Technical Report on critical concentrations for drug susceptibility testing of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine)

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Because diagnosis matters

WHO Collaborating Centre for
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Major data contributors

Acknowledgement of financial support

Provision of publications

Declaration and management of conflict of interest

All the contributors completed a WHO Declaration of Interest form. All stated declarations of interest were evaluated by members of the Steering Group for the existence of any possible financial conflict of interest which might warrant exclusion from membership of the Technical Expert Consultation Group or from the discussions as part of the consensus process. Intellectual conflict of interest was not considered for exclusion from membership of the Group, as broader expertise on DST methods for MTBC was considered as criteria for the selection. In addition, the diversity and representation in the Groups was large enough to balance and overcome any potential intellectual conflict of interest. During the consensus development process and the meeting, any emergence of intellectual conflict of interest was monitored by the Chair and there was no perceived intellectual conflict of interest identified during the meeting.

Abbreviations

7H10 = Middlebrook 7H10 medium

7H11 = Middlebrook 7H11 medium

ATU = area of technical uncertainty

BACTEC = BACTEC™ 460

CB = clinical breakpoint

CC = critical concentration

CI = exact binomial confidence interval

CLSI = Clinical & Laboratory Standards Institute

DST = drug-susceptibility testing

ECOFF = epidemiological cut-off value

ELI = European Laboratory Initiative

EUCAST = European Committee on Antimicrobial Susceptibility Testing

FIND = Foundation for Innovative New Diagnostics

gDST = genotypic drug susceptibility testing

gNWT = genotypically non-wild type

GLI = Global Laboratory Initiative

GTB = Global TB Program

gWT = genotypically wild type

HLR = high-level resistance/resistant

INH = isoniazid

ISO = International Organization for Standardization

LLR = low-level resistance/resistant

LJ = Löwenstein-Jensen medium

LPA = line probe assay

MDR = multidrug-resistant

MGIT = BACTEC™ Mycobacterial Growth Indicator Tube™ 960

MIC = minimum inhibitory concentration

MTBC = *Mycobacterium tuberculosis* complex

NGS = next generation sequencing

pDST = phenotypic drug susceptibility testing

pNWT = phenotypically non-wild type

PK/PD = pharmacokinetic/pharmacodynamic

PMID = PubMed ID

pWT = phenotypically wild type

R = resistance/resistant

RFB = rifabutin

RIF = rifampicin

RPT = rifapentine

RRDR = rifampicin resistance-determining region

S = susceptible/susceptibility

TB = tuberculosis

TEG = Technical Expert Group

WHO = World Health Organization

Glossary of terms

Antimicrobial susceptibility test interpretive category – a classification based on an *in vitro* response of an organism to an antimicrobial agent. For mycobacteria, two different categories, "critical concentration" and "minimum inhibitory concentration," have been used to categorise the *in vitro* results. For isolates of *Mycobacterium tuberculosis* complex, when tested against the lower concentration of some agents, the "critical concentration" category is applied. Testing of an additional higher concentration (a clinical breakpoint concentration) may also be recommended for some agents. However, there is no "intermediate" interpretive category, even when testing is performed both at the critical concentration and the clinical breakpoint concentration.

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

Clinical breakpoint – is the concentration or concentrations of an antimicrobial agent which defines an MIC above the critical concentration that separates isolates that will likely respond to treatment from those which will likely not respond to treatment. This concentration is determined by correlation with available clinical outcome data, MIC distributions, genetic markers, and pharmacokinetic/pharmacodynamic data including drug dose. A dose increase can be used to overcome resistance observed at lower dosing, up until the maximum tolerated dose, and therefore a higher clinical breakpoint above which the particular drug is not recommended for use. The clinical breakpoint is used to guide individual clinical decisions in patient treatment. The clinical breakpoint is not applicable for drug resistance surveillance purposes.

Critical proportion – is the proportion of resistant organisms within a particular cultured isolate that is used to determine resistance to a particular drug. A 1% critical proportion is used to differentiate susceptible and resistant isolates. Any culture that shows less than 1% growth on a medium containing a critical concentration of the agent being tested when compared with the growth on a control without the agent is considered to be susceptible; a culture that has 1% or more growth on the medium containing the critical concentration of the agent is considered to be resistant, and the patient whose sample is being tested may not respond to the agent. The critical concentration and proportion criteria are used for testing most first-line and second-line anti-TB agents.

Cross-resistance is resistance to multiple anti-tuberculosis agents caused by a single genetic change (or multiple changes, in case the given resistance mechanisms requires several genetic alterations), although in practice, such mutations may not be known.

Epidemiological cut-off value (ECOFF), phenotypically wild type (pWT) and non-wild type (pNWT) isolates

- Typically, when MICs that are tested using a standardised method are aggregated for one species, a single Gaussian-shaped MIC distribution is formed, which corresponds to the **pWT** distribution for that species (i.e. the distribution for organisms that lack phenotypically detectable resistance mechanisms). Additional distributions with higher overall MICs are sometimes identified, even prior to the clinical use of the particular drug in question (or prior to the clinical use of another, related drug that shares the same resistance mechanism), that

correspond to intrinsically or naturally resistant organisms. In this case, the distribution with the lowest MICs corresponds to the pWT distribution and the other distributions correspond to one or more **pNWT** distributions.

- The **ECOFF** corresponds to the upper end of the pWT distribution (i.e. it typically encompasses 99% of pWT isolates).
- Excluding the scenario where it is difficult to distinguish pWT and pNWT isolates because of methodological variation in MIC testing (i.e. where both distributions overlap), pWT isolates are, by definition, genotypically WT (gWT). However, this does not mean that gWT isolates are identical genotypically since they may harbour mutations in genes associated with resistance that do not change the MIC (e.g. the *gyrA* S95T mutation does not affect the MICs of fluoroquinolones).
- Conversely, organisms with MICs above the ECOFF are by definition pNWT. Again, excluding the possibility of methodological testing variation close to the ECOFF, there should be a genetic basis for this phenotype (i.e. the isolates should be genotypically NWT (gNWT)). Yet in practice, these gNWT isolates may appear to be gWT if:
  - The gene conferring the phenotype was not interrogated.
  - The gene was interrogated, but the genetic change conferring the phenotype was not detected, as it occurred at a frequency below the level of detection of the molecular test (i.e. heteroresistance).
  - The genetic change was detected but could not be interpreted because of an incomplete understanding of the genotype-phenotype relationship.

Indirect susceptibility test – a procedure based on inoculation of drug-containing media using organisms grown in culture.

Minimum inhibitory concentration (MIC) – the lowest concentration of an antimicrobial agent that prevents growth of more than 99% a microorganism in a solid medium or broth dilution susceptibility test.

Potency – All antimicrobial agents are assayed for standard units of activity or potency. The assay units may differ widely from the actual weight of the powder and often may differ between drug production lots. Thus, a laboratory must standardise its antimicrobial solutions based on assays of the antimicrobial powder lots that are being used.

The value for potency supplied by the manufacturer should include consideration for:
- Purity measures (usually by high-performance liquid chromatography assay)
- Water content (e.g. by Karl Fischer analysis or by weight loss on drying)
- Salt/counter-ion fraction (if the compound is supplied as a salt instead of free acid or base)

The potency may be expressed as a percentage, or in units of micrograms per milligrams (w/w).

Proportion method: The proportion method was originally proposed by Canetti and colleagues, and modified later; it is the most common method used for testing the susceptibility of *M. tuberculosis* complex isolates. In this method, the inoculum used is monitored by testing two dilutions of a culture suspension, and the growth (that is, the number of colonies) on a control medium without an anti-TB agent is compared with the growth (the number of colonies) present on a medium containing the critical concentration of the anti-TB agent being tested; the ratio of the number of colonies on the medium containing the anti-TB agent to the number of colonies on the medium without the anti-TB

agent is calculated, and the proportion is expressed as a percentage. A 1% critical proportion is used to differentiate the proportion of resistant organisms within a particular sample that is used to determine resistance to a particular drug.

Executive summary

The effective management of multidrug-resistant tuberculosis (MDR-TB) relies upon the rapid diagnosis and treatment of resistant infections. Growth-based phenotypic drug susceptibility testing (pDST) methods are currently the gold standard for drug resistance detection, but these methods are time-consuming, need strict quality control and are not always reproducible.

Traditionally, pDST for the *Mycobacterium tuberculosis* complex (MTBC) has relied on the testing of anti-TB agents at a single, critical concentration (CC) particular to each drug.[1] Traditionally, CCs were set based on expert opinion, taking into account the culture medium, reading time, and minimum inhibitory concentrations (MICs) of the phenotypically wild type (pWT) populations. In 2017, the World Health Organization (WHO) Global TB Programme commissioned FIND to perform a systematic review to inform a Technical Expert Group (TEG) tasked with evaluating the evidence base to establish or revise the CCs for 13 second-line anti-TB drugs, including bedaquiline and delamanid. The following media were considered: Löwenstein-Jensen (LJ), Middlebrook 7H10 (7H10), Middlebrook 7H11 (7H11) and BACTEC™ Mycobacterial Growth Indicator Tube™ 960 (MGIT). Based upon the TEG's review of the systematic review data, revised CCs were established for many of those compounds.[2]

In 2020, the WHO Global TB Program (GTB) convened a second TEG meeting to review the results of an equivalent systematic review of the published literature for the first-line anti-TB drugs isoniazid (INH) and the rifamycins (rifampicin (RIF), rifabutin (RFB) and rifapentine (RPT)). The quality and quantity of MIC data were limited for most drugs and media. Therefore, the TEG adopted a pragmatic approach by lowering CCs that were clearly above the epidemiological cut-off value (i.e. the 7H10 and MGIT CCs for RIF) but maintaining the remaining CCs (Table 1).[3] However, the validity of the 7H11 CC for RIF was questioned and the need for more data to evaluate the RIF CC on LJ was apparent. These findings underscored the need for greater standardization and validation of pDST.[4,5]

The TEG recommended that seven "borderline resistance" *rpoB* mutations, which have been referred to as "discordant", "disputed", "occult" or "(sub-breakpoint) low-level resistance" mutations in the literature (e.g. L430P (L511P) and I491F (I572F)) need to be treated with an MDR-TB regimen according to the latest WHO guidelines. [6,7] In addition, the interpretation of the remaining mutations in the RIF resistance-determining region (RRDR) was clarified (Section 3.3).

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[1] Ängeby K, Juréen P, Kahlmeter G, Hoffner S, Schön T. Challenging a dogma: antimicrobial susceptibility testing breakpoints for *Mycobacterium tuberculosis*. *Bull World Health Organ*. 2012. doi:10.2471/blt.11.096644.

[2] World Health Organization. Technical report on critical concentrations for TB drug susceptibility testing of medicines used in the treatment of drug-resistant TB; 2018 (https://apps.who.int/iris/bitstream/handle/10665/260470/WHO-CDS-TB-2018.5-eng.pdf, accessed 12 December 2018).

[3] Köser CU, Maurer FP, Kranzer K. 'Those who cannot remember the past are condemned to repeat it': Drug-susceptibility testing for bedaquiline and delamanid. *Int J Infect Dis*. 2019;80S:S32-S35. doi:10.1016/j.ijid.2019.02.027.

[4] Schön T, Köser CU, Werngren J, *et al*. What is the role of the EUCAST reference method for MIC testing of the *Mycobacterium tuberculosis* complex? *Clin Microbiol Infect*. 2020;26(11):1453-1455. doi:10.1016/j.cmi.2020.07.037.

[5] Schön T, Matuschek E, Mohamed S, *et al*. Standards for MIC testing that apply to the majority of bacterial pathogens should also be enforced for *Mycobacterium tuberculosis* complex. *Clin Microbiol Infect*. 2019;25(4):403-405. doi:10.1016/j.cmi.2019.01.019.

[6] Please refer to Section 3.2 for more details regarding the different *rpoB* numbering systems.

[7] World Health Organization. WHO consolidated guidelines on tuberculosis. Module 4: treatment – drug-resistant tuberculosis treatment (https://apps.who.int/iris/rest/bitstreams/1280998/retrieve, accessed 15 June 2020).

Changes to the CCs for INH were not warranted based on the available evidence. A detailed review of pharmacokinetic/pharmacodynamic and clinical outcome data for different INH resistance mechanisms is planned for 2021 to potentially set a clinical breakpoint for INH to stratify the level of resistance.

Table 1. Critical concentrations for INH and the rifamycins.

Drug	LJ	7H10	7H11	MGIT
Isoniazid	0.2	0.2	0.2	0.1
Rifampicin[a]	40	0.5	1.0	0.5
Rifabutin[b]	–	–	–	–
Rifapentine[c]	–	–	–	–

All concentrations are in mg/L and apply to the proportion method with 1% as the critical proportion. Changes to the previous version of the table are highlighted in red.[8]

[a] Additional data are needed to clarify whether the RIF CC for LJ is set correctly. The RIF CC for 7H11 was based on limited data and might be too high in light of the fact that the RIF CC for 7H10 had to be lowered to 0.5 mg/L.

[b] No CCs were adopted as RFB is not currently recommended for TB treatment by WHO, but the validity of the current CCs of 0.5 mg/L for 7H10, 7H11 and MGIT set by the Clinical and Laboratory Standards Institute could not be confirmed in this review.[9] As a conservative approach, gDST and, where applicable, pDST for RIF should serve as surrogate for RFB DST (Section 3.3).

[c] gDST and, where applicable, pDST for RIF should serve as surrogate for RPT DST (see Section 3.3).

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[8] World Health Organization. Updated interim critical concentrations for first-line and second-line DST (as of May 2012) (http://www.stoptb.org/wg/gli/assets/documents/Updated critical concentration table_1st and 2nd line drugs.pdf, accessed 7 June 2019).

[9] Clinical & Laboratory Standards Institute. Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 3rd edition approved standard. CLSI Document M24; 2018.

SECTION 1: Introduction

1.0 Background

Tuberculosis (TB) causes 10 million cases and 1.4 million deaths annually and it is estimated that 2.9 million cases go undiagnosed by public health services each year.[10] The global declines in TB incidence, as well as the global reduction in the total number of TB deaths observed in recent years, fall far short of the End TB Strategy milestones for 2020. Ending the global TB epidemic will only be achievable given intensive action by all countries, including a commitment to enhanced, multisectoral actions that have been demonstrated to drive down the epidemic at a rapid pace.

TB drug resistance is a major global public health problem that has threatened global progress made in TB care and prevention in recent decades. Drug resistance in the *Mycobacterium tuberculosis* complex (MTBC) is caused by selection of naturally occurring mutants. There are two ways that people get drug-resistant TB (DR-TB). Firstly, acquired DR-TB occurs when TB treatment is suboptimal due to inadequate policies and failures of health systems and care provision, poor quality of TB drugs, poor prescription practices, patient non-adherence, or a combination of the above. Secondly, primary DR-TB results from the direct transmission of DR-TB from one person to another, which is responsible for most multidrug-resistant TB (MDR-TB) in high-burden settings. In 2019, approximately half a million people worldwide developed TB that was resistant to rifampicin (RIF), the most effective first-line drug.[10] 78% of these patients were infected with TB that was additionally resistant to isoniazid (INH) and, consequently, had MDR-TB.[10]

The End TB Strategy calls for early diagnosis and prompt treatment of all persons of all ages with any form of drug-susceptible or -resistant TB. This requires ensuring access to rapid diagnostics recommended by the World Health Organization (WHO) and universal access to drug susceptibility testing (DST) for all patients with signs and symptoms of TB and no longer only prioritizing persons at greater risk of MDR-TB and-or HIV-associated TB. WHO defines universal access to DST as rapid DST for at least RIF, and further DST for at least fluoroquinolones among all TB patients with RIF resistance.[11]

The effective management of both drug-susceptible and -resistant TB relies upon the rapid diagnosis and treatment of infections. Culture-based phenotypic DST (pDST) methods are currently the gold standard for drug resistance detection, but these methods are time-consuming and require sophisticated laboratory infrastructure, qualified staff and strict quality control.

Traditionally, DST for MTBC has relied on the testing of a single, critical concentration (CC), which is used to differentiate resistant from susceptible isolates of MTBC, and is specific for each anti-TB agent and test method. However, the definition of the CC for MTBC DST has evolved over time and now considers more explicitly phenotypically wild type (pWT) vs. phenotypically non-wild type (pNWT) isolates, as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[12]

Laboratory tests of the susceptibility of tubercle bacilli to anti-TB agents serve three main purposes. Firstly, they can be used as guidance in the choice of chemotherapy to be given to a patient. Secondly,

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[10] World Health Organization. Global tuberculosis report 2020 (https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf, accessed 14 October 2020).

[11] World Health Organization. WHO operational handbook on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection (https://apps.who.int/iris/rest/bitstreams/1284635/retrieve, accessed 30 June 2020).

[12] Kahlmeter G. The 2014 Garrod Lecture: EUCAST - are we heading towards international agreement? *J Antimicrob Chemother*. 2015;70(9):2427-2439. doi:10.1093/jac/dkv145.

they are of value in confirming that drug resistance has emerged when a patient has failed to show a satisfactory response to treatment and, thirdly, they can be used for the surveillance of emerging drug resistance.

1.1 Scope of the Technical Expert Consultation Meeting

The WHO Global TB Program (GTB) initiated and provided oversight to the process of evidence retrieval and analysis, was responsible for selection of members for the Technical Expert Group (TEG), for management of declarations of interest, and, finally, conducting the face-to-face TEG meeting.

As a part of evidence retrieval and analysis, the WHO GTB commissioned the systematic review, which was performed by FIND in 2018-2019. The aim of the review was to collect the available data on minimum inhibitory concentrations (MICs) of pWT and pNWT isolates, including associated sequencing data for relevant gene regions, for the following anti-TB drugs:

- INH
- Rifamycins (RIF, rifabutin (RFB), and rifapentine (RPT))

The following media were considered:

- Löwenstein-Jensen (LJ)
- Middlebrook 7H10 (7H10)
- Middlebrook 7H11 (7H11)
- BACTEC™ Mycobacterial Growth Indicator Tube™ 960 (MGIT)

The objectives of the TEG were to revise and update the CCs for pDST for INH and the rifamycins.

The TEG meeting was convened by the Global TB Programme, WHO on 24 February 2020 in Geneva, Switzerland. During that meeting, the group assessed the MIC and sequencing data for each drug-medium combination, with a particular focus on potential sources of bias. Depending on the quality and quantity of the data, CCs were either established, maintained or revised. The decisions on the breakpoints for all anti-TB drugs in the review were based on majority view of TEG members.

The outcome of the TEG was an updated table of the CCs for INH and rifamycin DST (Table 1).

1.2 Systematic review

1.2.1 Search methodology

A MEDLINE/PubMed search without date restrictions was conducted of all publications reporting quantitative DST results for the selected antibiotics. The search terms for each drug or group of drugs, which can be found in the supplement of this report, were intentionally broad since the titles or abstracts of papers do not necessarily mention MIC data. Moreover, MIC data were also solicited from the WHO Supranational Reference Laboratory Network and directly from key researchers, as identified through the literature search and laboratory network.

Studies in the following languages were reviewed independently by one or more people:
1. English: Sophia Georghiou and Mikashmi Kohli or Vaidehi Nafade
2. Chinese: Hairong Huang, Suan Wen or Tingting Zhang
3. Croatian and Serbian: Ivan Barilar
4. Danish and Swedish: Thomas Schön or Erik Svensson
5. Dutch: Jakko van Ingen
6. French: Alexandra Aubry or Nicolas Veziris
7. German: Claudio Köser
8. Italian: Paolo Miotto
9. Japanese: Kiyohiko Izumi, Satoshi Mitarai or Akiko Takaki
10. Norwegian: Céline Cunen
11. Portuguese: Miguel Viveiros
12. Polish: Tomas Jagielski
13. Russian: Alexei Korobitsyn or Natalia Shubladze
14. Spanish: Victoria Furio
15. Turkish: Ferda Yılmaz
16. Ukrainian: Vlad Nikolayevskyy

1.2.2 Inclusion criteria

Studies identified as containing any MIC data through the full-text screening were further reviewed in detail by Sophia Georghiou and/or Claudio Köser. Studies that met the following criteria were included in the review:

1. The MICs for at least one of the anti-TB compounds of interest (with at least three concentrations tested per drug) were determined using the proportion method with a critical proportion of 1%, using LJ, 7H10, 7H11 or MGIT.
2. The drug concentrations tested were clearly defined (i.e. to assess potential truncations of the MIC results).
3. The number of isolates tested at each concentration was given (i.e. to evaluate the shape of the MIC distributions and determine the mode of the distributions).
4. The MIC data were available for at least 10 isolates per drug.

