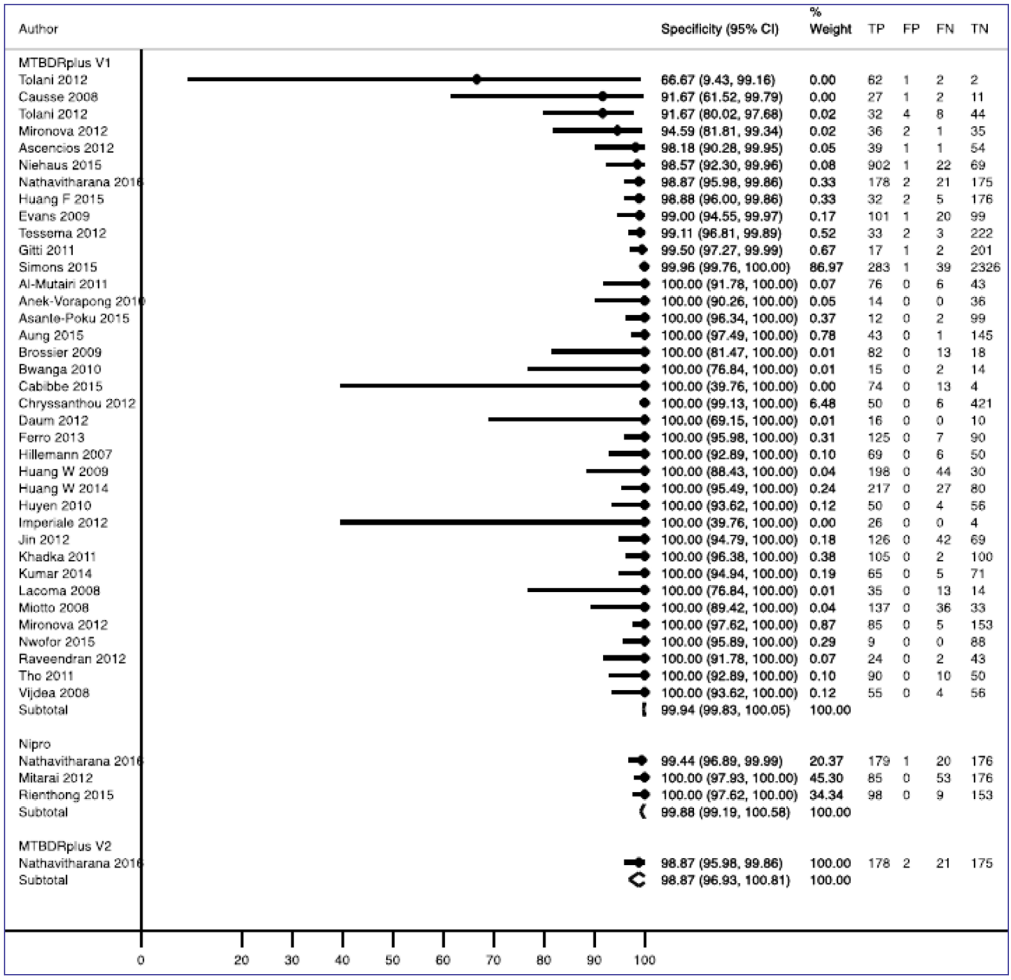
Figure 14. Forest plot for the sensitivity of all line probe assays evaluated for detecting isoniazid resistance by indirect testing of cultures of *Mycobacterium tuberculosis* complex[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are ordered by decreasing sensitivity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

Figure 15. Forest plot for the specificity of all line probe assays evaluated for detecting isoniazid resistance by indirect testing of cultures of *Mycobacterium tuberculosis* complex[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are ordered by decreasing sensitivity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

4.3.3 Comparison of diagnostic accuracy: direct versus indirect testing

The pooled sensitivity estimates of using LPA to detect isoniazid resistance were almost identical for LPA performed directly on sputum specimens and indirectly on culture isolates (89.2% and 91.0%, respectively). The pooled specificity estimate was slightly increased for indirect testing (99.7% compared with 98.4%).

No studies performed LPA testing on specimens and culture isolates from the same patients, precluding direct within-study comparisons. Only one study (Nathavitharana et al. 2016) performed a head-to-head comparison of all three LPAs, and this study found that Hain version 2 and Nipro were equivalent to Hain version 1 for detecting isoniazid resistance for both direct and indirect testing.[7] In this study, for all three LPAs the sensitivities for detecting isoniazid

resistance were lower for the indirect testing of MTBC culture isolates than for the direct testing of sputum specimens (indirect testing 89.1–89.6% compared with 94.4–95.4% for direct testing). The reduced sensitivity was due to the use of strains with resistance-conferring mutations that had been pre-selected to challenge the performance of the LPAs and were not intended to represent a typical population-based frequency of the distribution of resistance-conferring mutations. In this study, specificity was higher for the indirect testing of culture isolates than for the direct testing of sputum specimens (indirect testing 99.4–100% compared with 96.4–98.8% for direct testing).

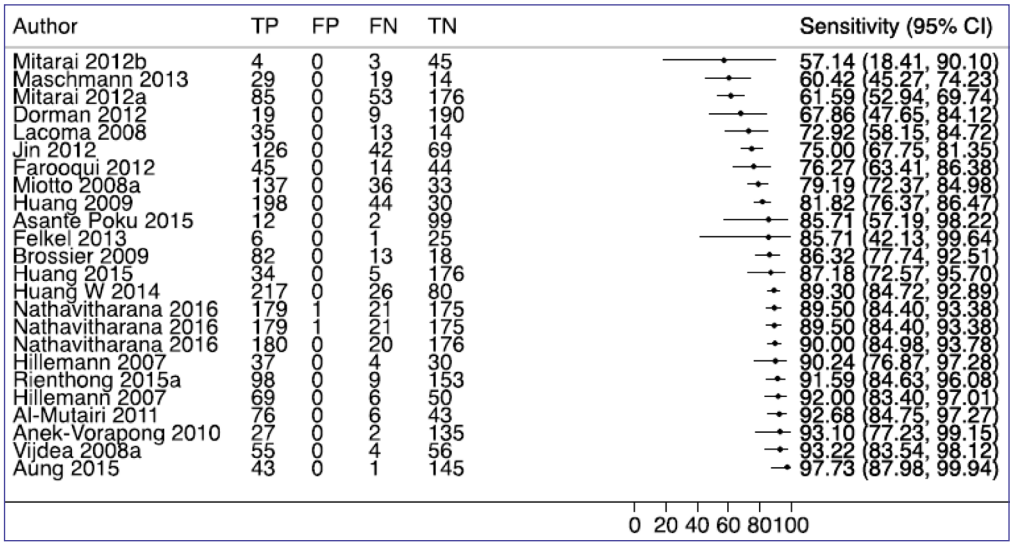
4.3.4 Accuracy for detecting isoniazid resistance compared with a composite reference standard

A total of 24 datasets were identified that included data on the accuracy of LPAs in detecting isoniazid resistance compared with a composite reference standard that included both DNA sequencing of the inhA gene promoter and the katG gene as well as phenotypic culture-based DST. Altogether, 4 516 cultures of MTBC that included 2 346 (52%) isoniazid-resistant strains were evaluated. Bivariate analysis of these studies revealed a pooled sensitivity of 85.0% (95% CI: 80.5–88.6) and a pooled specificity of 99.5% (95% CI: 99.1–99.8).