For studies that reported only MIC ranges (i.e. did not meet the third criterion), raw study data were solicited directly from the corresponding authors and/or their co-authors. These studies were excluded if detailed MIC data could not be obtained. In exceptional circumstances, studies that did not meet all of these criteria were still included if they presented data that were particularly valuable, such as studies with sequencing data for anti-TB drugs.

1.2.3 Studies identified through the systematic review

For the INH review, a total of 1,408 records were identified for possible inclusion, along with 79 additional datasets from other sources. As shown in Figure 1, 70 of these studies were included in the review, which were stratified further by medium (NB: the sum of the studies for individual media does not correspond to 70 as some studies featured MICs for multiple media).

For the rifamycins review, a total of 7,359 records were identified for possible inclusion, along with 23 additional datasets from other sources. As shown in Figure 2, 72 of these studies were included in the review, which were stratified further by medium (NB: the sum of the studies for individual media does not correspond to 72 as some studies featured MICs for multiple media).

Figure 1. PRISMA diagram for isoniazid search results and exclusion criteria

```
┌──────────────────────────────┐   ┌──────────────────────────────────┐
│  Records identified through  │   │ Additional datasets identified   │
│  PubMed/MEDLINE search       │   │ through supranational reference  │
│  (n = 1,408)                 │   │ laboratory network or personal   │
│                              │   │ communications (n = 79)          │
└──────────────────────────────┘   └──────────────────────────────────┘

                ┌──────────────────────────────┐
                │  Total records (n = 1,487)   │────────►┌──────────────────────────┐
                └──────────────────────────────┘         │ Studies excluded:        │
                                                         │ No access (n = 3)        │
                                                         │ Foreign language (n = 1) │
                ┌──────────────────────────────┐         └──────────────────────────┘
                │ Records screened (n = 1,483) │────────►┌──────────────────────────┐
                └──────────────────────────────┘         │ Studies excluded:        │
                                                         │ No INH MICs performed    │
                                                         │ (n = 1,132)              │
                ┌──────────────────────────────┐         └──────────────────────────┘
                │ Studies that performed MIC   │
                │ testing (n = 351)            │────────►┌──────────────────────────┐
                └──────────────────────────────┘         │ Studies excluded:        │
                                                         │ MIC method not included  │
                                                         │ in remit of review       │
                ┌──────────────────────────────┐         │ (n = 210)                │
                │ Studies with relevant MIC    │         │ Experimental details     │
                │ data (n = 70)                │         │ missing (n = 52)         │
                └──────────────────────────────┘         │ <10 isolates tested      │
                                                         │ (n = 19)                 │
                                                         └──────────────────────────┘
```

MICs on LJ	MICs on 7H10	MICs on 7H11	MICs in MGIT
(n = 16)	(n = 25)	(n = 10)	(n = 21)

Figure 2. PRISMA diagram for rifamycin search results and exclusion criteria
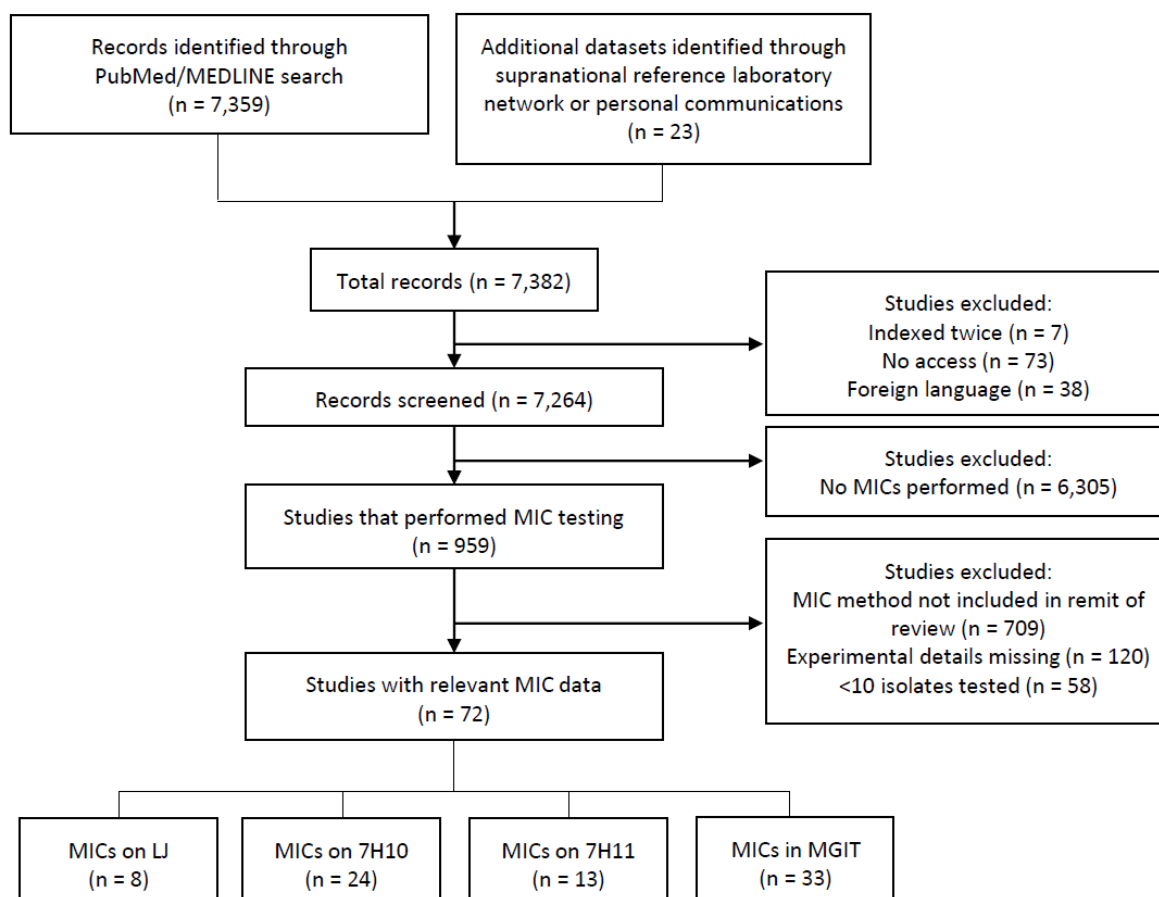
1.3 Data presentation

1.3.1 Format of this report

Each chapter in the report covers a single drug or group of drugs that share at least one resistance mechanism (i.e. the rifamycins). The results in each chapter are grouped by different media (LJ, 7H10, 7H11 and MGIT). For each medium, data are organised into three sections: (1) MICs for pWT isolates, including laboratory control isolates (e.g. H37Rv), (2) MICs for isolates with mutations in relevant resistance genes (i.e. MICs from *in vitro*, animal or clinical isolates as well as allelic exchange experiments, where available), (3) conclusion for CC for each combination of drug and medium, including the rationale for the revised or existing breakpoints.

1.3.2 Format of MIC tables

This report contains abridged versions of the full Excel MIC data files, which are included in the supplement. Details for the information provided in each column of these files can be found below. However, only essential columns were included in this report. For example, the column with the "total [number of] MICs" performed was included only if these numbers differed from the numbers of unique isolates tested (i.e. when isolates were tested repeatedly, as was the often the case for H37Rv).

The following points are relevant for the interpretation of the data:

- If a cell is empty, no information regarding the particular category were available (i.e. in the case of the "genotypic results" column, blank cells are not equivalent to gWT (where sequencing or another genotypic method was carried out but no relevant genetic changes were found)).
- MICs from different studies cannot be compared unless the concentrations and ranges of concentrations tested are considered. Shaded cells therefore designate the concentrations tested for each group of isolates (NB: some studies tested a wide range of concentrations. Table 2 provides an overview of how MIC data are displayed.

Table 2. Overview of MIC data presentation.

Studies	RIF MIC [mg/L]					
	0.25	0.5	1	2	4	8
study A			15	2	2	
study B	20		15			2

Shaded cells correspond to the concentrations tested in a particular study (e.g. concentrations of 0.5, 1 and 2 mg/L were tested for study A, whereas 0.5 and 2 mg/L were not tested in study B, which means that MICs of 1 mg/L in both studies are not equivalent). Truncated MIC values were highlighted in red. If red was used in a shaded cell, the MIC was either ≤ or ≥ the concentration in question. For example, the lowest MIC value for study B was ≤0.25 mg/L, whereas the highest MICs were 8 mg/L. If red was used in an unshaded cell, the MIC was > the last concentration tested (for study A, the highest MICs were >2 mg/L, as opposed to 4 mg/L). The mode of the putative pWT MIC distribution was indicated by highlighting the corresponding number of MICs in **bolded** text (e.g. 1 mg/L for study A). In the case of study B, the truncation of the MIC values meant that a mode could not be identified (e.g. it was possible that the MICs of all 20 isolates with MICs ≤0.25 mg/L were actually 0.25 mg/L, in which case 0.25 mg/L would be the mode of the MIC distribution).

The following information are provided in each data column.

"Studies" column:
- The names of the studies with notable limitations were highlighted in red (e.g. if the same laboratory participated in multiple studies that used the same medium or a method other than sequencing was used for genotypic DST (gDST)). The corresponding limitations were detailed below the tables in the footnotes in this report and in the 'comment' column in red in the supplementary MIC file.

"Lab" column:
- The laboratories that participated in multiple studies using the same medium were highlighted in red.

"Unique isolates" & "total MICs" columns:
- Red entries correspond to isolates that were tested multiple times.

"Comment" column:

- Additional remarks regarding the study in question were included in this column. Important limitations were highlighted in red.

SECTION 2: Isoniazid

2.0 INH resistance mechanisms

Despite the simple structure of INH, a large number of genes have been implicated in resistance to this drug, but the clinical relevance of many of these mechanisms remains poorly understood.[13] The two WHO-endorsed line probe assays (LPAs) by Hain (Hain Lifescience, Nehren, Germany) and Nipro (NIPRO Corporation, Osaka, Japan) rely upon the detection of well-characterized INH resistance mutations in *katG* and the promoter of *inhA* (Table 3). The S315T mutation in *katG* reduces but does not fully abolish the function of this catalase-peroxidase which is needed for the activation of the pro-drug INH.[14,15] By contrast, *inhA* encodes an enoyl acyl carrier protein reductase that represents the shared target of INH as well as the second-line compounds ethionamide and prothionamide.[14] The promoter-up mutations upstream of the *fabG1-inhA* operon interrogated by both LPAs, therefore, confer cross-resistance to all three compounds. The same effect is achieved by a mutation in *fabG1*, which creates an alternative promoter for *inhA*.[16] Finally, some gDST assays interrogate mutations upstream of *ahpC*, which compensate for loss-of-function mutations in *katG* and, thus, serve as indirect markers for INH resistance.[17] Numerous studies have found that mutations in *inhA* confer lower MIC increases to INH than *katG* mutations.[18,19] However, no study has been conducted to date to compare all four WHO-endorsed media systematically. Such a review would guide the interpretation of molecular tests by confirming the level of INH resistance conferred by the aforementioned mechanisms and could inform the setting of a clinical breakpoint (CB).

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[13] Merker M, Kohl TA, Barilar I, et al. Phylogenetically informative mutations in genes implicated in antibiotic resistance in *Mycobacterium tuberculosis* complex. *Genome Med*. 2020;12(1):27. doi:10.1186/s13073-020-00726-5.

[14] Vilchèze C, Jacobs JR. WR. Resistance to isoniazid and ethionamide in *Mycobacterium tuberculosis*: Genes, mutations, and causalities. *Microbiol Spectr*. 2014;2(4):MGM2-0014-02913. doi:10.1128/microbiolspec.mgm2-0014-2013.

[15] Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in *Mycobacterium tuberculosis*: A systematic review. *PLoS One*. 2015;10(3):e0119628. doi:10.1371/journal.pone.0119628.

[16] Ando H, Miyoshi-Akiyama T, Watanabe S, Kirikae T. A silent mutation in *mabA* confers isoniazid resistance on *Mycobacterium tuberculosis*. *Mol Microbiol*. 2014;91(3):538-547. doi:10.1111/mmi.12476.

[17] Sherman DR, Mdluli K, Hickey MJ, *et al*. Compensatory *ahpC* gene expression in isoniazid-resistant *Mycobacterium tuberculosis*. *Science.* 1996;272(5268):1641-3. doi:10.1126/science.272.5268.1641.

[18] Lempens P, Meehan CJ, Vandelannoote K, *et al*. Isoniazid resistance levels of *Mycobacterium tuberculosis* can largely be predicted by high-confidence resistance-conferring mutations. *Sci Rep*. 2018;8(1):3246. doi:10.1038/s41598-018-21378-x.

[19] Ghodousi A, Tagliani E, Karunaratne E, *et al*. Isoniazid resistance in *Mycobacterium tuberculosis* is a heterogeneous phenotype composed of overlapping MIC distributions with different underlying resistance mechanisms. *Antimicrob Agents Chemother*. 2019;63(7):e00092-19. doi:10.1128/AAC.00092-19.

Table 3. Probe-binding regions and *katG* and *inhA* promoter mutation coverage of WHO-endorsed LPAs.

Assay	Probe(s)	Codons/nucleotides analysed or specific mutation detected[a]	Mutations covered as per package insert
Hain GenoType MTBDR*plus* V2[20,21]	*katG* WT	315 region	S315T
	katG MUT1	agc/acc S315T[b]	
	katG MUT2	agc/aca S315T[b]	
	katG WT1, MUT1 and MUT2 all negative	*katG* deletion	
	inhA WT1	-15 region	c-15t, a-16g
	inhA WT2	-8 region	t-8c, t-8a
	inhA MUT1	c-15t[b]	
	inhA MUT2	a-16g	
	inhA MUT3A	t-8c	
	inhA MUT3B	t-8a	
Nipro NTM+MDRTB II[22]	*katG* S7	294-299	
	katG S8	313-317	
	katG S9	323-327	
	katG S10	325-330	
	katG R8a	S315T[b]	
	katG R8b	S315N[b]	
	inhA S6	-17 to -3	
	inhA R6a	a-16g	
	inhA R6b	c-15t	
	inhA R6c	t-8c	
	inhA R6d	t-8a	

[a] Hain has not disclosed which precise *katG* or *inhA* codons are covered by the corresponding "WT" or "MUT" probes. The same applies to the "R" probes of the Nipro LPA. The regions covered by the Hain "WT" or Nipro "S" probes may not be covered completely and not all mutations in these regions, particularly if they occur at the edges of the probes, prevent binding.[21]

[b] Mutations associated with INH resistance according to Miotto *et al.* (see Section 2.1).[23]

2.1 Current statements and policies regarding genotypic markers of INH resistance

The following statements, policy recommendations and practices regarding genotypic INH resistance are relevant to this review:

1. Miotto *et al*. conducted a comprehensive analysis of the association between INH resistance mutations and pDST results, which yielded only *katG* S315N and S315T and the c-15t *inhA* promoter mutations as markers for INH resistance.[23] No or insufficient evidence was found for the remaining *inhA* promoter mutations interrogated by the WHO-endorsed LPAs (Table 3).

[20] Hain Lifescience. GenoType MTBDR*plus* VER 2.0. Instructions for use. IFU-304A-09. (https://www.hain-lifescience.de/include_datei/kundenmodule/packungsbeilage/download.php?id=2877, accessed 1 Nov 2020).

[21] World Health Organization. Regional Office for Europe. Interpretation guide for GenoType MTBDR*plus* VER 2.0 and GenoType MTBDR*sl* VER 2.0. A technical guidance document developed by the European Laboratory Initiative. Version 1.0. (https://openwho.org/courses/multi-drug-resistant-tb, accessed 9 May 2020).

[22] FIND. Report for WHO: Non-inferiority evaluation of Nipro NTM+MDRTB and Hain GenoType MTBDR*plus* V2 line probe assays. Version 4.1. Geneva, Switzerland; 2015 (https://www.finddx.org/wp-content/uploads/2016/04/LPA-report_noninferiority-study_oct2015.pdf, accessed 22 May 2020).

[23] Miotto P, Tessema B, Tagliani E, *et al*. A standardised method for interpreting the association between mutations and phenotypic drug resistance in *Mycobacterium tuberculosis*. *Eur Respir J*. 2017;50(6):1701354. doi:10.1183/13993003.01354-2017.

2. For the purposes of surveillance, WHO has adopted a composite reference standard for INH resistance.[24] Specifically, the three aforementioned mutations from Miotto *et al*. were considered to be true markers for INH resistance (i.e. the presence of any of these mutations was deemed to be necessary and sufficient to confirm INH resistance). Any susceptible pDST result for an isolate with one of these mutations was thereby corrected to resistant.[24] Without this correction, 3.8% (exact binomial 95% confidence interval (CI) 2.9-4.8%) of INH-R isolates would have been characterized as INH-S in a recent multi-country surveillance study conducted by WHO.

3. The Global Laboratory Initiative (GLI) has recommended that the INH results for the Hain LPA are stratified into low-level resistance (LLR) and high-level resistance (HLR).[25,26] This has been implemented in a recently published guide by the European Laboratory Initiative (ELI), where isolates with a *katG* mutation or deletion are reported as HLR, whereas those with only an *inhA* promoter mutation are reported as "at least LLR" ("at least" was used to signal that higher MICs are possible due to mechanisms that are not interrogated by the LPA).[21]

2.2 INH MIC data stratification and current breakpoints

All mutations within the INH resistance-associated gene regions *katG*, *fabG1-inhA*, *inhA*, *oxyR-ahpC*, *ahpC*, *kasA*, *ndh*, *nat*, *mshA* and *fabG1* were noted for this report and included whenever reported. *oxyR-ahpC* mutations were numbered relative to the start of the *ahpC* gene (e.g. g-48a corresponds to g-6a relative to the transcriptional start site). Synonymous mutations were considered to be gWT for this report with the exception of the INH resistance-associated mutation *fabG1* L203L.[27] Additionally, polymorphisms that are likely not associated with phenotypic INH resistance (*inhA* g-102a, t-80g, g-47c, and T4I, and *katG* A110V, R463L and L499M, *mshA* A187V and N111S) were considered to be gWT and not reported herein.[28] For the purposes of this report, MIC data were stratified based upon the most common mutations reported in *katG*, the *inhA* promoter, the *inhA* coding regions, and the *oxyR-ahpC* intergenic region as well as mutations in more than one of these gene regions. For the correlation between resistance mutations and phenotypic results, binomial 95% CIs were calculated, where applicable (i.e. including all isolates where sufficient concentrations were tested to determine whether the isolates were resistant or susceptible at a particular concentration).

Table 4 provides an overview of the current WHO and CLSI CCs and CBs for INH.

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[24] Zignol M, Cabibbe AM, Dean AS, *et al*. Genetic sequencing for surveillance of drug resistance in tuberculosis in highly endemic countries: a multi-country population-based surveillance study. *Lancet Infect Dis*. 2018;18(6):675-683. doi:10.1016/S1473-3099(18)30073-2. doi:10.1016/S1473-3099(18)30073-2.

[25] Global Laboratory Initiative. Line probe assays for drug-resistant tuberculosis detection: interpretation and reporting guide for laboratory staff and clinicians; 2019 (http://www.stoptb.org/wg/gli/assets/documents/LPA_test_web_ready.pdf, accessed 22 May 2020).

[26] World Health Organization. WHO operational handbook on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection (https://apps.who.int/iris/rest/bitstreams/1284635/retrieve, accessed 30 June 2020).

[27] Ando H, Miyoshi-Akiyama T, Watanabe S, Kirikae T. A silent mutation in *mabA* confers isoniazid resistance on *Mycobacterium tuberculosis*. *Mol Microbiol*. 2014;91(3):538-547. doi:10.1111/mmi.12476.

[28] Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in *Mycobacterium tuberculosis*: A systematic review. *PLoS One*. 2015;10(3):e0119628. doi:10.1371/journal.pone.0119628.

Table 4. Overview of current INH CCs.

Drug	LJ		7H10		7H11		MGIT	
	WHO	CLSI	WHO	CLSI	WHO	CLSI	WHO	CLSI
INH	0.2	0.25/1.0	0.2/1.0		0.2/1.0		0.1/0.4	

Green CCs were set by both the WHO and CLSI; red CCs were set by WHO; blue CCs and CBs were set by CLSI.[29,30] All concentrations are in mg/L.

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[29] Clinical & Laboratory Standards Institute. Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 3rd edition approved standard. CLSI Document M24; 2018.
[30] World Health Organization. Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. (http://apps.who.int/iris/bitstream/handle/10665/275469/9789241514842-eng.pdf, accessed 17 November 2018).

2.A.1 INH MIC data on LJ

2.A.1.1 INH MICs for pWT isolates on LJ

Eleven studies were identified that reported INH MIC data for the pWT population on LJ (Table 5). Most of these studies were enriched for INH-R isolates and all identified pWT MIC distributions were severely truncated at the lower end, precluding an assessment of the shape of the distributions.