Sensitivity did not change when a composite reference standard was used because no false-negative LPA results were reclassified based on the results of sequencing. Even when sequencing results matched the LPA results – that is, when sequencing detected wild-type strains or silent mutations – the phenotypic culture-based DST result was considered to be correct. In these cases, it was assumed that there could be mutations outside of the hotspots targeted by the LPA and sequencing that were responsible for resistance (no studies performed whole-genome sequencing). Sequencing also revealed resistance mutations that were not detected by LPA. In one study (Jin et al. 2012), 10 of 11 strains with a rarer katG mutation, S315N, were not detected by LPA due to the absence of a specific probe for this mutation in the assay, and the wild-type probe for this region failed to disappear. Although seven LPA false-positive results (from six datasets) were reclassified as true positives based on sequencing confirming a known resistance mutation (four katG S315T mutations and three inhA C15T mutations), the specificity barely increased when a composite standard was used.

Fig. 16 and Fig. 17 show the forest plots for the sensitivity and specificity of all LPAs evaluated for detecting isoniazid resistance by indirect testing of MTBC culture isolates compared against a composite reference standard; the plots demonstrate homogeneous results for specificity, which was largely also the case for sensitivity, aside from a few outliers. The lowest sensitivity observed was in a study in Japan (Mitarai et al. 2012), which showed a sensitivity of 61.6% (95% CI: 52.9–69.7). Of the 53 isolates incorrectly identified as susceptible by LPA, 24 had a range of rare katG mutations not identified by any of the katG probes; 17 had inhA mutations; and 12 were identified as wild type by sequencing. Another study (Maschmann et al. 2013) reported that all 19 strains misclassified as susceptible by LPA were found to have wild-type katG and inhA genes according to targeted sequencing, indicating that there may have been mutations in other genes associated with isoniazid resistance or efflux systems that could not be detected by LPA.

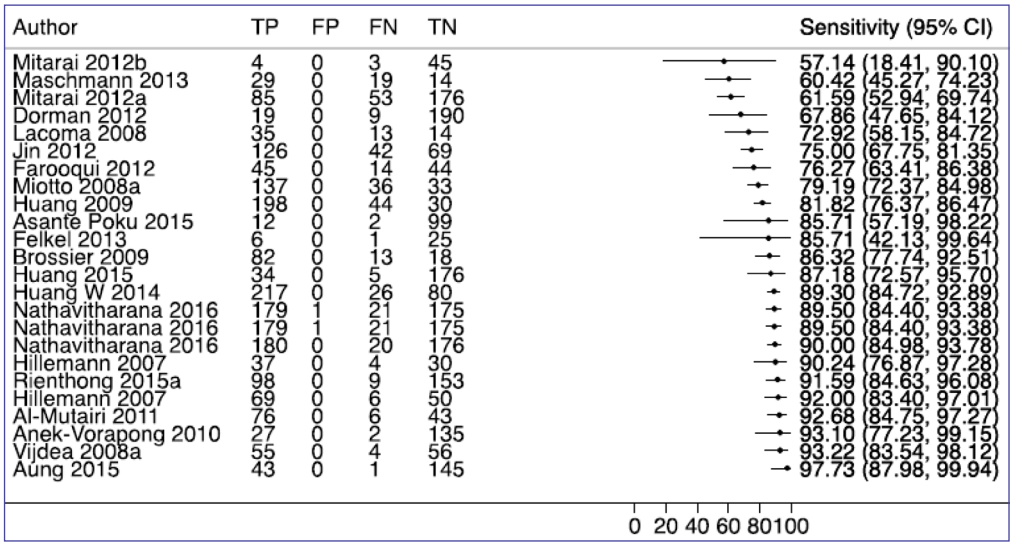
Figure 16. Forest plot for the sensitivity of all line probe assays evaluated for detecting isoniazid resistance by indirect testing of *Mycobacterium tuberculosis* complex culture isolates compared against a composite reference standard[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are ordered by decreasing sensitivity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

Figure 17. Forest plot for the specificity of all line probe assays evaluated for detecting isoniazid resistance by indirect testing of *Mycobacterium tuberculosis* complex culture isolates compared against a composite reference standard[a]



CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive.

a The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are ordered by decreasing sensitivity. Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

4.4 Accuracy in diagnosing MDR-TB

Altogether, 57 datasets included data on the diagnostic accuracy of LPA (direct and indirect testing) for detecting MDR-TB, with a total of 13 033 specimens that included 4 248 confirmed MDR-TB cases (33%). Bivariate meta-analysis of these datasets revealed a pooled sensitivity of 92.9% (95% CI: 90.4–94.8) and a pooled specificity of 99.3% (95% CI: 98.7–99.6). Three additional datasets contributed data only towards sensitivity but not specificity, increasing the total to 13 806 samples. A meta-analysis including these studies demonstrated a sensitivity of 91.4% (95% CI: 89.4–93.4). Specificity estimates were largely homogeneous except for a few outliers in which the number of sensitive (non-MDR) strains was fewer than 15, which was largely also the case for these sensitivity outliers.

Twelve datasets contained data comparing LPA with a composite reference standard (using the results from sequencing and phenotypic DST), with a total of 2 745 samples that included 1 315 MDR-TB cases (48%) (Fig. 15). Bivariate meta-analysis of these studies revealed a pooled sensitivity of 86.6% (95% CI: 81.9–90.3) and a pooled specificity of 99.6% (95% CI: 98.9–99.9). Bivariate analysis of the same 12 datasets compared with phenotypic culture-based DST revealed a pooled sensitivity of 86.9% (95% CI: 82.1–90.7) and a pooled specificity of 99.5% (95% CI: 97.9–99.9).

4.5 Diagnosing *Mycobacterium tuberculosis* complex infection

Data on diagnosing MTBC infection were limited because the majority of LPA studies identified by the systematic review did not report results for MTBC detection. Of the 21 datasets that did report data on MTBC detection, 15 studies were excluded because either they tested patients who were being treated (which can lead to false-positive LPA results due to the detection of dead bacilli) or did not specify that patients who were being treated were excluded.

Six datasets were included in the bivariate analysis, with a total of 3 451 samples that included 1 277 confirmed MTBC cases (37%). In all six of the datasets, LPA was performed directly on sputum specimens. Only one study (Crudu et al. 2012) evaluated Hain version 2. The other five studies evaluated Hain version 1 (Dorman et al. 2012, Felkel et al. 2012, Friedrich et al. 2011, Luetkemeyer et al. 2014, Scott et al. 2011). A meta-analysis of the datasets that reported both sensitivity and specificity revealed a pooled sensitivity of 85.0% (95% CI: 70.0–93.3) and a pooled specificity of 98.0% (95% CI: 96.2–99.0). In individual studies, the point estimates for sensitivity ranged from 49% to 100%, and the point estimates for specificity ranged from 52% to 100%. Moderate heterogeneity was seen among the sensitivity estimates for MTBC detection by LPA, with the point estimates ranging from 49% to 100%. The specificity estimates were homogeneous.

Five of the six datasets that evaluated LPAs on the direct testing of clinical specimens also reported smear status. Among sputum smear-positive specimens for all five studies – which accounted for 802 samples, of which 781 were confirmed MTBC cases – there was a pooled sensitivity of 94.4% (95% CI: 89.4 –99.4). Five studies included sputum smear-negative specimens, which accounted for 961 samples, of which 487 were confirmed MTBC cases. Of these, only four studies contributed data to both sensitivity and specificity, and a bivariate meta-analysis revealed a pooled sensitivity of 44.4% (95% CI: 20.2–71.7) and a specificity of 98.9% (95% CI: 95.4–99.7). The comparative study of all three LPA assays (Nathavitharana et al. 2016) found that smear grade affected rates of indeterminate results, and other studies also mentioned that smear grade affected the number of valid results, often resulting in studies evaluating only smear-positive specimens or selecting for analysis the specimens with the highest smear grade.

5. Summary: from evidence to recommendations

In patients with signs and symptoms consistent with TB, a positive LPA result for rifampicin resistance, isoniazid resistance or MDR-TB, or a combination of these, can be treated with confidence. Phenotypic resistance to rifampicin and isoniazid correlates strongly with resistance-conferring mutations detected by LPA. The diagnostic accuracy of LPA is similar when performed directly on sputum specimens or indirectly on cultured isolates of *M. tuberculosis*.

Given the confidence in a positive result showing rifampicin resistance and the ability of the test to provide results within a matter of days (compared with up to 3 weeks for phenotypic culture-based DST), LPA may be considered for use as an initial test for detecting resistance to first-line anti-TB agents. However, when the test shows a negative result for isoniazid, phenotypic culture-based DST can be performed, especially in persons with a high pre-test probability of isoniazid resistance, such as patients with rifampicin-resistant TB.

The use of LPAs in routine care should improve the time to diagnosis of drug-resistant TB, especially when used for the direct testing of a smear-positive sputum specimen from a patient with signs and symptoms consistent with TB who is at risk of MDR-TB. Early detection of resistance to rifampicin, isoniazid, or both, can allow for earlier initiation of appropriate therapy and improve patients' health outcomes. The accuracy of the assay in testing sputum smear-positive specimens is very good and interpretable results can be achieved in almost 95% of cases. In contrast, the limited data on the use of LPAs in sputum smear-negative specimens suggest that with direct testing only 44% of results will be interpretable. The GDG felt that the yield of LPA if used for all persons with signs and symptoms of TB would be suboptimal because the large majority of persons tested would have a negative LPA result. As a consequence, direct testing of sputum smear-negative specimens is not recommended.

Online annex 2 contains the GRADE summary of findings tables that summarize the review findings for direct and indirect testing of LPAs by applying the results to a hypothetical cohort of 1 000 individuals with signs and symptoms consistent with TB.

5.1 Using line probe assays to detect resistance to rifampicin

When used for direct testing, LPAs detect 96% of people whose TB has rifampicin resistance and rarely give a positive result for people whose TB is susceptible (see Online annex 2, Tables 5–7). Sensitivity did not change when a composite reference standard was used because no false-negative LPA results were reclassified based on the results of sequencing. However, specificity increased when a composite standard was used, as 37 LPA false-positive results (from 11 datasets) were reclassified as true positives based on sequencing, confirming a known resistance-conferring mutation. Commercial liquid DST using the BACTEC MGIT failed to detect some clinically relevant strains with *rpo*B mutations,[16] which suggests that sequencing of the *rpo*B gene may serve as a better reference method than phenotypic culture-based DST for detecting rifampicin resistance.

In a population of 1 000 people in which 150 have TB with rifampicin resistance (15% pre-test probability, the lower limit of rifampicin resistance among previously treated TB cases), LPAs will correctly identify 144 people with rifampicin resistance and miss 6 people. In this same population of 1 000 people in which 850 people have TB without rifampicin resistance, the test

[16] Van Deun A, Aung KJ, Bola V, Lebeke R, Hossain MA, de Rijk WB, et al. Rifampicin drug resistance tests for tuberculosis: challenging the gold standard. J Clin Microbiol. 2013;51(8):2633–40. doi:10.1128/JCM.00553-13.

will correctly classify 835 people as not having rifampicin resistance and misclassify 15 people as having resistance.

In a situation in which the pre-test probability is 5% (which is considered to be the upper limit of the prevalence of rifampicin resistance among new TB cases), the number of missed people with TB (false negatives) will decrease to 2, whereas the number of persons misclassified as having resistance (false positives) will increase to 17. Hence, as the pre-test probability for detecting rifampicin resistance increases, fewer patients will be misclassified as having resistance.

The GDG felt that both false-positive and false-negative rifampicin resistance results may cause harms to a patient. The consequences for patients wrongly diagnosed with rifampicin resistance (false positives) are likely to be anxiety, possible delays in further diagnostic evaluation, and prolonged and unnecessary treatment with anti-TB agents that may have additional serious adverse effects or less efficacy, or both. The consequences of the false-negative results for patients in the hypothetical scenario of 1 000 persons are the potential for increased risks of morbidity and mortality for the patient and the continued risk of community transmission of drug-resistant TB.

Given the speed of the test and its accuracy, and considering the balance of harms and benefits, the GDG made a conditional recommendation in favour of using an LPA as the initial test to detect rifampicin resistance among patients with signs and symptoms consistent with TB (see Online annex 3, Tables 12 and 13).

5.2 Using line probe assays to detect resistance to isoniazid

When used for direct testing, LPAs detect 89% of people with isoniazid resistance and rarely give a positive result for people without resistance (Online annex 2, Tables 8–10). Neither the sensitivity nor the specificity for detecting isoniazid resistance changed significantly when a composite reference standard was used.

A prevalence of 5% is considered the lower end of observed isoniazid monoresistance in some countries (for example, in Bangladesh, Malawi and Myanmar). A prevalence of 15% would be considered the higher end of observed isoniazid monoresistance in other countries (for example, in Azerbaijan, Latvia and Viet Nam). In a theoretical population of 1 000 people in which 150 of them have TB with isoniazid resistance (15% pre-test probability), LPA will correctly identify 134 people with isoniazid resistance and miss 16 people. In this same population of 1 000 people in which 850 have TB without isoniazid resistance, the test will correctly classify 836 people as not having isoniazid resistance and misclassify 14 people as having resistance.