Table 5. INH MICs for pWT isolates on LJ.

Studies	Lab	Isolate origin	Unique isolates	Type of isolates	Genotypic summary	0.05	0.1	0.2	0.25	0.5	0.8	1	1.6	2	2.5	3.2	4	5	8	10
2) Vincent 2012	2	clinical	98	Mix of first-line resistance profiles	gWT	46		44			4		1				1	2		
4) Lempens 2018	2	clinical	52	Mix of first-line resistance profiles	gWT	22		29			1									
	2		282			99		56			56					26	45			
	2		80			5		2			22					12	2	37		
11) Rigouts 2013 & Van Deun (unpublished)	2	clinical	59	Mix of first-line resistance profiles				9				5					21	24		
	2		27					6								4	17			
	2		124				9	17			20		1			11	18	48		
	2		84				9	14			12		5			9	1	34		
12) Farhat 2019	2	clinical	46	Mostly MDR	gWT	27		13			4						2			
6) Beckers 1985	5		1	H37Rv						1										
	5	clinical	17						15									2		
9) Brossier 2009 &	9	clinical	20	Mix of first-line resistance profiles	gWT			17			3									
10) Brossier 2016	9		1	H37Rv				1												
	9	clinical	4	Mostly INH-R	gWT			3			1									
7) Alame-Emane 2015	6, 7	clinical	50	Mix of first-line resistance profiles	gWT			49												1
1) Jagielski 2015 &	1		1	H37Rv				1												
Jagielski 2013 & Jagielski	1	clinical	66	Mix of MDR, INH mono-resistant and pan-S	gWT			48		12	4				2					
3) Lee 2000	3		1	H37Rv				1												
8) Thai 2018	8	clinical	214	Mix of first-line resistance profiles				26			81	94					8		2	3

The red line denotes the current WHO CC for INH DST on LJ (0.2 mg/L), whereas the blue lines denote the CLSI CC and CB (0.25 and 1 mg/L). Notable limitations: studies I2, I4, I11, and studies I9 and I10 were conducted in the same laboratory; study I7 presented data for isolates only characterized by LPA; and studies I12 and I10 presented data mostly for INH-R isolates.

2.A.1.2 INH MICs for mutated isolates on LJ
katG S315 mutants

Seven studies reported MIC data for 352 clinical isolates with *katG* S315 mutations and no co-occurring *inhA* promoter or coding mutations (Table 6). Based on the current INH CCs, all but one (99.7%, 95% CI 98-100%) of these mutated isolates were phenotypically INH-R. The modes of most MIC distributions were 2.5-10 mg/L on this medium; above the current CCs and CLSI CB.

Table 6. INH MICs for clinical *katG* S315 mutants on LJ.

Table 6. INH MICs for clinical *katG* S315 mutants on LJ.

Studies	Lab	Isolate origin	Unique isolates	Genotypic summary	0.05	0.1	0.2	0.25	0.5	0.8	1	1.6	2.5	3.2	5	6.4	10	12.8	19.2
2) Vincent 2012	2	clinical	1	katG S315R											1				
	2	clinical	6	katG S315N						2				3	1				
	2	clinical	1	katG S315I											1				
	2	clinical	79	katG S315T									3	35	41				
12) Farhat 2019	2	clinical	1	katG S315I											1				
	2	clinical	1	katG S315R											1				
	2	clinical	2	katG S315N						1				1					
	2	clinical	44	katG S315T	1								2	15	26				
4) Lempens 2018	2	clinical	63	katG S315T										9	50		4		
	2	clinical	3	katG S315T + kasA G269S										2	1				
	2	clinical	1	katG S315T + oxyR-ahpC c-52t														1	
	2	clinical	1	katG S315T + T275A														1	
	2	clinical	1	katG S315T + G192A											1				
	2	clinical	1	katG S315T + T625A									1						
	2	clinical	1	katG S315T + nat A210R											1				
	2	clinical	1	katG S315T + M84T + nat A210R											1				
	2	clinical	1	katG S315N									1						
	2	clinical	1	katG S315N + kasA G269S											1				
	2	clinical	1	katG S315N + S140N						1									
	2	clinical	1	katG S315N								1							
	2	clinical	1	katG S315T + nat deletion											1				
	2	clinical	1	katG S315T + ndh frameshift													1		
	2	clinical	1	katG S315T + T275A															1
7) Alame-Emane 2015	6, 7	clinical	2	katG S315N											2				
	6, 7	clinical	27	katG S315T											27				
9) Brossier 2009 & Brossier 2006	9	clinical	51	katG S315T						3							44	4	
	9	clinical	2	katG S315N						2									
10) Brossier 2016	9	clinical	2	katG S315T											2				
1) Jagielski 2015 & Jagielski 2013 & Jagielski 2014	1	clinical	30	katG S315T						1			25	2	2				
	1	clinical	1	katG S315T + mabA G48V + V75A										1					
	1	clinical	1	katG S315T + ahpC I38M										1					
	1	clinical	12	katG S315T + kasA G269S						2			9	1					
	1	clinical	1	katG S315T + ndh L50V									1						
	1	clinical	2	katG S315T + ndh 18A + nat G207R									1			1			
	1	clinical	1	katG S315T + oxyR-ahpC c-54t									1						
	1	clinical	1	katG S315T + ndh V18A + G207R + mshA frameshift												1			
	1	clinical	1	katG S315N + nat A103G									1						
	1	clinical	1	katG S315T + kasA G269S + mshA A362V									1						
	1	clinical	2	katG S315T + kasA G269S						1			1						
	1	clinical	1	katG S315T + kasA A321T									1						

The red line denotes the current WHO CC for INH DST on LJ (0.2 mg/L), whereas the blue lines denote the CLSI CC and CB (0.25 and 1 mg/L). Notable limitations: studies I9 and I10, and studies I2, I4 and I12 were conducted in the same laboratory and study I7 presented data for isolates only characterized by LPA and real-time PCR melting curve analysis.

inhA promoter mutants

Seven studies reported MIC data for 83 clinical isolates with *inhA* promoter mutations and no co-occurring *katG* S315 or *inhA* coding mutations (Table 7). Based on the current INH CC, all (100%, 95% CI 95-100%) of these mutated isolates were phenotypically INH-R. The modes of most MIC distributions were 0.8-1 mg/L on this medium (i.e. equivalent to the CLSI CB), though these INH MICs may be higher than the true MICs, given that these were usually the first concentrations tested above the CC.

Table 7. INH MICs for clinical *inhA* promoter mutants on LJ.

Studies	Lab	Isolate origin	Unique isolates	Genotypic summary	0.1	0.2	0.25	0.5	0.8	1	1.6	2.5	3.2	5	6.4	10	12.8
7) Alame-Emane 2015	6, 7	clinical	1	mabA-inhA t-8c						1							
2) Vincent 2012	2	clinical	1	mabA-inhA g-9a					1								
2) Vincent 2012	2	clinical	18	mabA-inhA c-15t					11		3		1	3			
4) Lempens 2018	2	clinical	1	mabA-inhA c-15t + kasA G312S					1								
4) Lempens 2018	2	clinical	1	katG T251K + mabA-inhA c-15t													1
4) Lempens 2018	2	clinical	1	katG G127P + mabA-inhA c-15t									1				
4) Lempens 2018	2	clinical	6	mabA-inhA c-15t					6								
12) Farhat 2019	2	clinical	6	mabA-inhA c-15t					5		1						
1) Jagielski 2015 &	1	clinical	1	mabA-inhA c-15t						1							
1) Jagielski 2015 &	1	clinical	1	katG W91R + mabA-inhA c-15t						1							
1) Jagielski 2015 &	1	clinical	1	katG P131Q + mabA-inhA c-15t						1							
1) Jagielski 2015 &	1	clinical	1	katG M126I + mabA-inhA c-15t								1					
3) Lee 2000	3	clinical	14	mabA-inhA c-15t					11					3			
7) Alame-Emane 2015	6, 7	clinical	15	mabA-inhA c-15t					14							1	
9) Brossier 2009 &	9	clinical	14	mabA-inhA c-15t					11							3	
4) Lempens 2018	2	clinical	1	katG L458R + mabA-inhA g-17t											1		

The red line denotes the current WHO CC for INH DST on LJ (0.2 mg/L), whereas the blue lines denote the CLSI CC and CB (0.25 and 1 mg/L). Notable limitations: studies I2, I4 and I12 were conducted in the same laboratory and study I7 presented data for isolates only characterized by LPA and real-time PCR melting curve analysis.

inhA coding mutants

Four studies reported MIC data for 10 clinical isolates with *inhA* coding mutations and no co-occurring *katG* S315 or *inhA* promoter mutations (Table 8). Where it could be assessed, 4 (57%, 95% CI 18-90%) of these mutated isolates were phenotypically INH-R based on the current INH CC. The three isolates with an *inhA* S94A mutation that tested phenotypically INH-S had an MIC at the CC. These mutations have been previously shown to result only in modest MIC increases.[31] By contrast, the *inhA* I194T mutation correlated with MICs above the CLSI CB.

Table 8. INH MICs for clinical *inhA* coding mutants on LJ.

Studies	Lab	Isolate origin	Unique isolates	Genotypic summary	0.1	0.2	0.25	0.5	0.8	1	1.6	2.5	3.2	5
4) Lempens 2018	2	clinical	1	inhA S94A		1								
4) Lempens 2018	2	clinical	1	katG M126I + inhA S94A									1	
12) Farhat 2019	2	clinical	2	inhA S94A			2							
9) Brossier 2009 &	9	clinical	3	inhA S94A						3				
4) Lempens 2018	2	clinical	1	inhA I194T									1	
1) Jagielski 2015 &	1	clinical	1	inhA I194T								1		
1) Jagielski 2015 &	1	clinical	1	katG K537E + inhA I194T								1		

The red line denotes the current WHO CC for INH DST on LJ (0.2 mg/L), whereas the blue lines denote the CLSI CC and CB (0.25 and 1 mg/L). Notable limitations: studies I2 and I12 were conducted in the same laboratory.

oxyR-ahpC intergenic region mutants

Only 2 studies were identified that reported MIC data for 8 clinical isolates with *oxyR-ahpC* intergenic region mutations and no co-occurring *katG* S315, *inhA* coding or *inhA* promoter mutations (Table 9). Where it could be assessed, 6 (86%, 95% CI 42-100%) of these mutated isolates were phenotypically INH-R based on the current INH CC.

Table 9. INH MICs for clinical *oxyR-ahpC* intergenic region mutants on LJ.

Studies	Lab	Isolate origin	Unique isolates	Genotypic summary	0.2	0.25	0.5	0.8	1	1.6	2.5	3.2	5	6.4	10	12.8	20	25	40	60	64
1) Jagielski 2015 &	1	clinical	1	katG T394P + oxyR-ahpC g-48a																	1
1) Jagielski 2015 &	1	clinical	1	katG R128Q + oxyR-ahpC g-48a + nat STOP127			1														
1) Jagielski 2015 &	1	clinical	1	katG R128Q + oxyR-ahpC g-48a + ndh A300P + nat STOP127			1														
4) Lempens 2018	2	clinical	1	oxyR-ahpC g-48a	1																
4) Lempens 2018	2	clinical	1	katG D612G + 970_971delACinsCT + T324L + oxyR-ahpC c-52t						1											
1) Jagielski 2015 &	1	clinical	1	katG STOP46 + A235G + oxyR-ahpC c-57t																	1
4) Lempens 2018	2	clinical	1	katG Y229C + oxyR-ahpC c-81t															1		
1) Jagielski 2015 &	1	clinical	1	oxyR-ahpC tca-82/-80atc + ndh S281S												1					

The red line denotes the current WHO CC for INH DST on LJ (0.2 mg/L), whereas the blue lines denote the CLSI CC and CB (0.25 and 1 mg/L).

Double mutants

katG S315 and inhA promoter double mutants

Six studies reported MIC data for 43 clinical isolates with *katG* S315 and *inhA* promoter double mutations (Table 10). Based on the current INH CC, all (100%, 95% CI 92-100%) of these mutated isolates were phenotypically INH-R, with MICs from 1->25 mg/L, slightly higher than those MIC distributions reported for the single mutants (Table 6 and Table 7).

katG S315 and inhA coding double mutants

Only one study reported MIC data for 1 clinical isolate with a *katG* S315 and *inhA* coding mutation (Table 10). The isolate was INH-R, with an MIC of 5 mg/L.

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[31] Vilchèze C, Wang F, Arai M, *et al*. Transfer of a point mutation in *Mycobacterium tuberculosis inhA* resolves the target of isoniazid. *Nat Med.* 2006;12(9):1027-9. doi:10.1038/nm1466.

inhA promoter and *inhA* coding double mutants

Four studies reported MIC data for 12 clinical isolates with *inhA* promoter and *inhA* coding mutations (Table 10). Based on the current INH CC, all (100%, 95% CI 63-100%) of these mutated isolates were phenotypically INH-R. The reported MICs were in line with the MICs reported for the single mutants tested on LJ (Table 7 and Table 8).

Table 10. INH MICs for clinical *katG* S315, *inhA* promoter and *inhA* coding double mutants on LJ.

Studies	Lab	Isolate origin	Unique isolates	Genotypic summary	0.1	0.2	0.25	0.5	0.8	1	1.6	2.5	3.2	5	6.4	10	12.8	19.2	25	30
9) Brossier 2009 &	9	clinical	1	katG S315T + mabA-inhA t-8c												1				
1) Jagielski 2015 &	1	clinical	1	katG S315T + mabA-inhA t-8c												1				
2) Vincent 2012	2	clinical	7	katG S315T + mabA-inhA c-15t											7					
2) Vincent 2012	2	clinical	1	katG S315T + mabA-inhA c-15t											1					
4) Lempens 2018	2	clinical	12	katG S315T + mabA-inhA c-15t															1	11
7) Alame-Emane 2015	6, 7	clinical	5	katG S315T + mabA-inhA c-15t						1						4				
9) Brossier 2009 &	9	clinical	10	katG S315T + mabA-inhA c-15t												3	7			
1) Jagielski 2015 &	1	clinical	1	katG S315T + mabA-inhA c-15t										1						
12) Farhat 2019	2	clinical	2	katG S315T + mabA-inhA c-15t										2						
4) Lempens 2018	2	clinical	3	katG S315T + mabA-inhA g-47a + nat G78D											3					
1) Jagielski 2015 &	1	clinical	1	katG S315T + inhA K57R + mshA G446S										1						
4) Lempens 2018	2	clinical	1	katG P367L + mabA-inhA c-15t + inhA I16T + kasA G269S									1							
4) Lempens 2018	2	clinical	1	mabA-inhA c-15t + inhA S94A									1							
4) Lempens 2018	2	clinical	1	mabA-inhA c-15t + inhA S94A + kasA G312S									1							
4) Lempens 2018	2	clinical	1	mabA-inhA c-15t + inhA S94A + ndh V317A + fabG1 E102D																1
12) Farhat 2019	2	clinical	2	mabA-inhA c-15t + inhA S94A							1			1						
9) Brossier 2009 &	9	clinical	4	mabA-inhA c-15t + inhA S94A						4										
1) Jagielski 2015 &	1	clinical	1	mabA-inhA c-15t + inhA S94A								1								
1) Jagielski 2015 &	1	clinical	1	mabA-inhA c-15t + inhA I194T						1										

The red line denotes the current WHO CC for INH DST on LJ (0.2 mg/L), whereas the blue lines denote the CLSI CC and CB (0.25 and 1 mg/L). Notable limitations: studies I2, I4 and I12 were conducted in the same laboratory and study I7 presented data for isolates only characterized by LPA and real-time PCR melting curve analysis.

2.A.1.3 Conclusion for INH CC for LJ

Owing to the limited quantity and quality of MICs for pWT isolates, it was not possible to assess whether the current CC of 0.2 mg/L corresponds to the epidemiological cut-off (ECOFF) for INH. However, given that the CC appeared to adequality detect known resistance mechanisms, with the exception of *inhA* S94A, **0.2 mg/L** was reaffirmed. Some experts noted that 0.25 mg/L should have been adopted in accordance with the standards of the International Organization for Standardization (ISO) and the CLSI CC.

2.A.2 INH MIC data on 7H10

2.A.2.1 INH MICs for pWT isolates on 7H10

Eighteen studies were identified that reported INH MIC data for the pWT population on 7H10 (Table 11). Five of these studies were conducted in the same laboratories, and 14 of these studies featured MIC data for 10 or more pWT isolates. Given that not all studies sequenced all INH resistance genes and many studies only tested a few concentrations, it was difficult to assess the shapes of many of the MIC distributions. Considering that the modes of most gWT distributions were between 0.05 and 0.125 mg/L, and that the MICs reported for the H37Rv control strain were 0.06-0.2 mg/L, 0.2 mg/L or the corresponding ISO concentration of 0.25 mg/L likely represents the tentative ECOFF for 7H10.

Table 11. INH MICs for pWT isolates on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic summary	0.0125	0.016	0.025	0.03	0.05	0.06	0.1	0.12	0.15	0.2	0.25	0.4	0.5	0.613	0.8	1	2	3	4	5	8	10	16	20	32	64	128
24) Rancoita 2018	19		2	2	H37Rv											2																	
	19	clinical	13	26	Mix of first-line resistance profiles	gWT	4		2		12		4				4																
29) Schön 2009	24		1	4	H37Rv ATCC27294								1		3																		
	24		1	1	BTB 08-049									1																			
	24	clinical	106	106								1	43	35		1		1				1	2		9		8		3			1	1
34) Gygli 2019	29		1	2	H37Rv ATCC27294								2																				
	29		1	2	Erdman							1	1																				
	29	clinical	55	57	Mix of first-line resistance profiles	gWT						15	17	18			7																
14) de Steenwinkel 2012, de Steenwinkel 2012 &	11		1	2	H37Rv ATCC27294									2																			
	11	clinical	10	20									1	19																		
32) Pholwat 2011	26		1	1	H37Rv								1																				
	26	clinical	11	11	Mix of first-line resistance profiles	gWT					3		7		1																		
13) Alonso 2013	10		1	1	H37Rv ATCC25618								1																				
	10		1	1	ATCC35822												1																
	10		36	36		gWT							30		6																		
	10		12	12	INH-S								12																				
26) Abe 2008	21	clinical	15	15	Mostly INH-S	gWT										2		4			9												
33) Farhat 2019	27	clinical	42	42		gWT										36						1				5							
	22		17	17	Mostly MDR	gWT							1						1			3		1						2	9		
	28		203	203		gWT					24		100	15				11	7			4	3	2	1	36							
28) Wedajo 2014	23	clinical	1	13	H37Rv ATCC27294								11	2																			
	23		46	46	MTBDRplus WT	gWT						7	29	5								3	2										
27) van Klingeren 2007	22	clinical	7956	7956	Mostly INH-S	gWT										6985	374		159			48	32	358									
25) Gali 2006	20	clinical	7	7	Mix of first-line resistance profiles	gWT											3	2				1											1
19) Pretorius 1995	16		1	1	H37Rv										1																		
15) Campbell 2011 &	12	clinical	122	122		gWT										102						5					5	10					
17) Cavusoglu 2006	14	clinical	9	9	Mostly RIF-R	gWT																6	1				2						
21) DeCoster 2005	18	clinical	26	26	Mix of first-line resistance profiles											7						9					6	4					
22) Moore 1999	18	clinical	17	17	Mix of first-line resistance profiles											6						7					2	2					
23) Kirk 1998	18	clinical	35	35	Mix of first-line resistance profiles											17						9					4	5					
31) Karunaratne 2018	25		1	1	H37Rv											1																	

The green line denotes the current WHO and CLSI CC for INH DST on 7H10 (0.2 mg/L), whereas the blue line denotes the CLSI CB (1 mg/L). Notable limitations: studies I21, I22 and I23, and studies I33 and I27 were conducted in the same laboratory; study I28 presented data for isolates only characterized by LPA; and studies I17 and I33 presented data for mostly INH-R isolates.

2.A.2.2 INH MICs for mutated isolates on 7H10

katG S315 mutants

Twelve studies reported 1113 MICs for isolates with *katG* S315 mutations and no co-occurring *inhA* promoter or coding mutations (Table 12). Based on the current INH CC, 1085 (97%, 95% CI 96-98%) of these mutated isolates were phenotypically INH-R. Most isolates that tested INH-S at the current CC were from one of two studies by Farhat *et al*. or Karunaratne *et al*., which reported lower MICs for mutants than reported by other studies. Although many studies only tested a few concentrations in a non-standard dilution series to establish MICs, the modes of the distributions appeared to be 5-10 mg/L, when they could be defined (i.e. above the CLSI CB).

Table 12. INH MICs for clinical *katG* S315 mutants on 7H10.