In a population of 1 000 people in which 50 of them have TB with isoniazid resistance (5% pre-test probability), LPA will correctly identify 45 people with isoniazid resistance and miss 5 people. In this same population of 1 000 people in which 950 have TB without isoniazid resistance, the test will correctly classify 935 people as not having isoniazid resistance and misclassify 15 people as having resistance.

Isoniazid resistance is highly correlated with resistance to rifampicin. A pre-test probability for isoniazid resistance of 90% is considered the lower limit for rifampicin-associated isoniazid resistance in the majority of settings. Hence, in a population of 1 000, 803 TB patients out of 900 patients will be correctly identified. Under this pre-test probability, the number of patients incorrectly classified as not having isoniazid resistance (false negatives) and the number of patients without isoniazid resistance (true negatives) will be almost the same: 97 and 98 patients, respectively. Thus, a negative LPA result for isoniazid resistance in patients with rifampicin resistance is not considered to be reliable, and phenotypic culture-based DST is necessary to confirm or exclude resistance to isoniazid.

Resistance-conferring mutations in *inh*A and *kat*G genes account for approximately 90% of isoniazid resistance detected by phenotypic DST

methods. Different mutations are associated with different levels (MICs) of resistance to isoniazid. Mutations in the promoter region of the *inh*A gene are normally associated with low-level resistance to isoniazid and with cross-resistance to the thioamides (ethionamide and prothionamide).[17, 18] The presence of a *katG* 315 mutation alone is associated with elevated MICs.[19, 20] Although resistance associated with *katG* is almost always encoded by the same mutation, (that is Ser315Thr), MICs vary considerably, with a mean of around 5 µg/ml or the peak serum concentration after a normal dose of isoniazid. With high-dose isoniazid, this peak increases proportionally. In only a minority of strains with this mutation are therapeutically achievable levels exceeded.[21]

Mutations outside the hotspot regions are uncommon, but the presence of double mutations in the coding region and promoter regions of the *inh*A gene have been reported to be associated with high-level isoniazid resistance.[22]

The GDG felt that both false-positive and false-negative isoniazid resistance results may cause harms to a patient. The consequences for patients wrongly diagnosed with isoniazid resistance (false positives) are likely patient anxiety, possible delays in further diagnostic evaluation, and prolonged and unnecessary treatment with anti-TB agents that may have additional serious adverse effects. The consequences of false-negative results for patients in the hypothetical scenario of 1 000 persons are the potential for increased risks of morbidity and mortality for the patient and a continued risk of community transmission of drug-resistant TB.

Given the speed of performing the test and considering the balance of harms and benefits, the GDG made a conditional recommendation in favour of using an LPA as the initial test to detect isoniazid resistance among patients at risk of MDR-TB. Conventional culture-based DST should be used in the follow-up evaluation of patients with a high risk for isoniazid resistance and a negative LPA result, especially in settings with a high pre-test probability of resistance to isoniazid (Online annex 3, Tables 14 and 15).

5.3 Using line probe assays to detect MDR-TB

When used for direct testing, LPAs detect 93% of people with MDR-TB and rarely give a positive result for people without resistance. In a population of 1 000 people in which 100 have MDR-TB, LPA will correctly identify 93 people with MDR-TB and miss 7 people. In this same population of 1 000 people in which 900 do not have MDR-TB, the test will correctly classify 894 people as not having MDR-TB and misclassify 6 people as having MDR-TB (Online annex 2, Table 11).

The consequences for patients wrongly diagnosed with MDR-TB (false positives) and the MDR-TB patients who are missed (false negatives) are similar to those that occur with the detection of rifampicin resistance and isoniazid resistance (see Sections 5.1 and 5.2).

LPAs have varying accuracy for detecting resistance-conferring mutations to rifampicin and isoniazid, the combination of which lower their overall accuracy for detecting MDR-TB. As a

[17] Vilchèze C, Wang F, Arai M, Hazbón MH, Colangeli R, Kremer L, et al. Transfer of a point mutation in *Mycobacterium tuberculosis inh*A resolves the target of isoniazid. Nat Med, 2006;12:1027–9. doi:10.1038/nm1466.

[18] Morlock G, Metchock B, Sikes D, Crawford JT, Cooksey RC. *eth*A, *inh*A, and *kat*G loci of ethionamide-resistant clinical *Mycobacterium tuberculosis* isolates. Antimicrob Agents Chemother. 2003;47(12):3799–805. doi:10.1128/AAC.47.12.3799-3805.2003.

[19] Kambli P, Ajbani K, Sadani M, Nikam C, Shetty A, Udwadia Z, et al. Defining multidrug-resistant tuberculosis: correlating GenoType MTBDR*plus* assay results with minimum inhibitory concentrations. Diagn Microbiol Infect Dis. 2015:82(1);49–53. doi:10.1016/j.diagmicrobio.2015.01.009.

[20] Böttger EC. The ins and outs of *Mycobacterium tuberculosis* drug susceptibility testing. Clin Microbiol Infect. 2011;17(8):1128–34. doi:10.1111/j.1469-0691.2011.03551.x.

[21] Rieder H, Van Deun A. Rationale for high-dose isoniazid in the treatment of multidrug-resistant tuberculosis. Int J Tuberc Lung Dis. In press.

[22] Machado D, Perdigão J, Ramos J, Couto I, Portugal I, Ritter C, et al.. High-level resistance to isoniazid and ethionamide in multidrug-resistant *Mycobacterium tuberculosis* of the Lisboa family is associated with inhA double mutations. J Antimicrob Chemother. 2013 Aug;68(8):1728–32. doi:10.1093/jac/dkt090.

consequence, the GDG decided that LPAs can be used for diagnosing MDR-TB but acknowledged that the diagnostic accuracy is suboptimal. The GDG did feel that LPAs could be used for the surveillance of MDR-TB, given statistical approaches that can adjust for its lower sensitivity and specificity during surveillance studies.

6. WHO's policy recommendations

For persons with a sputum smear-positive specimen or a cultured isolate of MTBC, commercial molecular LPAs may be used as the initial test instead of phenotypic culture-based DST to detect resistance to rifampicin and isoniazid (conditional recommendation, moderate certainty in the evidence for the test's accuracy).

Remarks

a. These recommendations apply to the use of LPAs for testing sputum smear-positive specimens (direct testing) and cultured isolates of MTBC (indirect testing) from both pulmonary and extrapulmonary sites.

b. LPAs are not recommended for the direct testing of sputum smear-negative specimens.

c. These recommendations apply to the detection of MTBC and the diagnosis of MDR-TB acknowledge that the accuracy of detecting resistance to rifampicin and isoniazid differs and, hence, the overall accuracy of a diagnosis of MDR-TB is reduced overall.

d. These recommendations do not eliminate the need for conventional culture-based DST, which will be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance.

e. Conventional culture-based DST for isoniazid may still be used to evaluate patients when the LPA result does not detect isoniazid resistance. This is particularly important for populations with a high pre-test probability of resistance to isoniazid.

f. These recommendations apply to the use of LPA in children based on the generalization of data from adults.

7. Implementation considerations

Adopting LPAs for detecting rifampicin resistance and isoniazid resistance does not eliminate the need for capacity for conventional culture and DST. Culture and phenotypic culture-based DST have critical roles in monitoring patients' responses to treatment and detecting additional resistance to second-line agents.

- The adoption of LPA should be phased in, starting at national or central reference laboratories or those with proven capability to conduct molecular testing. Expansion could be considered, within the context of a country's plans for laboratory strengthening, the availability of suitable personnel in peripheral centres and the quality of

specimen transport systems.

- Adequate and appropriate laboratory infrastructure and equipment should be provided to ensure that the required precautions for biosafety and the prevention of contamination are met: specimen processing for culture and procedures for manipulating cultures must be performed in biological safety cabinets in TB-containment laboratories.
- Laboratory facilities for LPAs require at least three separate rooms, one each for DNA extraction, pre-amplification procedures, and amplification and post-amplification procedures. To avoid contamination, access to molecular facilities must be restricted, a unidirectional work flow must be implemented, and stringent cleaning protocols must be established.
- Appropriate laboratory staff should be trained to conduct LPA procedures. It is strongly recommended that staff are supervised by a senior staff member with adequate training and experience in molecular assays. A programme for the external quality assessment of laboratories using LPAs should be developed as a priority.
- Mechanisms for rapidly reporting LPA results to clinicians must be established to provide patients with the benefit of early diagnosis. The same infrastructure used for performing LPAs can be used also to perform second-line LPAs.
- LPAs are designed to detect TB and resistance to rifampicin and isoniazid in the direct testing of processed sputum samples and in the indirect testing of culture isolates of MTBC. The use of LPAs with other respiratory samples (for example, from bronchoalveolar lavage or gastric aspiration) or extrapulmonary samples (such as tissue samples, cerebrospinal fluid or other body fluids) have not been adequately evaluated.
- The availability of second-line agents is critical in the event that resistance to rifampicin or isoniazid, or both, is detected.
- For patients with confirmed rifampicin-resistant TB or MDR-TB, second-line LPAs are recommended to detect additional resistance to second-line anti-TB agents.

7.1 Plans for disseminating WHO's policy guidance on using line probe assays

This WHO guidance will be published online (http://www.who.int/tb/laboratory/policy_statements/en/) and disseminated through WHO's Global TB Department LISTSERV to all WHO Regional and Country Offices and Member States, the Global Laboratory Initiative, the TB/HIV Working Group and New Diagnostics Working Groups of the Stop TB Partnership, and to donors, technical agencies and other stakeholders.

8. Research needs

Current recommendations on LPAs should not prevent or restrict further research on new, rapid molecular drug-susceptibility tests, especially for assays that can be used as close as possible to where patients with a presumptive diagnosis of TB are identified and where treatment can be initiated. Further operational research on LPAs should focus on the following priorities:

- developing and improving understanding of the correlation between the detection of resistance-conferring mutations using culture-based DST and patients' outcomes;
- reviewing evidence to confirm or revise

different critical concentrations used in culture-based DST methods;

- determining the limit of detection for LPA in detecting heteroresistance;
- determining needs for training, assessing competency and ensuring quality assurance;
- gathering more evidence on the impact on mortality of initiating appropriate treatment for MDR-TB ;
- meeting the Standards for reporting Diagnostic Accuracy studies (known as STARD) for future diagnostic studies (see http://www.equator-network.org/reporting-guidelines/stard/);
- performing country-specific cost–effectiveness and cost–benefit analyses of LPA use in different programmatic settings.

9. Online annexes

Online annexes are available at http://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/.

Annex 2. GRADE summary of findings tables

Annex 3. Evidence to recommendations tables

Annex 4. References to studies excluded from the review

Annex 1. Studies included in the review of the diagnostic accuracy of line probe assays

Al-Mutairi NM, Ahmad S, Mokaddas E (2011). Performance comparison of four methods for detecting multidrug-resistant *Mycobacterium tuberculosis* strains. Int J Tuberc Lung Dis. 2011;15(1):110–15.

Albert H, Bwanga F, Mukkada S, Nyesiga B, Ademun JP, Lukyamuzi G, et al (2010). Rapid screening of MDR-TB using molecular line probe assay is feasible in Uganda. BMC Infect Dis.10:41. doi:10.1186/1471-2334-10-41.

Anek-Vorapong R, Sinthuwattanawibool C, Podewils LJ, McCarthy K, Ngamlert K, Promsarin B, et al (2010). Validation of the GenoType MTBDR*plus* assay for detection of MDR-TB in a public health laboratory in Thailand. BMC Infect Dis. 10:123. doi:10.1186/1471-2334-10-123.

Asante-Poku A, Otchere ID, Danso E, Mensah DD, Bonsu F, Gagneux S, et al (2015). Evaluation of GenoType MTBDR*plus* for the rapid detection of drug-resistant tuberculosis in Ghana. Int J Tuberc Lung Dis.19(8):954–59. doi:10.5588/ijtld.14.0864.

Asencios L, Galarza M, Quispe N, Vásquez L, Leo E, Valencia E, et al (2012). [Molecular test Genotype® MTBDR*plus*, an alternative to rapid detection of multidrug resistance tuberculosis.] Rev Peru Med Exp Salud Publica. 29(1):92–98. doi:10.1590/S1726-46342012000100014.

Aung WW, Ei PW, Nyunt WW, Swe TL, Lwin T, Htwe MM, et al (2015). Phenotypic and genotypic analysis of anti-tuberculosis drug resistance in *Mycobacterium tuberculosis* Isolates in Myanmar. Ann Lab Med. 35(5):494–99. doi:10.3343/alm.2015.35.5.494.

Aurin TH, Munshi SK, Kamal SM, Rahman MM, Hossain MS, Marma T, et al (2014). Molecular approaches for detection of the multi-drug resistant tuberculosis (MDR-TB) in Bangladesh. PLOS ONE. 9(6):e99810. doi:10.1371/journal.pone.0099810.

Bablishvili N, Tukvadze N, Avaliani Z, Blumberg HM, Kempker RR (2015). A comparison of the Xpert® MTB/RIF and GenoType® MTBDR*plus* assays in Georgia. Int J Tuberc Lung Dis. 19(6):676–78. doi:10.5588/ijtld.14.0867.

Banu S, Rahman SMM, Khan MSR, Ferdous SS, Ahmed S, Gratz J, et al (2014). Discordance across several methods for drug susceptibility testing of drug-resistant *Mycobacterium tuberculosis* isolates in a single laboratory. J Clin Microbiol. 52(1):156–63. doi:10.1128/JCM.02378-13.