Studies	Isolate origin	Unique isolates	Total MICs	Genotypic summary	0.05	0.1	0.2	0.4	0.5	0.61	0.8	1	1.6	2	3	3.2	4	5	6.4	8	10	15	16	20	32	35
24) Rancoita 2018	clinical	7	14	katG S315T												6				8						
33) Farhat 2019	clinical	4	4	katG S315N															4							
		1	1	katG S315G															1							
		2	2	katG S315R															1							
		531	531	katG S315T	2	11	2	6		4		3		10			32	27	434							
		1	1	katG S315N															1							
		83	83	katG S315T										7	5			47			19				2	3
		2	2	katG S315N															2							
		46	46	katG S315T			1					1						2	42							
28) Wedajo 2014	clinical	16	16	katG MUT inhA WT									16													
32) Pholwat 2011	clinical	22	22	katG S315T													2			3			2		1	14
34) Gygli 2019	clinical	43	49	katG S315T								1		2			15			18			2		5	6
18) van Doorn 2003	clinical	4	4	katG S315T													3				1					
		1	1	katG S315N													1									
		16	16	katG S315T					1			1					5				4	5				
25) Gali 2006	clinical	11	11	katG S315T													1			9			1			
		1	1	katG S315R																1						
		1	1	katG S315N																1						
		1	1	katG S315T + A234G																1						
30) Otto-Knapp 2016	clinical	34	34	katG S315T													8			14	12					
		46	46	katG S315T							3							18	25							
		3	3	katG S315T																	3					
15) Campbell 2011 & Ramirez 2010	clinical	2	2	katG S315N														1	1							
		1	1	katG S315I														1								
		151	151	katG S315T								1						48	102							
		2	2	katG S315T + I335V															2							
16) Abbadi 2009	clinical	7	7	katG S315T							7															
		1	1	katG S315R							1															
17) Cavusoglu 2006	clinical	27	27	katG S315T														27								
31) Karunaratne 2018	clinical	15	15	katG S315N			1				4							4			1	1		4		
		1	1	katG S315N			1																			
		1	1	katG S315T + S275A																				1		
		1	1	katG S315N + G212D + ahpC C37T			1																			
		1	1	katG S315T + oxyR-ahpC c-52t							1															
		1	1	katG S315T + oxyR-ahpC g-88a			1																			
		1	1	katG S315T + ahpC T39S							1															
		8	8	katG S315T + ahpC C37T			5				2															
		3	3	katG S315N + ahpC C37T			3																			
		1	1	katG S315R + ahpC C37T																		1				

The green line denotes the current WHO and CLSI CC for INH DST on 7H10 (0.2 mg/L), whereas the blue line denotes the CLSI CB (1 mg/L). Notable limitations: study I28 presented data for isolates only characterized by LPA; studies I33 and I31 reported lower MICs for mutants than reported for other studies.

inhA promoter mutants

Nine studies reported 257 MICs for clinical isolates with *inhA* promoter mutations and no co-occurring *katG* S315 or *inhA* coding mutations (Table 13). Where it could be assessed, 238 (95%, 95% CI 92-98%) of these mutated isolates were phenotypically INH-R based on the current INH CC. Most isolates that tested INH-S at the current CC were from one of two studies by Farhat *et al*. or Karunaratne *et al*., which reported lower MICs for mutants than reported by other studies. Excluding these studies and the study by Pholwat *et al.*, for which MICs were higher than reported for other studies, the modes of the distributions appeared to be 0.25-1 mg/L, when they could be defined (i.e. below the CLSI CB).

Table 13. INH MICs for clinical *inhA* promoter mutants on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Genotypic summary	0.05	0.1	0.12	0.2	0.25	0.4	0.5	0.61	0.8	1	2	4	5	8	10	16	32	35
33) Farhat 2019	28	clinical	1	1	mabA-inhA t-8a														1				
33) Farhat 2019	27	clinical	1	1	mabA-inhA t-8a													1					
33) Farhat 2019	28	clinical	4	4	mabA-inhA t-8c				1							1	1	1					
25) Gali 2006	20	clinical	2	2	mabA-inhA t-8c					2													
32) Pholwat 2011	26	clinical	1	1	mabA-inhA t-8c																		1
33) Farhat 2019	28	clinical	1	1	mabA-inhA t-8g													1					
33) Farhat 2019	27	clinical	1	1	mabA-inhA t-8g										1								
24) Rancoita 2018	19	clinical	1	2	mabA-inhA c-15t						2												
26) Abe 2008	21	clinical	12	12	mabA-inhA c-15t						5			7									
26) Abe 2008	21	clinical	1	1	katG W204R + mabA-inhA c-15t									1									
33) Farhat 2019	28	clinical	138	138	mabA-inhA c-15t	1	4		1		12		11		9	20	8	2	70				
33) Farhat 2019	22	clinical	9	9	mabA-inhA c-15t							3			1	3		1		1			
34) Gygli 2019	29	clinical	14	18	mabA-inhA c-15t					2		13			3								
25) Gali 2006	20	clinical	10	10	mabA-inhA c-15t					3		5			2								
25) Gali 2006	20	clinical	1	1	katG Y678C + mabA-inhA c-15t										1								
25) Gali 2006	20	clinical	1	1	katG D189H + mabA-inhA c-15t											1							
32) Pholwat 2011	26	clinical	4	4	mabA-inhA c-15t														1			3	
34) Gygli 2019	29	clinical	1	1	katG Y155S + mabA-inhA c-15t											1							
15) Campbell	12	clinical	11	11	mabA-inhA c-15t										7		4						
31) Karunaratne 2018	25	clinical	1	1	mabA-inhA c-15t										1								
31) Karunaratne 2018	25	clinical	2	2	mabA-inhA c-15t + ahpC C37T					2													
33) Farhat 2019	27	clinical	5	5	mabA-inhA c-15t										1		3	1					
33) Farhat 2019	28	clinical	23	23	mabA-inhA g-17t	1	2				4		5		1	3	2	5					
33) Farhat 2019	22	clinical	1	1	mabA-inhA g-17t							1											
25) Gali 2006	20	clinical	1	1	mabA-inhA g-17t							1											
34) Gygli 2019	29	clinical	1	1	mabA-inhA g-17t					1													
33) Farhat 2019	27	clinical	3	3	mabA-inhA g-17t										1		1	1					
28) Wedajo 2014	23	clinical	1	1	katG WT inhA MUT										1								

The green line denotes the current WHO and CLSI CC for INH DST on 7H10 (0.2 mg/L), whereas the blue line denotes the CLSI CB (1 mg/L). Notable limitations: study I28 presented data for isolates only characterized by LPA; studies I33 and I31 reported lower MICs for mutants than reported for other studies.

inhA coding mutants

Two studies were identified that reported 17 MICs for clinical isolates with *inhA* coding mutations and no co-occurring *katG* S315 or *inhA* promoter mutations (Table 14). The data were insufficient to confirm whether these mutations conferred resistance, as the data were few and conflicting even for the S94A mutations, for which a greater number of MICs were identified.

Table 14. INH MICs for clinical *inhA* coding mutants on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Genotypic summary	0.1	0.12	0.2	0.25	0.4	0.5	0.613	1	2	4	5	6.4
34) Gygli 2019	29	clinical	1	2	inhA I21V		1		1								
33) Farhat 2019	28	clinical	13	13	inhA S94A			1						1	2	1	8
33) Farhat 2019	27	clinical	1	1	inhA S94A												1
34) Gygli 2019	29	clinical	1	1	inhA S94A		1										
33) Farhat 2019	28	clinical	1	1	inhA I194T												1

The green line denotes the current WHO and CLSI CC for INH DST on 7H10 (0.2 mg/L), whereas the blue line denotes the CLSI CB (1 mg/L). Notable limitations: study I33 reported lower MICs for mutants than reported for other studies.

oxyR-ahpC intergenic region mutants

No studies presenting MIC data for *oxyR-ahpC* intergenic region mutants on 7H10 were identified.

Double mutants

katG S315 and *inhA* promoter double mutants

Six studies reported 92 MICs for clinical isolates with *katG* S315 and *inhA* promoter double mutations tested on 7H10 (Table 15). Based on the current INH CC, all (100%, 95% CI 96-100%) of these mutated isolates were phenotypically INH-R. Most studies tested insufficiently high concentrations to enable a comparison of the MICs for these double mutations with those of *katG* S315 single mutants (Table 12).

katG S315 and *inhA* coding double mutants

Three studies reported MIC data for clinical isolates with both a *katG* S315 and *inhA* coding mutation (Table 15). The 13 MICs reported for the 11 clinical isolates were all >5 mg/L.

inhA promoter and *inhA* coding double mutants

Two studies presenting MIC data for *inhA* promoter and *inhA* coding double mutants on 7H10 were identified (Table 15). Based on the current INH CC, all 50 (100%, 95% CI 93-100%) of these mutated isolates were phenotypically INH-R, with MICs ranging from 0.6 to >32 mg/L.

Table 15. INH MICs for clinical *katG* S315, *inhA* promoter and *inhA* coding double mutants on 7H10.

Studies	Isolate origin	Unique isolates	Total MICs	Genotypic summary	0.2	0.4	0.5	0.613	0.8	1	1.6	2	3	4	5	6.4	8	10	15	16	20	32	35
34) Gygli 2019	clinical	4	4	katG S315T + mabA-inhA t-8a																1		3	
33) Farhat 2019	clinical	2	2	katG S315T + mabA-inhA t-8c														2					
33) Farhat 2019	clinical	7	7	katG S315T + mabA-inhA t-8c												7							
34) Gygli 2019	clinical	8	8	katG S315T + mabA-inhA t-8c													1			5			2
34) Gygli 2019	clinical	1	1	katG S315T + K557N + mabA-inhA t-8c																1			
33) Farhat 2019	clinical	2	2	katG S315T + mabA-inhA t-8c												2							
15) Campbell 2011 &	clinical	3	3	katG S315T + mabA-inhA t-8c												3							
33) Farhat 2019	clinical	1	1	katG S315T + mabA-inhA t-8g												1							
33) Farhat 2019	clinical	7	7	katG S315T + mabA-inhA c-15t									1						1		2	3	
34) Gygli 2019	clinical	6	8	katG S315T + mabA-inhA c-15t																1	1	1	6
15) Campbell 2011 &	clinical	20	20	katG S315T + mabA-inhA c-15t												20							
15) Campbell 2011 &	clinical	1	1	katG S315T + I335V + mabA-inhA c-15t												1							
31) Karunaratne 2018	clinical	1	1	katG S315T + mabA-inhA c-15t + ahpC C37T											1								
33) Farhat 2019	clinical	7	7	katG S315T + mabA-inhA c-15t												7							
33) Farhat 2019	clinical	1	1	katG S315T + mabA-inhA g-17t												1							
24) Rancoita 2018	clinical	2	4	katG S315T + mabA-inhA -34c deletion							4												
31) Karunaratne 2018	clinical	7	7	katG S315T + mabA-inhA -34c deletion																	7		
33) Farhat 2019	clinical	1	1	katG S315T + mabA-inhA c-34t												1							
34) Gygli 2019	clinical	1	1	katG S315T + mabA-inhA c-34t																		1	
31) Karunaratne 2018	clinical	1	1	katG S315T + mabA-inhA c-34t + oxyR-ahpC g-88a											1								
24) Rancoita 2018	clinical	2	4	katG S315T + mabA-inhA c-60t												4							
28) Wedajo 2014	clinical	1	1	katG MUT inhA MUT							1												
33) Farhat 2019	clinical	1	1	katG S315T + inhA I21V												1							
33) Farhat 2019	clinical	1	1	katG S315T + inhA I21V												1							
33) Farhat 2019	clinical	1	1	katG S315T + inhA S94A												1							
33) Farhat 2019	clinical	1	1	katG S315G + inhA S94A											1								
33) Farhat 2019	clinical	1	1	katG S315N + inhA S94A												1							
31) Karunaratne 2018	clinical	3	3	katG S315T + inhA S94A															3				
33) Farhat 2019	clinical	1	1	katG S315T + inhA I194T												1							
24) Rancoita 2018	clinical	2	4	katG S315T + inhA N231D													4						
34) Gygli 2019	clinical	2	2	mabA-inhA c-15t + inhA I21T											2								
33) Farhat 2019	clinical	32	32	mabA-inhA c-15t + inhA I21T						1	2	2		8	4	15							
33) Farhat 2019	clinical	5	5	mabA-inhA c-15t + inhA I21T											1	4							
34) Gygli 2019	clinical	1	1	mabA-inhA c-15t + inhA I21V							1												
33) Farhat 2019	clinical	1	1	mabA-inhA c-15t + inhA I21V											1								
34) Gygli 2019	clinical	1	1	mabA-inhA c-15t + inhA S94A								1											
33) Farhat 2019	clinical	1	1	mabA-inhA c-15t + inhA S94A + A26T								1											
34) Gygli 2019	clinical	1	1	mabA-inhA g-17t + inhA S94A							1												
33) Farhat 2019	clinical	2	2	mabA-inhA g-17t + inhA S94A												2							
33) Farhat 2019	clinical	2	2	mabA-inhA g-17t + inhA S94A												2							
33) Farhat 2019	clinical	1	1	mabA-inhA c-15t + inhA A239V											1								
33) Farhat 2019	clinical	1	1	mabA-inhA c-15t + inhA I194T												1							

The green line denotes the current WHO and CLSI CC for INH DST on 7H10 (0.2 mg/L), whereas the blue line denotes the CLSI CB (1 mg/L). Notable limitation: study I28 presented data for isolates only characterized by LPA; studies I33 and I31 reported lower MICs for mutants than reported for other studies.

2.A.2.3 Conclusion for INH CC for 7H10

The current CC of **0.2 mg/L** likely corresponds to the tentative ECOFF for INH and was consequently reaffirmed, although some experts noted that 0.25 mg/L should have been adopted in accordance with ISO standards.

2.A.3 INH MIC data on 7H11

2.A.3.1 INH MICs for pWT isolates on 7H11

Eight studies were identified that reported INH MIC data for the pWT population on 7H11 (Table 16). Four of these studies reported MICs for at least 10 pWT isolates. The modes of most pWT MIC distributions appeared to be between 0.05 and 0.125 mg/L, suggesting that 0.2 mg/L or the corresponding ISO concentration of 0.25 mg/L likely represents the tentative ECOFF for 7H11.

Table 16. INH MICs for pWT isolates on 7H11.

Studies	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic summary	0.025	0.05	0.06	0.1	0.12	0.2	0.25	0.4	0.5	1	2	4	8	16	32	64	102.4
39) Rey-Jurado 2013 &		1		1 H37Rv			1															
Rey-Jurado 2012	clinical	10		10 INH-S		4	6															
43) Lee 1987	clinical	17	17	17 Pre-treatment						12	5											
38) Coban 2013		1		1 H37Rv						1												
		1		2 ATCC35822																		1
		1		1 ATCC35838						1												
		1		1 ATCC35820						1												
		1		1 ATCC35837					1													
	clinical	40		40 Mostly INH-S						15	10			1		2	2	1	3	4	2	
41) Rodriguez Diaz 2003	clinical	16	16	16				6		4									6			
42) Shishido 2007		1		3 BCG Tokyo seed lot							3											
		1		14 BCG Tokyo				14														
37) Cockerill 1995		1		1 H37Rv							1											
	clinical	1		1 mix of first-line resistance profiles gWT														1				
40) Fattorini 1999		1		1 H37Rv ATCC27294							1											
		1		1 ATCC35822																		1
36) Guo 2006		1		1 H37Rv								1										

The green line denotes the current WHO and CLSI CC for INH DST on 7H11 (0.2 mg/L), whereas the blue line denotes the CLSI CB (1 mg/L).

2.A.3.2 INH MICs for mutated isolates on 7H11

katG S315 mutants

Only two studies were identified that reported INH MICs for 8 clinical isolates with *katG* S315 mutations and no co-occurring *inhA* promoter or coding mutations (Table 17). Based on the current INH CC, all 8 (100%, 95% CI 63-100%) of these mutated isolates were phenotypically INH-R.

Table 17. INH MICs for clinical *katG* S315 mutants on 7H11.

Studies	Isolate origin	Unique isolates	Genotypic summary	0.2	0.25	0.4	0.5	1	2	4	5	8	16
37) Cockerill 1995	clinical		1 katG S315T + S302R									1	
			1 katG S315T + M609I										1
36) Guo 2006	clinical		5 katG S315T					5					
			1 katG S315N + A379T					1					

The green line denotes the current WHO and CLSI CC for INH DST on 7H11 (0.2 mg/L), whereas the blue line denotes the CLSI CB (1 mg/L).

inhA promoter mutants

Only one study reported MICs for 13 clinical isolates with *inhA* promoter mutations and no co-occurring *katG* S315 or *inhA* coding mutations on 7H11 (Table 18). Given that only one study was identified, no comments could be made as to whether the current CC for 7H11 adequately differentiated these mutations.

Table 18. INH MICs for clinical *inhA* promoter mutants on 7H11.

Studies	Isolate origin	Unique isolates	Genotypic summary	0.2	0.4	1	5
36) Guo 2006	clinical	13	mabA-inhA c-15t	12	1		

The green line denotes the current WHO and CLSI CC for INH DST on 7H11 (0.2 mg/L), whereas the blue line denotes the CLSI CB (1 mg/L).

inhA coding mutants

No studies presenting MIC data for *inhA* coding mutants on 7H11 were identified.

oxyR-ahpC intergenic region mutants

No studies presenting MIC data for *oxyR-ahpC* intergenic region mutants on 7H11 were identified.

Double mutants

katG S315 and *inhA* promoter double mutants

Only one study was identified that reported INH MICs for 3 clinical isolates with *katG* S315 and *inhA* promoter double mutations on 7H11. Based on the current INH CC, all of these mutated isolates were phenotypically INH-R, with MICs of 5 mg/L.

katG S315 and *inhA* coding double mutants

No studies presenting MIC data for *katG* S315 and *inhA* coding double mutants on 7H11 were identified.

inhA promoter and *inhA* coding double mutants

No studies presenting MIC data for *inhA* promoter and *inhA* coding double mutants on 7H11 were identified.

Table 19. INH MICs for clinical *katG* S315, *inhA* promoter and *inhA* coding double mutants on 7H11.

Studies	Isolate origin	Unique isolates	Genotypic summary	INH MIC [mg/L]			
				0.2	0.4	1	5
	clinical	1	katG S315T + mabA-inhA t-8a				1
36) Guo 2006	clinical	1	katG S315T + mabA-inhA g-22c				1
	clinical	1	katG S315T + mabA-inhA t-8c				1

The green line denotes the current WHO and CLSI CC for INH DST on 7H11 (0.2 mg/L), whereas the blue line denotes the CLSI CB (1 mg/L).

2.A.3.3 Conclusion for INH CC for 7H11

The data for 7H11 were more limited than 7H10. Nevertheless, **0.2 mg/L** was reaffirmed as the current CC given that it likely corresponds to the tentative ECOFF for INH on 7H11. As for 7H10, some experts noted that the ISO concentration of 0.25 mg/L should have been adopted.

2.A.4 INH MIC data in MGIT

2.A.4.1 INH MICs for pWT isolates in MGIT

Seventeen studies were identified that reported INH MIC data for the pWT population by MGIT (Table 20). Over half of these studies were conducted in overlapping laboratories. Ten of these studies reported MICs for at least 10 pWT isolates. The modes of most of the reported distributions appeared to be between 0.03 and 0.06 mg/L, supporting 0.1 mg/L or the corresponding ISO concentration of 0.125 mg/L as the tentative ECOFF for MGIT.

Table 20. INH MICs for pWT isolates in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic summary	0.016	0.02	0.025	0.03	0.04	0.05	0.06	0.1	0.25	0.4	0.5	1	3	4	8	10	16
60) Colangeli 2018	46	clinical	25	25	Validation cohort		6	5	3	3	6	1	1										
54) Heyckendorf 2018 & Sturegård 2015	36	clinical	1	1	H37Rv ATCC27294				1														
	36	clinical	1	3	H37Rv ATCC27294								3										
	36		14	14	INH-S	gWT						7	7										
57) Niward 2018	24		1	3	H37Rv ATCC27294								3										
	39		1	2	H37Rv ATCC27294								2										
	24	clinical	26	26	non-MDR	gWT							17	8							1		
58) Groenheit (unpublished)	39		1	2	H37Rv								2										
	39	clinical	22	22		gWT							14	8									
59) Ghodousi 2019	19		1	1	H37Rv ATCC27294								1										
	19	clinical	4	4		gWT							3	1									
53) Tessema 2017	36	clinical	29	29	Mostly MDR	gWT			11				11			1		1	3				2
47) Kambli 2015	41		1	1	H37Ra								1										
	41		30	30	INH-S	gWT							5		25								
50) Rockwood 2017	16		94	94								5	60	21		3		3	2				
	16	clinical	23	23	pre-treatment (paired)							1	14	6		2							
	16		24	24	post-treatment (paired)							1	15	6		2							
52) Chigutsa 2015	45	clinical	54	54								1	39	12	2								
56) Rockwood 2017	45	clinical	100	100	Xpert RIF-S							5	63	24		6		1	1				
48) Torres 2015	42	clinical	2	2	Mostly INH-R	gWT											1		1				
44) Machado 2018 and Machado	35		1	1	H37Rv ATCC27294									1									
	35	clinical	6	6	INH-S	gWT								6									
45) Machado 2013	35		1	1	H37Rv ATCC27294									1									
49) Rueda 2015	43	clinical	4	4	INH-S	gWT								4									
51) Bernardelli 2004	44		1	1	H37Rv									1									
	44		1	1	BCG										1								
	44		1	1	AN5										1								
	44	seals	7	7										3	4								
55) Andres 2014 &	36	clinical	12	12		gWT								4				5	1				2
61) Gygli 2019	29		1	1	H37Rv ATCC27294									1									
	29		1	1	Erdman									1									
	29	clinical	8	8	mix of first-line resistance profiles	gWT								8									

The green line denotes the current WHO and CLSI CC for INH DST in MGIT (0.1 mg/L), whereas the blue line denotes the CLSI CB (0.4 mg/L). Notable limitations: studies I53, I54 and I55, studies I57 and I58, studies I46 and I59, studies I44 and I45, and studies I52 and I56 were conducted in the same laboratory; study I47 presented data for isolates characterized by a mix of LPA and pyrosequencing and studies I48 and I53 presented data mostly for INH-R isolates.

2.A.4.2 INH MICs for mutated isolates in MGIT

katG S315 mutants

Eleven studies reported INH MIC data for 603 clinical isolates with *katG* S315 mutations tested by MGIT (Table 21). Based on the current INH CCs, all (100%, 95% CI 99-100%) of these mutated isolates were phenotypically INH-R at the current CC. The modes of the various distributions ranged from 3-10 mg/L (i.e. two dilutions above the CLSI CB).

Table 21. INH MICs for clinical *katG* S315 mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic summary	0.1	0.4	0.5	1	2	3	4	5	8	10	16	20
54) Heyckendorf 2018 & Sturegård 2015	36	clinical	2	katG S315T							1		1			
	36		16	katG S315T						9				6	1	
44) Machado 2018 and Machado (unpublished)	35	clinical	7	katG S315T										3	3	1
	35		1	katG S315N						1						
45) Machado 2013 and	35		1	katG S315T											1	
46) Cambau 2015	9, 19, 22, 35-40	clinical	42	katG S315T						14				26	2	
	9, 19, 22, 35-40		57	katG S315T				1		25				27	4	
	9, 19, 22, 35-40		2	katG S315N										1	1	
47) Kambli 2015	41		50	katG S315T						10				35	5	
49) Rueda 2015	43	clinical	4	katG S315T							3	1				
	43		7	katG S315T + I248M + mshA I460R							6	1				
	43		1	katG S315T + ndh V18A								1				
	43		1	katG S315T + ndh N316K							1					
55) Andres 2014 & unpublished data	36	clinical	22	katG S315T						11				7	4	
	36		1	katG S315G					1							
	36		1	katG S315N					1							
58) Groenheit (unpublished)	39	clinical	70	katG S315T					14	1	40	15				
	39		1	katG S315T + I317V							1					
	39		1	katG S315T + T677I							1					
	39		1	katG S315T + F657L									1			
61) Gygli 2019	29	clinical	23	katG S315T						8				13	2	
53) Tessema 2017	36	clinical	2	katG S315T + mshA N69S						1				1		
	36		1	katG S315T + mshA G106V + ndh A209V										1		
	36		3	katG S315T + N493S						3						
	36		1	katG S315G + ndh deletion		1										
	36		1	katG S315T + ndh L221R										1		
	36		4	katG S315T + ndh V18A										3	1	
	36		1	katG S315T + ahpC D73H						1						
	36		2	katG S315T + oxyR-ahpC g-48a						1				1		
	36		1	katG S315T + oxyR-ahpC c-52a										1		
	36		4	katG S315T + oxyR-ahpC c-52t										3	1	
	36		1	katG S315T + oxyR-ahpC c-57t										1		
	36		1	katG S315N + oxyR-ahpC c-72t										1		
	36		1	katG S315T + oxyR-ahpC g-105a						1						
	36		1	katG T275A + S315T + oxyR-ahpC g-142a										1		
	36		3	katG S315T + oxyR-ahpC g-142a						3						
	36		2	katG S315T + oxyR-ahpC t-519c						1				1		
	36		2	katG S315T + oxyR-ahpC c-581g						2						
	36		2	katG S315N + I317V						1				1		
	36		1	katG S315T + I335V										1		
	36		1	katG D448A + S315T						1						
	36		1	katG S315T + T677P										1		
	36		2	katG S315T + G712A						2						
	36		1	katG S315N						1						
	36		226	katG S315T				2		185				38	1	
59) Ghodousi 2019	19	clinical	26	katG S315T							8	16		2		
	19		1	katG S315T + oxyR-ahpC g-47gt insertion								1				

The green line denotes the current WHO and CLSI CC for INH DST in MGIT (0.1 mg/L), whereas the blue line denotes the CLSI CB (0.4 mg/L). Notable limitations: studies I46, I53, I54, and I55, studies I46, I44 and I45, studies I46 and I58, and studies I46 and I59 were conducted in the same laboratory and study I47 presented data for isolates characterized by a mix of LPA and pyrosequencing.

inhA promoter mutants

Nine studies reported INH MIC data for 73 clinical isolates with *inhA* promoter mutations (Table 22). Of these, only two (3% (95% CI, 0-10%)) were INH-S at the current CC. Based on the current INH CCs, 71 (97%, 95% CI 90-100%) of these mutated isolates were phenotypically INH-R at the current CC. The current CC appeared to be sufficient to differentiate the vast majority of these mutants. Assuming equivalence between 0.4 and 0.5 mg/L, the current CLSI CB appears to divide the MIC distribution for *inhA* promoter mutants, which was not the case for LJ (Table 7) and 7H10 (Table 13), although more data are needed to support this observation.

Table 22. INH MICs for clinical *inhA* promoter mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic summary	0.1	0.12	0.25	0.4	0.5	1	2	3	4	8	10	16	64	102.4
58) Groenheit	39	clinical	1	katG G285D + mabA-inhA t-8c					1									
47) Kambli 2015	41		16	mabA-inhA c-15t					12	4								
53) Tessema 2017	36	clinical	1	mabA-inhA c-15t + fabG1 M98V				1										
53) Tessema 2017	36	clinical	1	mabA-inhA c-15t + ndh K78N				1										
53) Tessema 2017	36	clinical	2	oxyR-ahpC g-142a + mabA-inhA c-15t	1			1										
53) Tessema 2017	36	clinical	11	mabA-inhA c-15t					9	1					1			
54) Heyckendorf	36	clinical	1	mabA-inhA c-15t						1								
59) Ghodousi 2019	19	clinical	6	mabA-inhA c-15t			2		2	1	1							
44) Machado 2018	35	clinical	1	mabA-inhA c-15t				1										
46) Cambau 2015	9, 19, 22, 35-40	clinical	3	mabA-inhA c-15t						2					1			
46) Cambau 2015	9, 19, 22, 35-40	clinical	3	mabA-inhA c-15t						3								
49) Rueda 2015	43	clinical	1	katG V442G + mabA-inhA c-15t	1													
58) Groenheit	39	clinical	1	mabA-inhA c-15t						1								
61) Gygli 2019	29	clinical	11	mabA-inhA c-15t						11								
61) Gygli 2019	29	clinical	1	katG Y155S + mabA-inhA c-15t								1						
53) Tessema 2017	36	clinical	1	katG A109T + mabA-inhA c-15t + ahpC P44R						1								
53) Tessema 2017	36	clinical	1	katG A130E + mabA-inhA c-15t						1								
53) Tessema 2017	36	clinical	1	katG R249H + mabA-inhA c-15t						1								
53) Tessema 2017	36	clinical	1	katG insertion + mabA-inhA c-15t													1	
59) Ghodousi 2019	19	clinical	5	katG deletion + mabA-inhA c-15t														5
59) Ghodousi 2019	19	clinical	1	katG T271A + mabA-inhA c-15t						1								
59) Ghodousi 2019	19	clinical	1	katG insertion + mabA-inhA c-15t										1				
59) Ghodousi 2019	19	clinical	1	katG A172V + mabA-inhA c-15t + oxyR-ahpC g-51a											1			
53) Tessema 2017	36	clinical	1	mabA-inhA g-17t				1										

The green line denotes the current WHO and CLSI CC for INH DST in MGIT (0.1 mg/L), whereas the blue line denotes the CLSI CB (0.4 mg/L). Notable limitations: studies I46 and I58, studies I46, I53 and I54, studies I44 and I46, and studies I46 and I59 were conducted in the same laboratory and study I47 presented data for isolates characterized by a mix of LPA and pyrosequencing.

inhA coding mutants

Only two studies were identified that presented MIC data for *inhA* coding mutants without co-occurring *katG* S315 or *inhA* promoter mutations by MGIT (Table 23). MIC data were only available for 2 isolates, including 1 *inhA* S94A mutant with an MIC of 0.4 mg/L. The other *inhA* coding mutant, with an I21V mutation, had an MICs ≤0.1 mg/L, below the current CC.

Table 23. INH MICs for clinical *inhA* coding mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic summary	0.1	0.4	1	3	10
61) Gygli 2019	29	clinical	1	inhA I21V	1				
53) Tessema 2017	36	clinical	1	inhA S94A		1			

The green line denotes the current WHO and CLSI CC for INH DST in MGIT (0.1 mg/L), whereas the blue line denotes the CLSI CB (0.4 mg/L).

oxyR-ahpC intergenic region mutants

Two studies were identified that presented MIC data for *oxyR-ahpC* intergenic region mutants without co-occurring *katG* S315, *inhA* promoter, or *inhA* coding mutations by MGIT (Table 24). These studies were conducted in the same laboratory and presented MIC data for 7 clinical mutants. Six of the 7 mutants (86%, 95% CI 42-100%) had MICs above the current CC.

Table 24. INH MICs for clinical *oxyR-ahpC* intergenic region mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic summary	0.05	0.1	0.4	1	3	10	12.8
55) Andres 2014 &	36	clinical	1	oxyR-ahpC c-15t				1			
53) Tessema 2017	36	clinical	1	katG I87W + oxyR-ahpC c-52t							1
53) Tessema 2017	36	clinical	1	katG W191R + oxyR-ahpC c-72t			1				
55) Andres 2014 &	36	clinical	1	oxyR-ahpC g-115a							1
55) Andres 2014 &	36	clinical	1	oxyR-ahpC c-121t							1
53) Tessema 2017	36	clinical	1	oxyR-ahpC g-142a					1		
53) Tessema 2017	36	clinical	1	oxyR-ahpC g-552a	1						

The green line denotes the current WHO and CLSI CC for INH DST in MGIT (0.1 mg/L), whereas the blue line denotes the CLSI CB (0.4 mg/L). Notable limitations: both studies were conducted in the same laboratory.

Double mutants

katG S315 and *inhA* promoter double mutants

Nine studies were identified that reported INH MICs for 200 isolates with *katG* S315 and *inhA* promoter double mutations by MGIT (Table 25). Based on the current INH CC, all (100%, 95% CI 98-100%) of these mutated isolates were phenotypically INH-R, with MICs ≥3 mg/L, far above the current CC. Moreover, the majority of isolates appeared to have MICs >10 mg/L, above those MICs reported for single *katG* S315 mutants (Table 21).

katG S315 and *inhA* coding double mutants

Five studies were identified that reported INH MICs for six clinical isolates with *katG* S315 and *inhA* coding mutations by MGIT (Table 25). Based on the current INH CC, all of these mutated isolates were phenotypically INH-R, with MICs ≥3 mg/L.

inhA promoter and *inhA* coding double mutants

Six studies were identified that reported INH MICs for 67 clinical isolates with *inhA* promoter and *inhA* coding mutations by MGIT (Table 25). Based on the current INH CC, all of these mutated isolates were phenotypically INH-R, with MICs ≥0.5 mg/L.

Table 25. INH MICs for clinical *katG* S315, *inhA* promoter and *inhA* coding double mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic summary	0.1	0.4	1	1.25	2	2.5	3	4	5	7.5	8	10	15	16	24	25	32	35	48	64
55) Andres 2014 &	36	clinical	1	katG S315T + mabA-inhA t-8a													1							
53) Tessema 2017	36	clinical	4	katG S315T + mabA-inhA t-8a													4							
59) Ghodousi 2019	19	clinical	1	katG S315T + mabA-inhA t-8a																	1			
46) Cambau 2015	9, 19, 22, 35-40	clinical	1	katG S315T + mabA-inhA t-8c												1								
46) Cambau 2015	9, 19, 22, 35-40	clinical	1	katG S315T + mabA-inhA t-8c												1								
47) Kambli 2015	41		4	katG S315T + mabA-inhA t-8c												3	1							
49) Rueda 2015	43	clinical	1	katG S315T + mabA-inhA t-8c										1										
55) Andres 2014 &	36	clinical	1	katG S315T + mabA-inhA t-8c												1								
53) Tessema 2017	36	clinical	3	katG S315T + mabA-inhA t-8c								1				2								
59) Ghodousi 2019	19	clinical	1	katG S315T + mabA-inhA t-8c														1						
49) Rueda 2015	43	clinical	2	katG S315T + mabA-inhA t-8g										1	1									
44) Machado 2018 and	35	clinical	2	katG S315T + mabA-inhA c-15t													2							
46) Cambau 2015	9, 19, 22, 35-40	clinical	2	katG S315T + mabA-inhA c-15t													2							
46) Cambau 2015	9, 19, 22, 35-40	clinical	2	katG S315T + mabA-inhA g-17t													2							
46) Cambau 2015	9, 19, 22, 35-40	clinical	4	katG S315T + mabA-inhA c-15t													4							
47) Kambli 2015	41		20	katG S315T + mabA-inhA c-15t													20							
49) Rueda 2015	43	clinical	1	katG S315G + mabA-inhA c-15t									1											
49) Rueda 2015	43	clinical	2	katG S315T + mabA-inhA c-15t										2										
55) Andres 2014 &	36	clinical	11	katG S315T + mabA-inhA c-15t												1	10							
58) Groenheit	39	clinical	6	katG S315T + mabA-inhA c-15t									6											
61) Gygli 2019	29	clinical	5	katG S315T + mabA-inhA c-15t												1	4							
61) Gygli 2019	29	clinical	1	katG S315T + K155N+ mabA-inhA c-15t													1							
53) Tessema 2017	36	clinical	1	katG S315T + mabA-inhA g-17t + oxyR-ahpC g-142a												1								
53) Tessema 2017	36	clinical	107	katG S315T + mabA-inhA c-15t												3	104							
59) Ghodousi 2019	19	clinical	14	katG S315T + mabA-inhA c-15t											1			2		5				6
59) Ghodousi 2019	19	clinical	1	katG S315T + mabA-inhA -34c deletion																				1
59) Ghodousi 2019	19	clinical	1	katG S315N + mabA-inhA c-5t																				1
53) Tessema 2017	36	clinical	1	katG S315T + inhA I21V + oxyR-ahpC a-432c															1					
46) Cambau 2015	9, 19, 22, 35-40	clinical	1	katG S315T + inhA G40W															1					
49) Rueda 2015	43	clinical	2	katG S315T + inhA S94A														2						
58) Groenheit	39	clinical	1	katG S140N + S315N + inhA S94A													1							
59) Ghodousi 2019	19	clinical	1	katG S315T + inhA S94A																		1		
46) Cambau 2015	9, 19, 22, 35-40	clinical	1	mabA-inhA t-8c + inhA S94A								1												
58) Groenheit	39	clinical	1	mabA-inhA c-15t + inhA I21T										1										
59) Ghodousi 2019	19	clinical	2	mabA-inhA c-15t + inhA I21T						2														
53) Tessema 2017	36	clinical	1	mabA-inhA c-15t + ndh V18A + inhA I21T																	1			
53) Tessema 2017	36	clinical	1	mabA-inhA c-15t + inhA I21T											1									
58) Groenheit	39	clinical	1	mabA-inhA c-15t + inhA I21V								1												
59) Ghodousi 2019	19	clinical	1	mabA-inhA c-15t + inhA I21V						1														
58) Groenheit	39	clinical	1	mabA-inhA c-15t + inhA G40W						1														
44) Machado 2018 and	35	clinical	7	mabA-inhA c-15t + inhA S94A												6							1	
45) Machado 2013 and	35	clinical	10	mabA-inhA c-15t + inhA S94A												9					1			
46) Cambau 2015	9, 19, 22, 35-40	clinical	7	mabA-inhA c-15t + inhA S94A												6								
54) Heyckendorf 2018	36	clinical	2	mabA-inhA c-15t + inhA S94A								2												
58) Groenheit	39	clinical	3	mabA-inhA c-15t + inhA S94A								2		1										
61) Gygli 2019	29	clinical	1	mabA-inhA c-15t + inhA S94A								1												
59) Ghodousi 2019	19	clinical	1	mabA-inhA c-15t + inhA S94A										1										
44) Machado 2018 and	35	clinical	7	mabA-inhA c-15t + inhA I194T												7								
45) Machado 2013 and	35	clinical	6	mabA-inhA c-15t + inhA I194T												6								
46) Cambau 2015	9, 19, 22, 35-40	clinical	5	mabA-inhA c-15t + inhA I194T												5								
53) Tessema 2017	36	clinical	2	mabA-inhA c-15t + inhA I194T								2												
58) Groenheit	39	clinical	2	mabA-inhA c-15t + inhA I194T								2												
59) Ghodousi 2019	19	clinical	2	mabA-inhA c-15t + inhA I194T								1		1										
46) Cambau 2015	9, 19, 22, 35-40	clinical	1	mabA-inhA c-15t + inhA I200T												1								
59) Ghodousi 2019	19	clinical	1	mabA-inhA c-15t + inhA A239V						1														
61) Gygli 2019	29	clinical	1	mabA-inhA g-17t + inhA S94A								1												

The green line denotes the current WHO and CLSI CC for INH DST in MGIT (0.1 mg/L), whereas the blue line denotes the CLSI CB (0.4 mg/L). Notable limitations: studies I46, I53, I54 and I55, studies I46 and I59, studies I44, I45 and I46, and studies I46 and I58 were conducted in the same laboratory and study I47 presented data for isolates characterized by a mix of LPA and pyrosequencing.

2.A.4.3 Conclusion for INH CC in MGIT

The current CC of **0.1 mg/L** was reaffirmed as it likely corresponds to the tentative ECOFF based on the available data. ISO dilutions should be adopted for testing and future breakpoints.

2.3 INH conclusions and comments

The quality and quantity of MIC data for all media did not meet EUCAST standards to define ECOFFs.[32] Nevertheless, the current CCs were reaffirmed as they are likely close to the ECOFFs for INH and adequately identified most known resistance mechanisms. However, ISO concentrations should be used in the future for MIC testing and DST breakpoints.

INH resistance is a heterogenous phenotype, but the level of resistance conferred can be predicted, to some extent, based on the combination of *inhA* promoter and *katG* S315 mutations.[33] *inhA* promoter mutations confer only modest MIC increases (0.25-2 mg/L in MGIT) and, consequently, are at risk of being misclassified as susceptible because of the inherent technical variation in pDST.[34] By contrast, *katG* S315 mutations correlate with marked MIC increases (4-16 mg/L in MGIT) and test reliably resistant at the current CC.[34] The effects of the two mechanisms are likely additive, which means that double mutants typically have higher MICs than single mutants (8-64 mg/L in MGIT). [33,34] This is reflected in the time to sputum culture conversion for these three groups (i.e. isolates with only *inhA* promoter mutations have faster conversion times than *katG* S315 mutants, which, in turn, respond better than double mutants).[35] Finally, *katG* loss-of-function mutations, which can sometimes be inferred with the Hain LPA, typically correlate with very high INH MICs.[34,36,37]

The TEG considered clinical trial data from the AIDS Clinical Trials Group A5312 that provided some of the strongest evidence to date that isolates with only *inhA* promoter mutations, and corresponding modest MIC increases, may benefit from high-dose INH therapy.[38] Nevertheless, a CB was not set. As a result, INH resistance is now stratified genotypically into LLR and HLR by the Hain LPA but not by pDST.[37,39] This inconsistency was highlighted by the TEG as the topic for a future meeting that will consider detailed pharmacokinetic/pharmacodynamic (PK/PD) modelling and additional clinical outcome data (e.g. from the clinical trials NCT01589497, NCT01936831 and NCT02236078, registered at ClinicalTrials.gov).[35,38,40]

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[32] European Committee on Antimicrobial Susceptibility Testing. Standard Operating Procedure. MIC distributions and the setting of epidemiological cut-off (ECOFF) values. SOP 10.1. 30 November 2019 (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/EUCAST_SOPs/EUCAST_SOP_10.1_MIC_distributions_and_epidemiological_cut-off_value__ECOFF__setting_20191130.pdf, accessed 26 July 2020).