Barnard M, Albert H, Coetzee G, O'Brien R, Bosman ME (2008). Rapid molecular screening for multidrug-resistant tuberculosis in a high-volume public health laboratory in South Africa. Am J Respir Crit Care Med. 177(7):787–92. doi:10.1164/rccm.200709-1436OC.

Brossier F, Veziris N, Jarlier V, Sougakoff W (2009). Performance of MTBDRplus for detecting high/low levels of *Mycobacterium tuberculosis* resistance to isoniazid. Int J Tuberc Lung Dis. 13(2):260–65.

Bwanga F, Joloba ML, Haile M, Hoffner S (2010). Evaluation of seven tests for the rapid detection of multidrug-resistant tuberculosis in Uganda. Int J Tuberc Lung Dis. 14(7):890–95.

Cabibbe AM, Miotto P, Moure R, Alcaide F, Feuerriegel S, Pozzi G, et al (2015). A lab-on-chip based platform for fast molecular diagnosis of multi-drug resistant tuberculosis. J Clin Microbiol. 53(12):3876–80. doi:10.1128/JCM.01824-15.

Catanzaro A, Rodwell TC, Catanzaro DG, Garfein RS, Jackson RL, Seifert M, et al (2015). Performance comparison of three rapid tests for

the diagnosis of drug-resistant tuberculosis. PLOS ONE. 10(8):e0136861. doi:10.1371/journal.pone.0136861.

Causse M, Ruiz P, Gutierrez JB, Zerolo J, Casal M (2008). Evaluation of new GenoType® MTBDR*plus* for detection of resistance in cultures and direct specimens of *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis. 12(12):1456–60.

Chen C, Kong W, Zhu L, Zhou Y, Peng H, Shao Y, et al (2014). Evaluation of the GenoType® MTBDR*plus* line probe assay on sputum-positive samples in routine settings in China. Int J Tuberc Lung Dis. 18(9):1034–39. doi:10.5588/ijtld.13.0857.

Chryssanthou E, Angeby K (2012). The GenoType® MTBDR*plus* assay for detection of drug resistance in *Mycobacterium tuberculosis* in Sweden. APMIS. 120(5):405–9. doi:10.1111/j.1600-0463.2011.02845.x.

Crudu V, Stratan E, Romancenco E, Allerheiligen V, Hillemann A, Moraru N (2012). First evaluation of an improved assay for molecular genetic detection of tuberculosis as well as rifampin and isoniazid resistances. J Clin Microbiol. 50(4):1264–69. doi:10.1128/JCM.05903-11.

Daum LT, Rodriguez JD, Worthy SA, Ismail NA, Omar SV, Dreyer AW, et al (2012). Next-generation ion torrent sequencing of drug resistance mutations in *Mycobacterium tuberculosis* strains. J Clin Microbiol. 50(12):3831–37. doi:10.1128/JCM.01893-12.

Dorman SE, Chihota VN, Lewis JJ, van der Meulen M, Mathema B, Beylis N, et al (2012). Genotype MTBDR*plus* for direct detection of *Mycobacterium tuberculosis* and drug resistance in strains from gold miners in South Africa. J Clin Microbiol. 50(4):1189–94. doi:10.1128/JCM.05723-11.

Dubois Cauwelaert N, Ramarokoto H, Ravololonandriana P, Richard V, Rasolofo V (2011). DNA extracted from stained sputum smears can be used in the MTBDR*plus* assay. J Clin Microbiol. 49(10):3600–3. doi:10.1128/JCM.00745-11.

Eliseev PI, Maryandyshev AO, Nikishova EI, Tarasova IV, Gorina GP, Chryssanthou E, et al (2013). Epidemiological analyses of tuberculosis in Archangelsk, Russia, and implementation of a rapid assay for detection of resistance in this high burden setting. Int J Mycobacteriol. 2(2):103–8. doi:10.1016/j.ijmyco.2013.04.002.

Evans J, Stead MC, Nicol MP, Segal H (2009). Rapid genotypic assays to identify drug-resistant *Mycobacterium tuberculosis* in South Africa. J Antimicrob Chemother. 63(1):11–16. doi:10.1093/jac/dkn433.

Fabre M, Hauck Y, Pourcel C, Vergnaud G, Vong R, Soler C (2011). [Performances of the assay MTBDR*plus*® in the surveillance of rifampicin resistance in *Mycobacterium tuberculosis*.] Pathol Biol. 59(2):94–96. doi:10.1016/j.patbio.2010.07.006.

Farooqi JQ, Khan E, Alam SM, Ali A, Hasan Z, Hasan R (2012). Line probe assay for detection of rifampicin and isoniazid resistant tuberculosis in Pakistan. J Pak Med Assoc. 62(8):767–72.

Felkel M, Exner R, Schleucher R, Lay H, Autenrieth IB, Kempf VA, et al (2013). Evaluation of *Mycobacterium tuberculosis* drug susceptibility in clinical specimens from Nigeria using Genotype MTBDR*plus* and MTBDRsl assays. Eur J Microbiol Immunol. 3(4):252-257. doi:10.1556/EuJMI.3.2013.4.3.

Ferro BE, Garcia PK, Nieto LM, van Soolingen D (2013). Predictive value of molecular drug resistance testing of *Mycobacterium tuberculosis* isolates in Valle del Cauca, Colombia. J Clin Microbiol. 51(7):2220–24. doi:10.1128/JCM.00429-13.

Friedrich SO, Venter A, Kayigire XA, Dawson R, Donald PR, Diacon AH (2011). Suitability of Xpert MTB/RIF and genotype MTBDR*plus* for patient selection for a tuberculosis clinical trial. J Clin Microbiol. 49(8):2827–31. doi:10.1128/JCM.00138-11.

Gauthier M, Somoskovi A, Berland JL, Ocheretina O, Mabou MM, Boncy J, et al (2014). Stepwise implementation of a new diagnostic algorithm for multidrug-resistant tuberculosis in Haiti. Int J Tuberc Lung Dis. 18(2):220–26. doi:10.5588/ijtld.13.0513.

Gitti Z, Mantadakis E, Maraki S, Samonis G (2011). GenoType® MTBDR*plus* compared with conventional drug-susceptibility testing of *Mycobacterium tuberculosis* in a low-resistance locale. Future Microbiol. 6(3):357–62. doi:10.2217/fmb.11.8.

Hillemann D, Rusch-Gerdes S, Richter E (2007). Evaluation of the GenoType MTBDR*plus* assay for rifampin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens. J Clin Microbiol. 45(8):2635–40. doi:10.1128/JCM.00521-07.

Huang FL, Jin JL, Chen S, Zhou Z, Diao N, Huang HQ, et al (2015). MTBDR*plus* results correlate with treatment outcome in previously treated tuberculosis patients. Int J Tuberc Lung Dis. 19(3):319–25. doi:10.5588/ijtld.14.0406.