[33] Lempens P, Meehan CJ, Vandelannoote K, *et al*. Isoniazid resistance levels of *Mycobacterium tuberculosis* can largely be predicted by high-confidence resistance-conferring mutations. *Sci Rep*. 2018;8(1):3246. doi:10.1038/s41598-018-21378-x.

[34] Ghodousi A, Tagliani E, Karunaratne E, *et al*. Isoniazid resistance in *Mycobacterium tuberculosis* is a heterogeneous phenotype composed of overlapping MIC distributions with different underlying resistance mechanisms. *Antimicrob Agents Chemother*. 2019;63(7):e00092-19. doi:10.1128/AAC.00092-19.

[35] Click ES, Kurbatova EV, Alexander H, *et al*. Isoniazid and rifampin-resistance mutations associated with resistance to second-line drugs and with sputum culture conversion. *J Infect Dis*. 2020;221(12):2072-2082. doi:10.1093/infdis/jiaa042.

[36] Ando H, Kitao T, Miyoshi-Akiyama T, Kato S, *et al*. Downregulation of *katG* expression is associated with isoniazid resistance in *Mycobacterium tuberculosis*. *Mol Microbiol*. 2011;79(6):1615-28. doi:10.1111/j.1365-2958.2011.07547.x.

[37] World Health Organization. Regional Office for Europe. Interpretation guide for GenoType MTBDR*plus* VER 2.0 and GenoType MTBDR*sl* VER 2.0. A technical guidance document developed by the European Laboratory Initiative. Version 1.0. (https://openwho.org/courses/multi-drug-resistant-tb, accessed 9 May 2020).

[38] Dooley KE, Miyahara S, von Groote-Bidlingmaier F, *et al*. Early bactericidal activity of different isoniazid doses for drug resistant TB (INHindsight): A randomized open-label clinical trial. *Am J Respir Care Med*. 2020;201(11):1416-1424. doi:10.1164/rccm.201910-1960OC.

[39] World Health Organization. WHO operational handbook on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection (https://apps.who.int/iris/rest/bitstreams/1284635/retrieve, accessed 30 June 2020).

[40] Diacon A, Miyahara S, Dawson R, *et al*. Assessing whether isoniazid is essential during the first 14 days of tuberculosis therapy: a phase 2a, open-label, randomised controlled trial. *Lancet Microbe*. 2020;1(2):E84-E92. doi:10.1016/S2666-5247(20)30011-2.

2.4 References for INH MIC studies

1. (a) Jagielski T, Bakuła Z, Roeske K, *et al*. Mutation profiling for detection of isoniazid resistance in *Mycobacterium tuberculosis* clinical isolates. *J Antimicrob Chemother*. 2015;70(12):3214-3221. doi:10.1093/jac/dkv253.
   (b) Jagielski T, Grzeszczuk M, Kamiński M, *et al*. Identification and analysis of mutations in the *katG* gene in multidrug-resistant *Mycobacterium tuberculosis* clinical isolates. *Pneumonol Alergol Pol*. 2013;81(4):298-307.
   (c) Jagielski T, Bakuła Z, Roeske K, *et al*. Detection of mutations associated with isoniazid resistance in multidrug-resistant *Mycobacterium tuberculosis* clinical isolates. *J Antimicrob Chemother*. 2014;69(9):2369-2375. doi:10.1093/jac/dku161.
2. Vincent V, Rigouts L, Nduwamahoro E, *et al*. The TDR Tuberculosis Strain Bank: a resource for basic science, tool development and diagnostic services. *Int J Tuberc Lung Dis*. 2012;16(1):24-31. doi:10.5588/ijtld.11.0223.
3. Lee H, Cho SN, Bang HE, *et al*. Exclusive mutations related to isoniazid and ethionamide resistance among *Mycobacterium tuberculosis* isolates from Korea. *Int J Tuberc Lung Dis*. 2000;4(5):441-447.
4. Lempens P, Meehan CJ, Vandelannoote K, *et al*. Isoniazid resistance levels of *Mycobacterium tuberculosis* can largely be predicted by high-confidence resistance-conferring mutations. *Sci Rep*. 2018;8(1):3246. doi:10.1038/s41598-018-21378-x.
5. Schönfeld N, Vesenbeckh S, Rüssmann H, *et al.* Minimal inhibitory concentrations of first-line drugs of multidrug-resistant tuberculosis isolates. *Lung India*. 2012;29(4):309. doi:10.4103/0970-2113.102794.
6. Beckers B, Lang HRM, Schimke D, Lammers A. Evaluation of a bioluminescence assay for rapid antimicrobial susceptibility testing of mycobacteria. *Eur J Clin Microbiol*. 1985;4(6):556-561. doi:10.1007/BF02013394.
7. Alame-Emane AK, Xu P, Pierre-Audigier C, *et al*. Pyrazinamide resistance in *Mycobacterium tuberculosis* arises after rifampicin and fluoroquinolone resistance. *Int J Tuberc Lung Dis*. 2015;19(6):679-684. doi:10.5588/ijtld.14.0768.
8. Thai PVK, Ha DTM, Hanh NT, *et al*. Bacterial risk factors for treatment failure and relapse among patients with isoniazid resistant tuberculosis. *BMC Infect Dis*. 2018;18(1):112. doi:10.1186/s12879-018-3033-9.
9. (a) Brossier F, Veziris N, Jarlier V, Sougakoff W. Performance of MTBDR*plus* for detecting high/low levels of *Mycobacterium tuberculosis* resistance to isoniazid. *Int J Tuberc Lung Dis*. 2009;13(2):260-265.
   (b) Brossier F, Veziris N, Truffot-Pernot C, Jarlier V, Sougakoff W. Performance of the genotype MTBDR line probe assay for detection of resistance to rifampin and isoniazid in strains of *Mycobacterium tuberculosis* with low- and high-level resistance. *J Clin Microbiol*. 2006;44(10):3659-3664. doi:10.1128/JCM.01054-06.
10. Brossier F, Boudinet M, Jarlier V, Petrella S, Sougakoff W. Comparative study of enzymatic activities of new KatG mutants from low- and high-level isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis*. *Tuberculosis*. 2016;100:15-24. doi:10.1016/j.tube.2016.06.002.
11. (a) Rigouts L, Gumusboga M, de Rijk WB, *et al*. Rifampin resistance missed in automated liquid culture system for *Mycobacterium tuberculosis* isolates with specific *rpoB* mutations. *J Clin Microbiol*. 2013;51(8):2641-2645. doi:10.1128/JCM.02741-12.
    (b) Van Deun A. Personal communication. 2018.
12. Farhat MR, Freschi L, Calderon R, *et al*. GWAS for quantitative resistance phenotypes in *Mycobacterium tuberculosis* reveals resistance genes and regulatory regions. *Nat Commun*. 2019;10(1):2128. doi:10.1038/s41467-019-10110-6.
13. Alonso M, Martínez-Lirola M, Palacios JJ, *et al*. Evaluation of the potential role of a new mutation in *mabA* in modifying the response of *Mycobacterium tuberculosis* to isoniazid. *Tuberculosis*. 2013;93(6):664-667. doi:10.1016/j.tube.2013.07.003.
14. (a) de Steenwinkel JEM, ten Kate MT, de Knegt GJ, *et al*. Drug susceptibility of *Mycobacterium tuberculosis* Beijing genotype and association with MDR TB. *Emerg Infect Dis*. 2012;18(4):660-663. doi:10.3201/eid1804.110912.
    (b) de Steenwinkel JEM, ten Kate MT, de Knegt GJ, *et al*. Consequences of noncompliance for therapy efficacy and emergence of resistance in murine tuberculosis caused by the Beijing genotype of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2012;56(9):4937-4944. doi:10.1128/AAC.00124-12.

(c) Bax HI, Bakker-Woudenberg IAJM, de Vogel CP, *et al*. The role of the time-kill kinetics assay as part of a preclinical modeling framework for assessing the activity of anti-tuberculosis drugs. *Tuberculosis*. 2017;105:80-85. doi:10.1016/j.tube.2017.04.010.

15. (a) Campbell PJ, Morlock GP, Sikes RD, *et al*. Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2011;55(5):2032-2041. doi:10.1128/AAC.01550-10.
(b) Ramirez M V., Cowart KC, Campbell PJ, *et al*. Rapid detection of multidrug-resistant *Mycobacterium tuberculosis* by use of real-time PCR and high-resolution melt analysis. *J Clin Microbiol*. 2010;48(11):4003-4009. doi:10.1128/JCM.00812-10.

16. Abbadi SH, Sameaa GA, Morlock G, Cooksey RC. Molecular identification of mutations associated with anti-tuberculosis drug resistance among strains of *Mycobacterium tuberculosis*. *Int J Infect Dis*. 2009;13(6):673-678. doi:10.1016/j.ijid.2008.10.006.

17. Cavusoglu C, Turhan A, Akinci P, Soyler I. Evaluation of the Genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* isolates. *J Clin Microbiol*. 2006;44(7):2338-2342. doi:10.1128/JCM.00425-06.

18. Van Doorn HR, Claas ECJ, Templeton KE, *et al*. Detection of a point mutation associated with high-level isoniazid resistance in *Mycobacterium tuberculosis* by using real-time PCR technology with 3'-minor groove binder-DNA probes. *J Clin Microbiol*. 2003;41(10):4630-4635. doi:10.1128/JCM.41.10.4630-4635.2003.

19. Pretorius GS, Van Helden PD, Sirgel F, Eisenach KD, Victor TC. Mutations in *katG* gene sequences in isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis* are rare. *Antimicrob Agents Chemother*. 1995;39(10):2276-2281. doi:10.1128/AAC.39.10.2276.

20. Schechter MC, Bizune D, Kagei M, *et al*. Time to sputum culture conversion and treatment outcomes among patients with isoniazid-resistant tuberculosis in Atlanta, Georgia. *Clin Infect Dis*. 2017;65(11):1862-1871. doi:10.1093/cid/cix686.

21. DeCoster DJ, Vena RM, Callister SM, Schell RF. Susceptibility testing of *Mycobacterium tuberculosis*: Comparison of the BACTEC TB-460 method and flow cytometric assay with the proportion method. *Clin Microbiol Infect*. 2005;11(5):372-378. doi:10.1111/j.1469-0691.2005.01127.x.

22. Moore A V., Kirk SM, Callister SM, Mazurek GH, Schell RF. Safe determination of susceptibility of *Mycobacterium tuberculosis* to antimycobacterial agents by flow cytometry. *J Clin Microbiol*. 1999;37(3):479-483. doi:10.1128/jcm.37.3.479-483.1999.

23. Kirk SM, Schell RF, Moore A V., Callister SM, Mazurek GH. Flow cytometric testing of susceptibilities of *Mycobacterium tuberculosis* isolates to ethambutol, isoniazid, and rifampin in 24 hours. *J Clin Microbiol*. 1998;36(6):1568-1573. doi:10.1128/jcm.36.6.1568-1573.1998.

24. Rancoita PM V., Cugnata F, Gibertoni Cruz AL, *et al*. Validating a 14-drug microtiter plate containing bedaquiline and delamanid for large-scale research susceptibility testing of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2018;62(9):e00344-18. doi:10.1128/AAC.00344-18.

25. Galí N, Domínguez J, Blanco S, *et al*. Use of a mycobacteriophage-based assay for rapid assessment of susceptibilities of *Mycobacterium tuberculosis* isolates to isoniazid and influence of resistance level on assay performance. *J Clin Microbiol*. 2006;44(1):201-205. doi:10.1128/JCM.44.1.201-205.2006.

26. Abe C, Kobayashi I, Mitarai S, *et al*. Biological and molecular characteristics of *Mycobacterium tuberculosis* clinical isolates with low-level resistance to isoniazid in Japan. *J Clin Microbiol*. 2008;46(7):2263-2268. doi:10.1128/JCM.00561-08.

27. van Klingeren B, Dessens-Kroon M, van der Laan T, Kremer K, van Soolingen D. Drug susceptibility testing of *Mycobacterium tuberculosis* complex by use of a high-throughput, reproducible, absolute concentration method. *J Clin Microbiol*. 2007;45(8):2662-2668. doi:10.1128/JCM.00244-07.

28. Wedajo W, Schön T, Bedru A, *et al*. A 24-well plate assay for simultaneous testing of first and second line drugs against *Mycobacterium tuberculosis* in a high endemic setting. *BMC Res Notes*. 2014;7:512. doi:10.1186/1756-0500-7-512.

29. Schön T, Juréen P, Giske CG, *et al*. Evaluation of wild-type MIC distributions as a tool for determination of clinical breakpoints for *Mycobacterium tuberculosis*. *J Antimicrob Chemother*. 2009;64(4):786-793. doi:10.1093/jac/dkp262.

30. Otto-Knapp R, Vesenbeckh S, Schönfeld N, *et al*. Isoniazid minimal inhibitory concentrations of tuberculosis strains with *katG* mutation. *Int J Tuberc Lung Dis*. 2016;20(9):1275-1276. doi:10.5588/ijtld.16.0148.

31. Karunaratne GHRE, Wijesundera SS, Vidanagama D, Adikaram CP, Perera J. Significance of coexisting mutations on determination of the degree of isoniazid resistance in *Mycobacterium tuberculosis* strains. *Microb Drug Resist*. 2018;24(6):844-851. doi:10.1089/mdr.2017.0330.

32. Pholwat S, Heysell S, Stroup S, Foongladda S, Houpt E. Rapid first- and second-line drug susceptibility assay for *Mycobacterium tuberculosis* isolates by use of quantitative PCR. *J Clin Microbiol*. 2011;49(1):69-75. doi:10.1128/JCM.01500-10.

33. Farhat MR, Freschi L, Calderon R, *et al*. GWAS for quantitative resistance phenotypes in *Mycobacterium tuberculosis* reveals resistance genes and regulatory regions. *Nat Commun*. 2019;10(1):2128. doi:10.1038/s41467-019-10110-6.

34. Gygli SM, Keller PM, Ballif M, *et al*. Whole-genome sequencing for drug resistance profile prediction in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2019;63(4):e02175-18. doi:10.1128/AAC.02175-18.

35. Park S-W, Tasneen R, Converse PJ, Nuermberger EL. Immunodeficiency and intermittent dosing promote acquired rifamycin monoresistance in murine tuberculosis. *Antimicrob Agents Chemother*. 2017;61(11):e01502-17. doi:10.1128/AAC.01502-17.

36. Guo H, Seet Q, Denkin S, Parsons L, Zhang Y. Molecular characterization of isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis* from the USA. *J Med Microbiol*. 2006;55(11):1527-1531. doi:10.1099/jmm.0.46718-0.

37. Cockerill FR, Uhl JR, Temesgen Z, *et al*. Rapid identification of a point mutation of the *Mycobacterium tuberculosis* catalase-peroxidase (*katG*) gene associated with isoniazid resistance. *J Infect Dis*. 1995;171(1):240-245. doi:10.1093/infdis/171.1.240.

38. Coban AY. Blood agar validation for susceptibility testing of isoniazid, rifampicin, ethambutol, and streptomycin to *Mycobacterium tuberculosis* isolates. *PLoS One*. 2013;8(2):e55370. doi:10.1371/journal.pone.0055370.

39. (a) Rey-Jurado E, Tudó G, de la Bellacasa JP, Espasa M, González-Martín J. *In vitro* effect of three-drug combinations of antituberculous agents against multidrug-resistant *Mycobacterium tuberculosis* isolates. *Int J Antimicrob Agents*. 2013;41(3):278-280. doi:10.1016/j.ijantimicag.2012.11.011.
(b) Rey-Jurado E, Tudó G, Martínez JA, González-Martín J. Synergistic effect of two combinations of antituberculous drugs against *Mycobacterium tuberculosis*. *Tuberculosis*. 2012;92(3):260-263. doi:10.1016/j.tube.2012.01.005.

40. Fattorini L, Iona E, Ricc ML, *et al*. Activity of 16 Antimicrobial agents against drug-resistant strains of *Mycobacterium tuberculosis*. *Microb Drug Resist*. 1999;5(4):265-270. doi:10.1089/mdr.1999.5.265.

41. Rodriguez Díaz JC, Ruiz M, López M, Royo G. Synergic activity of fluoroquinolones and linezolid against *Mycobacterium tuberculosis*. *Int J Antimicrob Agents*. 2003;21(4):354-356. doi:10.1016/s0924-8579(02)00387-4.

42. Shishido Y, Mitarai S, Otomo K, *et al*. Anti-tuberculosis drug susceptibility testing of *Mycobacterium bovis* BCG Tokyo strain. *Int J Tuberc Lung Dis*. 2007;11(12):1334-1338.

43. Lee C-N, Heifets LB. Determination of minimal inhibitory concentrations of antituberculosis drugs by radiometric and conventional methods. *Am Rev Respir Dis*. 1987;136(2):349-352. doi:10.1164/ajrccm/136.2.349.

44. Machado D, Perdigão J, Portugal I, *et al*. Efflux activity differentially modulates the levels of isoniazid and rifampicin resistance among multidrug resistant and monoresistant *Mycobacterium tuberculosis* strains. *Antibiotics*. 2018;7(1):18. doi:10.3390/antibiotics7010018.

45. Machado D, Perdigão J, Ramos J, *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;68(8):1728-1732. doi:10.1093/jac/dkt090.

46. Cambau E, Viveiros M, Machado D, *et al*. Revisiting susceptibility testing in MDR-TB by a standardized quantitative phenotypic assessment in a European multicentre study. *J Antimicrob Chemother*. 2015;70(3):686-696. doi:10.1093/jac/dku438.

47. Kambli P, Ajbani K, Sadani M, *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.

48. Torres JN, Paul L V., Rodwell TC, *et al*. Novel *katG* mutations causing isoniazid resistance in clinical *M. tuberculosis* isolates. *Emerg Microbes Infect*. 2015;4(7):e42. doi:10.1038/emi.2015.42.

49. Rueda J, Realpe T, Mejia GI, *et al*. Genotypic analysis of genes associated with independent resistance and cross-resistance to isoniazid and ethionamide in *Mycobacterium tuberculosis* clinical isolates. *Antimicrob Agents Chemother*. 2015;59(12):7805-7810. doi:10.1128/AAC.01028-15.

50. Rockwood N, Sirgel F, Streicher E, *et al*. Low frequency of acquired isoniazid and rifampicin resistance in rifampicin-susceptible pulmonary tuberculosis in a setting of high HIV-1 infection and tuberculosis coprevalence. *J Infect Dis*. 2017;216(6):632-640. doi:10.1093/infdis/jix337.

51. Bernardelli A, Morcillo N, Loureiro J, Quse V, Davenport S. *In vitro* susceptibility testing of *Mycobacterium tuberculosis* complex strains isolated from seals to antituberculosis drugs. *Biomedica*. 2004;24 Supp 1:85-91.

52. Chigutsa E, Pasipanodya JG, Visser ME, *et al*. Impact of nonlinear interactions of pharmacokinetics and MICs on sputum bacillary kill rates as a marker of sterilizing effect in tuberculosis. *Antimicrob Agents Chemother*. 2015;59(1):38-45. doi:10.1128/AAC.03931-14.

53. Tessema B, Nabeta P, Valli E, *et al*. FIND Tuberculosis Strain Bank: A resource for researchers and developers working on tests to detect *Mycobacterium tuberculosis* and related drug resistance. Land GA, ed. *J Clin Microbiol*. 2017;55(4):1066-1073. doi:10.1128/JCM.01662-16.

54. (a) Heyckendorf J, Andres S, Köser CU, *et al*. What is resistance? Impact of phenotypic versus molecular drug resistance testing on therapy for multi- and extensively drug-resistant Tuberculosis. *Antimicrob Agents Chemother*. 2017;62(2):e01550-17. doi:10.1128/AAC.01550-17.
   (b) Sturegård E, Ängeby KA, Werngren J, *et al*. Little difference between minimum inhibitory concentrations of *Mycobacterium tuberculosis* wild-type organisms determined with BACTEC MGIT 960 and Middlebrook 7H10. *Clin Microbiol Infect*. 2015;21(2):148.e5-7. doi:10.1016/j.cmi.2014.08.021.