Huang WL, Chen HY, Kuo YM, Jou R (2009). Performance assessment of the GenoType MTBDR*plus* test and DNA sequencing in detection of multidrug-resistant *Mycobacterium tuberculosis*. J Clin Microbiol. 47(8):2520–24. doi:10.1128/JCM.02499-08.

Huang WL, Hsu ZJ, Chang TC, Jou R (2014). Rapid and accurate detection of rifampin and isoniazid-resistant *Mycobacterium tuberculosis* using an oligonucleotide array. Clin Microbiol Infect. 20(9):O542-549. doi:10.1111/1469-0691.12517.

Huyen MN, Tiemersma EW, Lan NT, Cobelens FG, Dung NH, Sy DN, et al (2010). Validation of the GenoType® MTBDR*plus* assay for diagnosis of multidrug resistant tuberculosis in South Vietnam. BMC Infect Dis. 10:149. doi:10.1186/1471-2334-10-149.

Imperiale BR, Zumarraga MJ, Weltman G, Zudiker R, Cataldi AA, Morcillo NS (2012). First evaluation in Argentina of the GenoType® MTBDR*plus* assay for multidrug-resistant *Mycobacterium tuberculosis* detection from clinical isolates and specimens. Rev Argent Microbiol. 44(4):283–89.

Jin J, Zhang Y, Fan X, Diao N, Shao L, Wang F, et al (2012). Evaluation of the GenoType® MTBDR*plus* assay and identification of a rare mutation for improving MDR-TB detection. Int J Tuberc Lung Dis. 16(4):521–26. doi:10.5588/ijtld.11.0269.

Kapata N, Mbulo G, Cobelens F, de Haas P, Schaap A, Mwamba P, et al (2015). The Second Zambian National Tuberculosis Drug Resistance survey – a comparison of conventional and molecular methods. Trop Med Int Health. 20(11):1492–1500. doi:10.1111/tmi.12581.

Khadka JB, Rai SK, Shrestha S, Maharjan B, Bhatta DR, Ghimire P (2011). Study of rifampicin and isoniazid resistance mutation genes of M. tuberculosis isolates in Nepal. Nepal Med Coll J. 13(3):147–51.

Kumar P, Balooni V, Sharma BK, Kapil V, Sachdeva KS, Singh S (2014). High degree of multi-drug resistance and hetero-resistance in pulmonary TB patients from Punjab state of India. Tuberculosis. 94(1):73–80. doi:10.1016/j.tube.2013.10.001.

Lacoma A, Garcia-Sierra N, Prat C, Ruiz-Manzano J, Haba L, Roses S, et al (2008). GenoType MTBDR*plus* assay for molecular detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* strains and clinical samples. J Clin Microbiol. 46(11):3660–67. doi:10.1128/JCM.00618-08.

Li Q, Dong HY, Pang Y, Xia H, Ou XC, Zhang ZY, et al (2015). Multicenter evaluation of the molecular line probe assay for multidrug resistant *Mycobacterium tuberculosis* detection in China. Biomed Environ Sci. 28(6):464–67. doi:10.3967/bes2015.066.

Luetkemeyer AF, Kendall MA, Wu X, Lourenco MC, Jentsch U, Swindells S, et al (2014). Evaluation of two line probe assays for rapid detection of *Mycobacterium tuberculosis*, tuberculosis (TB) drug resistance, and non-TB mycobacteria in HIV-infected individuals with suspected TB. J Clin Microbiol. 52(4):1052–59. doi:10.1128/JCM.02639-13.

Lyu J, Kim MN, Song JW, Choi CM, Oh YM, Lee SD, et al (2013). GenoType® MTBDR*plus* assay detection of drug-resistant tuberculosis in routine practice in Korea. Int J Tuberc Lung Dis. 17(1):120–24. doi:10.5588/ijtld.12.0197.

Macedo R, Amorim A, Pereira E (2009). Multidrug-resistant tuberculosis: rapid molecular detection with MTBDR*plus* assay in clinical samples. Rev Port Pneumol. 15(3):353–65.

Maschmann Rde A, Sá Spies F, Nunes Lde S, Ribeiro AW, Machado TR, Zaha A, et al (2013). Performance of the GenoType MTBDR*plus* assay directly on sputum specimens from Brazilian patients with tuberculosis treatment failure or relapse. J Clin Microbiol. 51(5):1606–8. doi:10.1128/JCM.00364-13.

Miotto P, Piana F, Cirillo DM, Migliori GB (2008). Genotype MTBDR*plus*: a further step toward rapid identification of drug-resistant *Mycobacterium tuberculosis*. J Clin Microbiol. 46(1):393–94. doi:10.1128/JCM.01066-07.

Miotto P, Saleri N, Dembele M, Ouedraogo M, Badoum G, Pinsi G, et al (2009). Molecular detection of rifampin and isoniazid resistance to guide chronic TB patient management in Burkina Faso. BMC Infect Dis. 9:142. doi:10.1186/1471-2334-9-142.

Mironova S, Pimkina E, Kontsevaya I, Nikolayevskyy V, Balabanova Y, Skenders G, et al (2012). Performance of the GenoType® MTBDR*plus* assay in routine settings: a multicenter study. Eur J Clin Microbiol Infect Dis. 31(7):1381–87. doi:10.1007/s10096-011-1453-1.

Mitarai S, Kato S, Ogata H, Aono A, Chikamatsu K, Mizuno K, et al (2012). Comprehensive multicenter evaluation of a new line probe assay kit for identification of Mycobacterium species and detection of drug-resistant *Mycobacterium tuberculosis*. J Clin Microbiol. 50(3):884–90. doi:10.1128/JCM.05638-11.

N'Guessan K, Assi JS, Ouassa T, Ahui-Brou JM, Tehe A, Keita Sow M, et al (2014). Assessment of the Genotype MTBDR*plus* assay for rifampin and isoniazid resistance detection on sputum samples in Côte d'Ivoire. Eur J Microbiol Immunol. 4(3):166–73. doi:10.1556/EUJMI-D-14-00014.

Nathavitharana RR, Hillemann D, Schumacher SG, Schlueter B, Ismail N, Vally Omar S, et al (2016). Multicenter noninferiority evaluation of Hain GenoType MTBDR*plus* version 2 and Nipro NTM+MDRTB line probe assays for the diagnosis of rifampin and isoniazid resistance. J Clin Microbiol. 54(6):1624–30. doi:10.1128/JCM.00251-16.

Niehaus AJ, Mlisana K, Gandhi NR, Mathema B, Brust JC (2015). High prevalence of inhA promoter mutations among patients with drug-resistant tuberculosis in KwaZulu-Natal, South Africa. PLOS ONE. 10(9):e0135003. doi:10.1371/journal.pone.0135003.