55. (a) Andres S, Hillemann D, Rüsch-Gerdes S, Richter E. Occurrence of *rpoB* mutations in isoniazid-resistant but rifampin-susceptible *Mycobacterium tuberculosis* isolates from Germany. *Antimicrob Agents Chemother*. 2014;58(1):590-592. doi:10.1128/AAC.01752-13.
   (b) Andres S. Personal communication. 2018.

56. Rockwood N, Pasipanodya JG, Denti P, *et al*. Concentration-dependent antagonism and culture conversion in pulmonary tuberculosis. *Clin Infect Dis*. 2017;64(10):1350-1359. doi:10.1093/cid/cix158.

57. Niward K, Davies Forsman L, Bruchfeld J, *et al*. Distribution of plasma concentrations of first-line anti-TB drugs and individual MICs: a prospective cohort study in a low endemic setting. *J Antimicrob Chemother*. 2018;73(10):2838-2845. doi:10.1093/jac/dky268.

58. Groenheit R. Personal communication. 2018.

59. Ghodousi A, Tagliani E, Karunaratne E, *et al*. Isoniazid resistance in *Mycobacterium tuberculosis* is a heterogeneous phenotype composed of overlapping MIC distributions with different underlying resistance mechanisms. *Antimicrob Agents Chemother*. 2019;63(7):e00092-19. doi:10.1128/AAC.00092-19.

60. Colangeli R, Jedrey H, Kim S, *et al*. Bacterial factors that predict relapse after tuberculosis therapy. *N Engl J Med*. 2018;379(9):823-833. doi:10.1056/NEJMoa1715849.

61. Gygli SM, Keller PM, Ballif M, *et al*. Whole-genome sequencing for drug resistance profile prediction in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2019;63(4):e02175-18. doi:10.1128/AAC.02175-18.

SECTION 3: Rifamycins

3.0 Rifamcyin resistance mechanisms

RIF, RFB and RPT belong to the rifamycin group, and inhibit bacterial DNA-dependent RNA synthesis.[41,42,43] The vast majority (~96%) of rifamycin resistance is caused by genetic changes in the RIF resistance-determining region (RRDR), a well-defined, 81-base-pair central region of the *rpoB* gene stretching from *M. tuberculosis* codons 426 to 452 (*Escherichia coli* numbering 507-533).[44,45,46,47,48,49] This RRDR forms the RIF drug-binding pocket, and, as a result, non-synonymous changes and, more rarely, in-frame deletions or insertions in this region confer resistance to rifamycins.

rpoB mutations well outside of the RRDR have also been associated with RIF resistance. These mutations have been most commonly reported at *rpoB* codons 170 (146 by *E. coli* numbering) and 491 (572 by *E. coli* numbering).[44] The V170F mutation has been shown to confer RIF resistance in *E. coli* through site-directed mutagenesis studies.[50] The same has been demonstrated for the I491F mutation in laboratory mutagenesis studies in *E. coli*.[51] Importantly, transformation experiments of both of these mutations confirmed that they also result in elevated RIF MICs in *M. tuberculosis*.[52,53] Each of these mutations was shown to be responsible for 0.5% (95% CI 0.2-1.2%) of RIF resistance respectively based on a recent WHO multi-country population-based surveillance study.[54] However, the I491F (I572F) mutation accounted for more than half of RIF resistance in Eswatini even though the frequency in neighboring South Africa was <1%.[55,56]

[41] Calvori C, Frontali L, Leoni L, Tecce G. Effect of rifamycin on protein synthesis. *Nature*. 1965;207(4995):417-418. doi:10.1038/207417a0.

[42] Wehrli W. Rifampin: Mechanisms of action and resistance. *Rev Infect Dis*. 1983;5:S407-11. doi:10.2307/4453139.

[43] Yang B, Koga H, Ohno H, *et al*. Relationship between antimycobacterial activities of rifampicin, rifabutin and KRM-1648 and rpoB mutations of *Mycobacterium tuberculosis*. *J Antimicrob Chemother*. 1998;42(5):621-628. doi:10.1093/jac/42.5.621.

[44] Please refer to Section 3.2 for more details regarding the different *rpoB* numbering systems.

[45] Telenti A, Imboden P, Marchesi F, *et al*. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. *Lancet*. 1993;341(8846):647-650. doi:10.1016/0140-6736(93)90417-f.

[46] Van Rie A, Warren R, Mshanga I, *et al.* Analysis for a limited number of gene codons can predict drug resistance of *Mycobacterium tuberculosis* in a high-incidence community. *J Clin Microbiol*. 2001;39(2):636-641. doi:10.1128/JCM.39.2.636-641.2001.

[47] Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. *Tuber Lung Dis*. 1998;79(1):3-29. doi:10.1054/tuld.1998.0002

[48] Zhang Y, Telenti A. Genetics of drug resistance in *Mycobacterium tuberculosis*. In: Hatfull GF, Jacobs WR, eds. *Molecular Genetics of Mycobacteria*. ASM Press; 2000:363 (https://library.villanova.edu/Find/Record/538689/TOC, accessed 7 June 2019).

[49] World Health Organization. Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis; 2018. (https://apps.who.int/iris/bitstream/handle/10665/275469/9789241514842-eng.pdf, accessed 7 June 2019).

[50] Severinov K, Soushko M, Goldfarb A, Nikiforov V. Rif^R mutations in the beginning of the *Escherichia coli rpoB* gene. *MGG Mol Gen Genet*. 1994;244:120-126. doi:10.1007/BF00283512.

[51] Rodríguez-Verdugo A, Gaut BS, Tenaillon O. Evolution of *Escherichia coli* rifampicin resistance in an antibiotic-free environment during thermal stress. *BMC Evol Biol*. 2013;13:50. doi:10.1186/1471-2148-13-50.

[52] Siu GK, Zhang Y, Lau TC, *et al*. Mutations outside the rifampicin resistance-determining region associated with rifampicin resistance in *Mycobacterium tuberculosis*. *J Antimicrob Chemother*. 2011;66(4):730-733. doi:10.1093/jac/dkq519.

[53] Lai LY, Hsu LY, Weng SH, *et al*. A glutamine insertion at codon 432 of RpoB confers rifampicin resistance in *Mycobacterium tuberculosis*. *Front Microbiol*. 2020;11:583194. doi:10.3389/fmicb.2020.583194.

[54] Zignol M, Cabibbe AM, Dean AS, *et al*. Genetic sequencing for surveillance of drug resistance in tuberculosis in highly endemic countries: a multi-country population-based surveillance study. *Lancet Infect Dis*. 2018;18(6):675-683. doi:10.1016/S1473-3099(18)30073-2. doi:10.1016/S1473-3099(18)30073-2.

[55] Sikhondze W, Dlamini T, Joloba Moses, *et al*. Xpert MTB/RIF miss es more than 50% of rifampicin resistant TB cases in Eswatini: results of the 2nd national anti-TB drug resistance survey (2017/2018). *Int J Tuberc Lung Dis*. 2019;23(10 Suppl 1):S585.

[56] Ismail NA, Omar SV, Mvusi L, Madhi SA. Prevalence of drug-resistant tuberculosis in South Africa - Authors' reply. *Lancet Infect Dis*. 2018;18(8):836-837. doi:10.1016/S1473-3099(18)30422-5.

A recent report by Huseby *et al*. demonstrated that a frameshift in the RRDR of *E. coli* is possible, despite *rpoB* being an essential gene, and also confers RIF resistance through spontaneous frameshift suppression.[57] Similar mutations could, in principle, occur throughout the entire length of *rpoB*. Frameshifts and nonsense mutations (i.e. premature stop codons) have been described in MTBC in the literature, but it is unclear what proportion of these may be due to experimental errors and, consequently, which percentage of RIF resistance in clinical isolates is caused by these mechanisms.

3.1 Current statements and policies regarding genotypic markers of rifamycin resistance

Currently, there is a lack of consensus on a list of genetic alterations that confer rifamycin resistance, and the degree of cross-resistance between the three rifamycins is unclear.[58] In particular, there is a need for alignment regarding the following statements, policy recommendations and practices regarding RIF resistance:

1. Miotto *et al*. conducted a comprehensive analysis of the association between *rpoB* mutations and pDST results, which yielded 24 high-, moderate-, or minimal-confidence mutations that were associated with RIF resistance after performing a p-value correction (Table 26).[59] A further 13 *rpoB* mutations (or combinations thereof) were associated with RIF resistance based on their nominal p-values, but were statistically indeterminate following a p-value correction.

2. For the purposes of surveillance, WHO has adopted a composite reference standard for RIF resistance.[54] Specifically, the aforementioned list of 24 p-value corrected *rpoB* mutations from Miotto *et al*. were considered to be true markers for RIF resistance (i.e. the presence of any of these mutations is necessary and sufficient to confirm RIF resistance). Any susceptible pDST result for an isolate with one of these mutations was thereby corrected to resistant.

3. In the recently published WHO technical manual for TB DST, DNA sequencing of the entire *rpoB* gene was proposed as a reference standard for RIF resistance. Furthermore, an expert rule was established, that "[a]ny mutation (excluding silent mutations) observed in the 81bp RRDR [as defined above] hotspot region of the *rpoB* gene are known or assumed to be associated with rifampicin resistance" (i.e. even mutations that were statistically indeterminate in Miotto *et al.* or have never been reported to date within the RRDR were considered RIF-R).[60] This expert rule was introduced to address the confusion surrounding the "borderline resistance" *rpoB* mutations, which are also referred to as "disputed", "discordant", "occult" or "(sub-breakpoint) low-level resistance" mutations. These account for 12% (95% CI 10-15%) of RIF resistance based on WHO surveillance data from seven countries but can be considerably more frequent in some settings

[57] Huseby DL, Brandis G, Praski Alzrigat L, Hughes D. Antibiotic resistance by high-level intrinsic suppression of a frameshift mutation in an essential gene. *Proc Natl Acad Sci U S A*. 2020;117(6):3185-3191. doi:10.1073/pnas.1919390117.

[58] Schön T, Juréen P, Chryssanthou E, *et al*. Rifampicin-resistant and rifabutin-susceptible *Mycobacterium tuberculosis* strains: a breakpoint artefact? *J Antimicrob Chemother*. 2013;68(9):2074-2077. doi:10.1093/jac/dkt150

[59] Miotto P, Tessema B, Tagliani E, *et al.* A standardised method for interpreting the association between mutations and phenotypic drug resistance in *Mycobacterium tuberculosis*. *Eur Respir J*. 2017;50(6):1701354. doi:10.1183/13993003.01354-2017.

[60] World Health Organization. Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. (http://apps.who.int/iris/bitstream/handle/10665/275469/9789241514842-eng.pdf, accessed 17 November 2018).

(e.g. in Eswatini or São Paulo state, Brazil).[54,55,61] These were originally defined as "disputed" given that they are more likely to test RIF-S by MGIT compared to LJ.[62] However, studies differ on which exact mutations are associated with borderline RIF resistance. Based on the published literature and a review of MIC data collected as part of this systematic review, six RRDR mutations as well as I491F (I572F) were classified as borderline resistance mutations for the purposes of this report, which are highlighted in Table 26.[62,63,64] In order to justify the expert rule that any RRDR mutation, apart from synonymous mutations, should be considered to be a valid marker for RIF-R, it is necessary to determine why these borderline resistance mutations are more likely to test RIF-S (see Section 3.3).

4. In the recently published WHO technical guide for the use of next-generation sequencing (NGS), an abridged review of MIC evidence for *rpoB* mutations from Miotto *et al*. was performed, though there were a few differences in the confidence grading of rare mutations.[65] For example, the N437 (N518) deletion in RRDR was listed as a high-confidence resistance mutation in the NGS guide even though it actually was statistically indeterminate in Miotto *et al*. following p-value correction.[59]

5. The WHO-endorsed LPAs and Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA) cover codons immediately downstream and/or upstream of RRDR (Table 27). Because the gDST results for these assays are not always confirmed in many settings, the aforementioned expert rule for RRDR is *de facto* extended to these adjacent codons (e.g. 424-425 (505-506) in the case of the Hain LPA).[60,66]

6. Even when confirmatory genotypic testing is conducted, the interpretation of sequencing results has not been well aligned. For example, the GLI recommends that sequencing results are interpreted according to the NGS technical guide but does not mention the RRDR expert rule from the DST manual. [60,66]

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[61] Brandao AP, Pinhata JMW, Simonsen V, *et al*. Transmission of *Mycobacterium tuberculosis* presenting unusually high discordance between genotypic and phenotypic resistance to rifampicin in an endemic tuberculosis setting. *Tuberculosis (Edinb)*. 2020;125:102004. doi:10.1016/j.tube.2020.102004.

[62] Torrea G, Ng K, Van Deun A. Variable ability of rapid tests to detect *Mycobacterium tuberculosis rpoB* mutations conferring phenotypically occult rifampicin resistance. *Sci Rep*. 2019;9(1):11826. doi:10.1038/s41598-019-48401-z.

[63] Rigouts L, Gumusboga M, de Rijk WB, *et al*. Rifampin resistance missed in automated liquid culture system for *Mycobacterium tuberculosis* isolates with specific *rpoB* mutations. *J Clin Microbiol*. 2013;51(8):2641-2645. doi:10.1128/JCM.02741-12.

[64] Miotto P, Cabibbe AM, Borroni E, Degano M, Cirillo DM. Role of disputed mutations in the *rpoB* gene in interpretation of automated liquid MGIT culture results for rifampin susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2018;56(5):e01599-17. doi:10.1128/JCM.01599-17.

[65] World Health Organization. The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in *Mycobacterium tuberculosis* complex: Technical guide; 2018 (https://apps.who.int/iris/bitstream/handle/10665/274443/WHO-CDS-TB-2018.19-eng.pdf, accessed 7 June 2019).

[66] Global Laboratory Initiative. Line probe assays for drug-resistant tuberculosis detection: interpretation and reporting guide for laboratory staff and clinicians; 2019 (http://www.stoptb.org/wg/gli/assets/documents/LPA_test_web_ready.pdf, accessed 22 May 2020).

Table 26. Overview of confidence-graded *rpoB* RIF resistance mutations, including the seven borderline resistance mutations.

High confidence *M. tuberculosis* resistance mutation (*E. coli* numbering)	Moderate confidence *M. tuberculosis* resistance mutation (*E. coli* numbering)	Minimal confidence *M. tuberculosis* resistance mutation (*E. coli* numbering)
Q432K (Q513K)	D435Y (D516Y)	L430P (L511P)
Q432L (Q513L)	S441L (S522L)	H445N (H526N)
Q432P (Q513P)	L452P (L533P)	I491F (I572F)
F433dupl (F514dupl)		
D435A (D516A)		
D435F (D516F)		
D435G (D516G)		
D435V (D516V)		
S441Q (S522Q)		
H445C (H526C)		
H445D (H526D)		
H445G (H526G)		
H445L (H526L)		
H445R (H526R)		
H445Y (H526Y)		
S450F (S531F)		
S450L (S531L)		
S450W (S531W)		

Cells with borderline resistance mutations are shaded in grey. Mutations that were statistically indeterminate after p-value correction are not listed, which is the case for H445S (H526S), the seventh borderline resistance mutation.[59]

Table 27. Probe-binding regions and *rpoB* mutation coverage of WHO-endorsed gDST assays.

Assay	*rpoB* probe	Codons analysed or specific mutation detected[a]	Mutations covered as per package insert
Hain GenoType MTBDR*plus* V2[67,68]	WT1	424-428 (505-509)[b]	F424L (F505L), T427A (T508A), S428T (S509T)
	WT2	429-432 (510-513)	Q429H (Q510H), L430P (L511P)
	WT3	432-436 (513-517)	Q432L (Q513L), Q432P (Q513P), del433-435 (del514-516)
	WT4	435-438 (516-519)	D435V (D516V), D435Y (D516Y), del434 (del515)
	WT5	437-441 (518-522)	del437 (del518), N437I (N518I)
	WT6	441-444 (522-525)	S441L (S522L), S441Q (S522Q)
	WT7	445-448 (526-529)	H445C (H526C), H445D (H526D), H445L (H526L), H445N (H526N), H445P (H526P), H445Q (H526Q), H445R (H526R), H445S (H526S), H445Y (H526Y)
	WT8	449-452 (530-533)	S450L (S531L), S450Q (S531Q), S450W (S531W), L452P (L533P)
	MUT1	gac/gtc D435V (D516V)	
	MUT2A	cac/tac H445Y (H526Y)	
	MUT2B	cac/gac H445D (H526D)	
	MUT3	tcg/ttg S450L (S531L)	
Nipro NTM+MDRTB II[69]	S1	428-433 (509-514)	
	S2	434-439 (515-520)	
	S3	439-444 (520-525)	
	S4	444-449 (525-530)	
	S5	449-454 (530-535)[b]	
	R2	D435V (D516V)	
	R4a	H445Y (H526Y)	
	R4b	H445D (H526D)	
	R5	S450L (S531L)	
Cepheid Xpert MTB/RIF G4[70]	A	426-431 (507-512)	
	B[c]	430-438 (511-519)	
	C	436-442 (517-523)	
	D	441-447 (522-528)	
	E	447-453 (528-534)[b]	
Cepheid Xpert Ultra[70]	1	426-435 (507-516)	
	2	433-443 (514-524)	
	3	441-450 (522-531)	
	4	446-455 (527-536)[b]	
Molbio Truenat MTB-RIF Dx	Not published to date		

[a] Hain has not published which precise codons are covered by "MUT" probes. The same applies to the "R" probes of the Nipro LPA. The codons covered by the Hain "WT" or Nipro "S" probes may not be covered completely and not all mutations

[67] Hain Lifescience. GenoType MTBDR*plus* VER 2.0. Instructions for use. IFU-304A-09. (https://www.hain-lifescience.de/include_datei/kundenmodule/packungsbeilage/download.php?id=2877, accessed 1 Nov 2020).

[68] World Health Organization. Regional Office for Europe. Interpretation guide for GenoType MTBDR*plus* VER 2.0 and GenoType MTBDR*sl* VER 2.0. A technical guidance document developed by the European Laboratory Initiative. Version 1.0. (https://openwho.org/courses/multi-drug-resistant-tb, accessed 9 May 2020).

[69] FIND. Report for WHO: Non-inferiority evaluation of Nipro NTM+MDRTB and Hain GenoType MTBDR*plus* V2 line probe assays. Version 4.1. Geneva, Switzerland; 2015 (https://www.finddx.org/wp-content/uploads/2016/04/LPA-report_noninferiority-study_oct2015.pdf, accessed 22 May 2020).

[70] Chakravorty S. Personal communication. 2020.

in these codons, particularly if they occur at the edges of the probes, prevent binding.[68] Similarly, the codons listed for Xpert and Ultra probes may only be covered partially.[70]

[b] Probes include nucleotides or codons outside of the RRDR: 426-452 (507-533).[71]

[c] Xpert G3 and earlier versions used a different probe B.[72,73,74]

3.2 Rifamycin MIC data stratification and current breakpoints

As the RRDR was originally defined in *E. coli*, it is standard practice to use the numbering system of this species even when describing specific features of *rpoB* from other bacteria.[75] For the purposes of this report, both the *M. tuberculosis* and *E. coli* numbering systems were used for reporting of *rpoB* mutations, with the *E. coli* sequence annotation noted in parentheses.[76] Specifically, the annotation adopted for the genome of the *M. tuberculosis* H37Rv reference genome is used throughout this report (GenBank accession AL123456.3).[77] The start of this annotation is six codons downstream of the one first proposed in 1994 (GenBank accession AAA21416.1), which is occasionally used in the literature.[78,79]

All *rpoB* mutations that were identified in codons 424-456 (505-537), as covered by WHO-endorsed molecular assays (Table 27), as well as codons 170 (146) and 491 (572), were recorded and reported in this analysis. MIC data were only excluded if *rpoB* mutations occurred in codons other than those of interest (*M. tuberculosis* codons 170, 424-456, and 491), or if multiple *rpoB* single nucleotide polymorphisms (SNPs) were reported in the same codon, as this might signify either mixtures or translation errors, or if the exact concentrations used for MIC testing of isolates or final MICs for any isolates could not be clarified after contacting the lead study authors. Synonymous *rpoB* mutations were considered to be gWT. MIC data were stratified based on mutation location: RRDR mutations were grouped as S450 (S531) codon mutations (because these confer large MIC increases, they can be used accordingly to assess the quality of MIC testing of a given study[80]); RRDR borderline resistance mutations (Table 26); other RRDR mutations; and *rpoB* mutations outside the RRDR. Only single mutants are included in this report, though MIC data for isolates with multiple mutations can be found in the corresponding Excel file. Similarly, *rpoB* indels were only recorded in the Excel files. For the

[71] World Health Organization. Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. (http://apps.who.int/iris/bitstream/handle/10665/275469/9789241514842-eng.pdf, accessed 17 November 2018).