Nikolayevskyy V, Balabanova Y, Simak T, Malomanova N, Fedorin I, Drobniewski F (2009). Performance of the Genotype® MTBDR*plus* assay in the diagnosis of tuberculosis and drug resistance in Samara, Russian Federation. BMC Clin Pathol. 9:2. doi:10.1186/1472-6890-9-2.

Nwofor AC, Nyamngee A, Nwabuisi C, Iwakun M, Gidado M, Mensah C, et al (2015). Performance of Genotype MTBDR*plus* in the detection of resistance to rifampicin and isoniazid among clinical mycobacteria isolates in Ilorin, Nigeria. Curr HIV Res. 13(4):308–14. doi:10.2174/1570162X13666150407143147.

Ocheretina O, Escuyer VE, Mabou MM, Royal-Mardi G, Collins S, Vilbrun SC, et al (2014). Correlation between genotypic and phenotypic testing for resistance to rifampin in *Mycobacterium tuberculosis* clinical isolates in Haiti: investigation

of cases with discrepant susceptibility results. PLOS ONE. 9(3):e90569. doi:10.1371/journal.pone.0090569.

Raizada N, Sachdeva KS, Chauhan DS, Malhotra B, Reddy K, Dave PV, et al (2014). A multi-site validation in India of the line probe assay for the rapid diagnosis of multi-drug resistant tuberculosis directly from sputum specimens. PLOS ONE. 9(2):e88626. doi:10.1371/journal.pone.0088626.

Raveendran R, Wattal C, Oberoi JK, Goel N, Datta S, Prasad KJ (2012). Utility of GenoType MTBDR*plus* assay in rapid diagnosis of multidrug resistant tuberculosis at a tertiary care centre in India. Indian J Med Microbiol. 30(1):58–63. doi:10.4103/0255-0857.93034.

Rienthong S, Boonin C, Chaiyasirinrote B, Satproedprai N, Mahasirimongkol S, Yoshida H, et al (2015). Evaluation of a novel line-probe assay for genotyping-based diagnosis of *Mycobacterium tuberculosis* in Thailand. Int J Tuberc Lung Dis. 19(7):817–22. doi:10.5588/ijtld.14.0311.

Rigouts L, Hoza AS, De Rijk P, Torrea G, Chonde TM, Basra D, et al (2011). Evaluation of the Genotype® MTBDR*plus* assay as a tool for drug resistance surveys. Int J Tuberc Lung Dis. 15(7):959–65. doi:10.5588/ijtld.10.0515.

Rufai SB, Kumar P, Singh A, Prajapati S, Balooni V, Singh S (2014). Comparison of Xpert MTB/RIF with line probe assay for detection of rifampin-monoresistant *Mycobacterium tuberculosis*. J Clin Microbiol. 52(6):1846–52. doi:10.1128/JCM.03005-13.

Sangsayunh P, Chuchothawon C (2014). Genotype MTBDR*plus* (Hain) test in suspected MDR-TB patients. J Med Assoc Thai. 97(10):1028–32.

Schön T, Jureen P, Chryssanthou E, Giske CG, Kahlmeter G, Hoffner S, et al (2013). Rifampicin-resistant and rifabutin-susceptible *Mycobacterium tuberculosis* strains: a breakpoint artefact? J Antimicrob Chemother. 68(9):2074–77. doi:10.1093/jac/dkt150.

Scott LE, McCarthy K, Gous N, Nduna M, Van Rie A, Sanne I, et al (2011). Comparison of Xpert MTB/RIF with other nucleic acid technologies for diagnosing pulmonary tuberculosis in a high HIV prevalence setting: a prospective study. PLOS Med. 8(7):e1001061. doi:10.1371/journal.pmed.1001061.

Shubladze N, Tadumadze N, Bablishvili N (2013). Molecular patterns of multidrug resistance of in Georgia. Int J Mycobacteriol. 2(2):73–78. doi:10.1016/j.ijmyco.2013.02.002.

Simons SO, van der Laan T, de Zwaan R, Kamst M, van Ingen J, Dekhuijzen PN, et al (2015). Molecular drug susceptibility testing in the Netherlands: performance of the MTBDR*plus* and MTBDRsl assays. Int J Tuberc Lung Dis. 19(7):828–33. doi:10.5588/ijtld.15.0043.

Singhal R, Arora J, Lal P, Bhalla M, Myneeedu VP, Behera D (2012). Comparison of line probe assay with liquid culture for rapid detection of multi-drug resistance in *Mycobacterium tuberculosis*. Indian J Med Res. 136(6):1044–47.

Tessema B, Beer J, Emmrich F, Sack U, Rodloff AC (2012). Analysis of gene mutations associated with isoniazid, rifampicin and ethambutol resistance among *Mycobacterium tuberculosis* isolates from Ethiopia. BMC Infect Dis. 12:37. doi:10.1186/1471-2334-12-37.

Tho DQ, Lan NT, Chau NV, Farrar J, Caws M (2011). Multiplex allele-specific polymerase chain reaction for detection of isoniazid resistance in *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis. 15(6):799–803. doi:10.5588/ijtld.10.0599.

Tolani MP, D'Souza DT, Mistry NF (2012). Drug resistance mutations and heteroresistance detected using the GenoType MTBDR*plus* assay and their implication for treatment outcomes in patients from Mumbai, India. BMC Infect Dis. 12:9. doi:10.1186/1471-2334-12-9.

Tukvadze N, Kempker RR, Kalandadze I, Kurbatova E, Leonard MK, Apsindzelashvili R, et al (2012). Use of a molecular diagnostic test in AFB smear positive tuberculosis suspects greatly reduces time to detection of multidrug resistant tuberculosis. PLOS ONE. 7(2):e31563. doi:10.1371/journal.pone.0031563.

Vijdea R, Stegger M, Sosnovskaja A, Andersen AB, Thomsen VO, Bang D (2008). Multidrug-resistant tuberculosis: rapid detection of resistance to rifampin and high or low levels of isoniazid in clinical specimens and isolates. Eur J Clin Microbiol Infect Dis. 27(11):1079–86. doi:10.1007/s10096-008-0548-9.

Yadav RN, Singh BK, Sharma SK, Sharma R, Soneja M, Sreenivas V, et al (2013). Comparative evaluation of GenoType MTBDR*plus* line probe assay with solid culture method in early diagnosis of multidrug resistant tuberculosis (MDR-TB) at a tertiary care centre in India. PLOS ONE. 8(9):e72036. doi:10.1371/journal.pone.0072036.

Yordanova S, Bachiyska E, Atanasova Y, Todorova Y, Baikova A, Kantardjiev T (2013). Multidrug resistant tuberculosis in Bulgaria – microbiological aspects. Probl Infect Parasit Dis. 41(1):5–8.

Global TB Programme
World Health Organization
Avenue Appia 20, CH-1211
Geneva-27, Switzerland

Information Resource Centre HTM/GTB:
Email: tbdocs@who.int
Website: www.who.int/tb

ISBN 978 92 4 151126 1