[72] FIND. Performance of Xpert MTB/RIF version G4 assay. Version and date: 1.0/30 Nov 2011. (http://www.stoptb.org/wg/gli/assets/documents/map/findg4cartridge.pdf, accessed 15 August 2020).

[73] Helb D, Jones M, Story E, *et al*. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol*. 2010;48(1):229-237. doi:10.1128/JCM.01463-09.

[74] Chakravorty S, Simmons AM, Rowneki M, *et al*. The new Xpert MTB/RIF Ultra: Improving detection of *Mycobacterium tuberculosis* and resistance to rifampin in an assay suitable for point-of-care testing. *mBio*. 2017;8(4) e00812-17.

[75] Calvori C, Frontali L, Leoni L, Tecce G. Effect of rifamycin on protein synthesis. *Nature*. 1965;207(4995):417-418. doi:10.1038/207417a0.

[76] Andre E, Goeminne L, Cabibbe A, *et al*. Consensus numbering system for the rifampicin resistance-associated *rpoB* gene mutations in pathogenic mycobacteria. *Clin Microbiol Infect*. 2017;23(3):167-172. doi:10.1016/j.cmi.2016.09.006.

[77] Camus JC, Pryor MJ, Médigue C, Cole ST. Re-annotation of the genome sequence of *Mycobacterium tuberculosis* H37Rv. *Microbiology (Reading)*. 2002;148(Pt 10):2967-2973. doi:10.1099/00221287-148-10-2967.

[78] Miller LP, Crawford JT, Shinnick TM. The *rpoB* gene of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 1994;38(4):805-811. doi:10.1128/AAC.38.4.805.

[79] Heep M, Brandstätter B, Rieger U, *et al*. Frequency of *rpoB* mutations inside and outside the cluster I region in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates. *J Clin Microbiol*. 2001;39(1):107-110. doi:10.1128/JCM.39.1.107-110.2001.

[80] Nebenzahl-Guimaraes H, Jacobson KR, Farhat MR, Murray MB. Systematic review of allelic exchange experiments aimed at identifying mutations that confer drug resistance in *Mycobacterium tuberculosis*. *J Antimicrob Chemother*. 2014;69(2):331-342. doi:10.1093/jac/dkt358.

correlation between resistance mutations and phenotypic results, binomial 95% CIs were calculated, where applicable.

Table 28 provides an overview of the current WHO and CLSI CCs for RIF, RFB and RPT.[81,82]

Table 28. Overview of current rifamycin CCs.

Drug	LJ		7H10		7H11		MGIT	
	WHO	**CLSI**	**WHO**	**CLSI**	**WHO**	**CLSI**	**WHO**	**CLSI**
RIF	40.0		1.0		1.0		1.0	
RFB	–	–	–	0.5	–	0.5	–	0.5
RPT	–	–	–	–	–	–	–	–

Green CCs were set by both the WHO and CLSI; blue CCs were set by CLSI. All concentrations are in mg/L.

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[81] Clinical & Laboratory Standards Institute. Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 3rd edition approved standard. CLSI Document M24; 2018.

[82] World Health Organization. Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. (http://apps.who.int/iris/bitstream/handle/10665/275469/9789241514842-eng.pdf, accessed 17 November 2018).

3.A.1 RIF MIC data on LJ

3.A.1.1 RIF MICs for pWT isolates on LJ

Four studies reported RIF MIC data for the pWT population on LJ, of which two were from the same laboratory (Table 29). All of these studies reported MICs for more than 10 pWT isolates, though the majority of the MIC distributions were truncated at the lower end, meaning that little insight could be gained about the shape of the pWT MIC distribution. It was, therefore, not clear whether the current CC of 40 mg/L or 32 mg/L (i.e. the equivalent ISO dilution for 30 mg/L) corresponds to the ECOFF.

Table 29. RIF MICs for pWT isolates on LJ.

Studies	Lab	Isolate origin	Unique isolates	Type of isolates	Genotypic results	2	8	10	20	30	32	40	60	80	100	120	128	160	200	320	400	800
4) Fabry 1995	4	clinical	20			2	17				1											
1) Vincent 2012	1	clinical	115	Mixture of first-line resistance profiles	gWT			74	32	5		3		1								
5) Rigouts 2013 & Van Deun 2009 & Van Deun (unpublished)	5	clinical	5		gWT			5														
	6		5		gWT			4	1													
	3		5		gWT			5														
	1		5		gWT			1	3	1												
	1	clinical	237		gWT			222	12	2						1						
	1	clinical	81		gWT			55	16	3		1		2		1	1			1	1	
6) Jagielski 2018	7		1	H37Rv								1										
	7	clinical	53	mixture of first-line resistance profiles	gWT							52										1

The green line denotes the current WHO and CLSI CC for RIF on LJ (40 mg/L). Notable limitations: studies RF1 and RF5 were conducted in the same laboratory.

3.A.1.2 RIF MICs for mutated isolates on LJ

rpoB 450 (531) mutants

Three studies reported MICs for 160 *rpoB* S450 (S531) codon mutants tested on LJ (Table 30). Based on the current RIF CCs, only 2 (1%, 95% CI 0-4%) of these mutated isolates tested phenotypically RIF-S. An area of technical uncertainty (ATU)[83] at 40 mg/L would lower this percentage to 1% (95% CI 0-3%).

Table 30. RIF MICs for *rpoB* S450 (S531) mutants on LJ.

Studies	Lab	Dataset	Isolate origin	Unique isolates	Genotypic results	10	20	30	40	48	60	64	80	100	120	128	160	200	320	400	640	800
1) Vincent 2012	1	1	clinical	55	*rpoB* S450L (S531L)								4		2	49						
	1	1	clinical	3	*rpoB* S450W (S531W)											3						
	1	1	clinical	1	*rpoB* S450F (S531F)											1						
5) Rigouts 2013 & Van Deun 2009 & Van Deun (unpublished)	5	5	clinical	1	*rpoB* S450L (S531L)									1								
	6	5	clinical	1	*rpoB* S450L (S531L)									1								
	3	5	clinical	1	*rpoB* S450L (S531L)									1								
	1	5	clinical	1	*rpoB* S450L (S531L)									1								
	1	6	clinical	22	*rpoB* S450L (S531L)				1				6	15								
	1	8	clinical	27	*rpoB* S450L (S531L)	1				1		1	1	13		2	1		7			
	1	8	clinical	1	*rpoB* S450W (S531W)											1						
	1	7	clinical	11	*rpoB* S450L (S531L)																1	10
	1	7	clinical	4	*rpoB* S450W (S531W)																	4
6) Jagielski 2018	7	9	clinical	31	*rpoB* S450L (S531L)									1					6			24
	7	9	clinical	1	*rpoB* S450W (S531W)																	1

The green line denotes the current WHO and CLSI CC for RIF on LJ (40 mg/L). Notable limitations: studies RF1 and RF5 were conducted in the same laboratory.

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[83] European Committee on Antimicrobial Susceptibility Testing. Area of Technical Uncertainty (ATU) in antimicrobial susceptibility testing (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Area_of_Technical_Uncertainty_-_guidance_2019-1.pdf, accessed 15 February 2020).

rpoB borderline RRDR mutants

Four studies reported MICs for 115 *rpoB* borderline RRDR mutants tested on LJ (Table 31). At the current RIF CC, 32 (28%, 95% CI 20-37%) of these 115 mutated isolates tested phenotypically RIF-S, which could be lowered to 22% (95% CI 15-30%) with an ATU at 40 mg/L.

Table 31. RIF MICs for *rpoB* borderline RRDR mutants on LJ.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	5	10	20	30	40	48	60	80	96	100	120	128	160	200	320	400	640	800
1) Vincent 2012	1	clinical	1	*rpoB* L430P (L511P)								1										
5) Rigouts 2013 & Van	5	clinical	2	*rpoB* L430P (L511P)		1	1															
5) Rigouts 2013 & Van	6	clinical	3	*rpoB* L430P (L511P)		1							2									
5) Rigouts 2013 & Van	3	clinical	3	*rpoB* L430P (L511P)		2						1										
5) Rigouts 2013 & Van	1	clinical	3	*rpoB* L430P (L511P)				1	2													
5) Rigouts 2013 & Van	1	clinical	1	*rpoB* L430P (L511P)									1									
5) Rigouts 2013 & Van	1	clinical	5	*rpoB* L430P (L511P)									1				1	1		2		
5) Rigouts 2013 & Van	1	clinical	6	*rpoB* L430P (L511P)								3						1	1		1	
1) Vincent 2012	1	clinical	2	*rpoB* D435Y (D516Y)								2										
5) Rigouts 2013 & Van	5	clinical	4	*rpoB* D435Y (D516Y)			2	1	1													
5) Rigouts 2013 & Van	6	clinical	4	*rpoB* D435Y (D516Y)					1			1	2									
5) Rigouts 2013 & Van	3	clinical	4	*rpoB* D435Y (D516Y)			2	2														
5) Rigouts 2013 & Van	1	clinical	2	*rpoB* D435Y (D516Y)			1		1													
5) Rigouts 2013 & Van	1	clinical	6	*rpoB* D435Y (D516Y)									3				2	1				
5) Rigouts 2013 & Van	1	clinical	6	*rpoB* D435Y (D516Y)															2		3	1
6) Jagielski 2018	7	clinical	2	*rpoB* D435Y (D516Y)											2							
5) Rigouts 2013 & Van	5	clinical	1	*rpoB* H445L (H526L)					1													
5) Rigouts 2013 & Van	6	clinical	1	*rpoB* H445L (H526L)									1									
5) Rigouts 2013 & Van	3	clinical	1	*rpoB* H445L (H526L)					1													
5) Rigouts 2013 & Van	1	clinical	1	*rpoB* H445L (H526L)						1												
5) Rigouts 2013 & Van	1	clinical	10	*rpoB* H445L (H526L)																	5	5
6) Jagielski 2018	7	clinical	1	*rpoB* H445L (H526L)														1				
5) Rigouts 2013 & Van	1	clinical	1	*rpoB* H445N (H526N)			1															
5) Rigouts 2013 & Van	1	clinical	5	*rpoB* H445N (H526N)								1					1	1		1	1	
1) Vincent 2012	1	clinical	1	*rpoB* H445S (H526S)												1						
5) Rigouts 2013 & Van	5	clinical	1	*rpoB* H445S (H526S)				1														
5) Rigouts 2013 & Van	6	clinical	1	*rpoB* H445S (H526S)									1									
5) Rigouts 2013 & Van	3	clinical	1	*rpoB* H445S (H526S)				1														
5) Rigouts 2013 & Van	1	clinical	1	*rpoB* H445S (H526S)									1									
3) Andres 2014	3	clinical	2	*rpoB* L452P (L533P)	1	1																
1) Vincent 2012	1	clinical	6	*rpoB* L452P (L533P)		1						1			1	3						
5) Rigouts 2013 & Van	5	clinical	2	*rpoB* L452P (L533P)			2															
5) Rigouts 2013 & Van	6	clinical	2	*rpoB* L452P (L533P)			1						1									
5) Rigouts 2013 & Van	3	clinical	2	*rpoB* L452P (L533P)			2															
5) Rigouts 2013 & Van	1	clinical	1	*rpoB* L452P (L533P)								1										
5) Rigouts 2013 & Van	1	clinical	1	*rpoB* L452P (L533P)								1										
5) Rigouts 2013 & Van	1	clinical	3	*rpoB* L452P (L533P)								2						1				
5) Rigouts 2013 & Van	1	clinical	15	*rpoB* L452P (L533P)														4	5		5	1
6) Jagielski 2018	7	clinical	1	*rpoB* L452P (L533P)														1				

The green line denotes the current WHO and CLSI CC for RIF on LJ (40 mg/L). Notable limitations: studies RF1 and RF5 and studies RF3 and RF5 were conducted in the same laboratory.

Other *rpoB* RRDR mutants

One hundred thirty isolates from 3 studies harboured *rpoB* RRDR mutations other than S450 (S531) and the borderline mutations (Table 32). Based on the current RIF CC on LJ, only 3 (2%, 95% CI 0-7%) of these 130 mutated isolates tested phenotypically RIF-S. This would be only slightly lowered to 2% (95% CI 0-5%) with an ATU at 40 mg/L.

Table 32. RIF MICs for other *rpoB* RRDR mutants on LJ.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	10	20	30	40	48	60	80	100	120	128	160	200	320	400	640	800
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* Q432K (Q513K)					1												
5) Rigouts 2013 & Van	1	clinical	2 *rpoB* Q432K (Q513K)																	2
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* Q432P (Q513P)														1			
5) Rigouts 2013 & Van	1	clinical	4 *rpoB* Q432P (Q513P)																	4
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* H445C (H526C)																1	
6) Jagielski 2018	7	clinical	2 *rpoB* H445C (H526C)														1	1		
1) Vincent 2012	1	clinical	5 *rpoB* H445D (H526D)											5						
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* H445D (H526D)		1															
5) Rigouts 2013 & Van	1	clinical	4 *rpoB* H445D (H526D)							1			2					1		
5) Rigouts 2013 & Van	1	clinical	10 *rpoB* H445D (H526D)																1	9
6) Jagielski 2018	7	clinical	2 *rpoB* H445D (H526D)															1		1
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* D435F (D516F)									1								
5) Rigouts 2013 & Van	1	clinical	4 *rpoB* D435F (D516F)																1	3
1) Vincent 2012	1	clinical	7 *rpoB* D435V (D516V)										1	6						
5) Rigouts 2013 & Van	1	clinical	2 *rpoB* D435V (D516V)								2									
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* D435V (D516V)															1		
5) Rigouts 2013 & Van	1	clinical	15 *rpoB* D435V (D516V)														1		1	13
6) Jagielski 2018	7	clinical	2 *rpoB* D435V (D516V)																	2
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* S441L (S522L)													1				
5) Rigouts 2013 & Van	1	clinical	2 *rpoB* S441L (S522L)																	2
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* S441Q (S522Q)		1															
5) Rigouts 2013 & Van	1	clinical	11 *rpoB* S441Q (S522Q)								1						1		1	8
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* H445P (H526P)									1								
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* H445P (H526P)																	1
1) Vincent 2012	1	clinical	5 *rpoB* H445R (H526R)										1	4						
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* H445R (H526R)															1		
5) Rigouts 2013 & Van	1	clinical	5 *rpoB* H445R (H526R)																	5
1) Vincent 2012	1	clinical	9 *rpoB* H445Y (H526Y)											9						
5) Rigouts 2013 & Van	1	clinical	3 *rpoB* H445Y (H526Y)								3									
5) Rigouts 2013 & Van	1	clinical	6 *rpoB* H445Y (H526Y)								5							1		
5) Rigouts 2013 & Van	1	clinical	14 *rpoB* H445Y (H526Y)																3	11
6) Jagielski 2018	7	clinical	3 *rpoB* H445Y (H526Y)														1	1		1
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* K446Q (K527Q)									1								
5) Rigouts 2013 & Van	1	clinical	1 *rpoB* R447L (R528L)							1										

The green line denotes the current WHO and CLSI CC for RIF on LJ (40 mg/L). Notable limitations: studies RF1 and RF5 were conducted in the same laboratory.

rpoB mutants outside the RRDR

Fourteen isolates from 2 studies harbored *rpoB* I491F (I572F) mutations. Based on the current RIF CC on LJ, 5 (36%, 95% CI 13-65%) of these 14 mutated isolates were classified as phenotypically RIF-S. This percentage would be lowered to 21% (95% CI 5-51%) given an ATU at 40 mg/L.

Table 33. RIF MICs for *rpoB* mutants outside the RRDR on LJ.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	10	20	30	40	60	80	120	160	320	640	800
1) Vincent 2012	1	clinical	1 *rpoB* I491F (I572F)							1					
5) Rigouts 2013 & Van Deun	5	clinical	1 *rpoB* I491F (I572F)			1									
5) Rigouts 2013 & Van Deun	6	clinical	1 *rpoB* I491F (I572F)						1						
5) Rigouts 2013 & Van Deun	3	clinical	1 *rpoB* I491F (I572F)					1							
5) Rigouts 2013 & Van Deun	1	clinical	1 *rpoB* I491F (I572F)		1										
5) Rigouts 2013 & Van Deun	1	clinical	1 *rpoB* I491F (I572F)		1										
5) Rigouts 2013 & Van Deun	1	clinical	1 *rpoB* I491F (I572F)									1			
5) Rigouts 2013 & Van Deun	1	clinical	7 *rpoB* I491F (I572F)					1		1			1	2	2

The green line denotes the current WHO and CLSI CC for RIF on LJ (40 mg/L). Notable limitations: studies RF1 and RF5 were conducted in the same laboratory.

3.A.1.3 Conclusion for RIF CC for LJ

Given that the MIC data for RIF on LJ were limited (Table 29), it was not clear whether 40 mg/L corresponds to the ECOFF. To avoid potentially misclassifying susceptible isolates as resistant, **40 mg/L** was reaffirmed as the CC. However, the TEG noted that this decision would be revisited should data

from appropriately designed studies become available.[84] The effect of introducing an ATU at this concentration was considered, though no ATU was endorsed (see Section 3.3 for more details).

Table 34. Effect of introducing an ATU for RIF DST on LJ.

Mutation type	Percentage of mutants classified RIF-S at	
	<u>CC 40 mg/L</u>	CC+ATU 40 mg/L
S450 (S531)	1% (95% CI 0-4%)	1% (95% CI 0-3%)
borderline RRDR	28% (95% CI 20-37%)	22% (95% CI 15-30%)
other RRDR	2% (95% CI 0-7%)	2% (95% CI 0-5%)
borderline I491F (I572F)	36% (95% CI 13-65%)	21% (95% CI 5-51%)

The current CC is underlined.

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[84] Schön T, Köser CU, Werngren J, *et al*. What is the role of the EUCAST reference method for MIC testing of the *Mycobacterium tuberculosis* complex? *Clin Microbiol Infect*. 2020;26(11):1453-1455. doi:10.1016/j.cmi.2020.07.037.

3.A.2 RIF MIC data on 7H10

3.A.2.1 RIF MICs for pWT isolates on 7H10

Fifteen studies were identified that reported RIF MIC data for the pWT population on 7H10 (Table 35). Nine of these studies featured MIC data for more than 10 pWT isolates. Seven of these studies reported MIC data from the same laboratories. Most distributions were not truncated and therefore provided a good understanding of the shape of the pWT MIC distributions. García *et al*. clearly had a systematic error with testing given that no on-scale MIC results were obtained for all presumably pWT isolates despite 0.016 mg/L being tested as the lowest concentration (this was also the case for RFB on 7H10 for the same study, as shown in Table 50). The modes of the pWT distributions for most of the remaining studies were all within plus or minus one dilution (i.e. 0.06-0.25 mg/L). Only 1 dataset (de Steenwinkel *et al*.) had an elevated mode of 0.5 mg/L, for which the current CC of 1 mg/L would be optimal. Therefore, the tentative ECOFF for 7H10 is likely 0.5 mg/L. Lowering the CC to 0.5 mg/L would misclassify at most 37 of 8537 isolates (0.4%, 95% CI 0.3-0.6%) that are currently regarded as RIF-S. However, this is likely an overestimate given that 32 of those 37 isolates were from a single laboratory from Farhat *et al*., and the quality of the results is questionable. This study stood out because of the unusually high number of isolates that either tested phenotypically INH-S despite harbouring classical INH resistance mutations (Table 12 and Table 13) or that were phenotypically RIF-S isolates despite having *rpoB* S450 (S531) mutations (Table 36). In addition, this study was enriched for MDR isolates and had an unusually long tail of isolates that were phenotypically RIF-R despite being characterized as gWT by whole genome sequencing. The pWT MIC distribution from this laboratory was strongly truncated and no MICs for H37Rv were available. Excluding this study would reduce the number of potentially misclassified isolates to just 5 of 8362 (0.1%, 95% 0.0-0.1%).

Table 35. RIF MICs for pWT isolates on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.02	0.03	0.04	0.06	0.08	0.1	0.12	0.2	0.25	0.3	0.5	0.7	1	1.25	2	3	4	5	8	10	16	25	32	50	64	128
19) Schön 2013	16		1	2	H37Rv ATCC25618			1					1																			
	16		1	1	BTB 08-049										1																	
	16		22	22	Mostly RIF-R	gWT	1					7	8		2		1										1	1				1
20) Schön 2009	16		1	4	H37Rv ATCC27294										4																	
	16		1	1	BTB 08-049						1																					
	16	clinical	92	92						8		11	24		33		14															2
13) Rancoita 2018	12		1	2	H37Rv										2																	
	12		17	34		gWT	10	4		4			10		4		2															
21) García 2010	17	reference	16	48			30																							18		
	17	clinical	63	189			138																							51		
26) Gygli 2019	21		1	3	H37Rv ATCC27294			1				1		1																		
	21		1	3	Erdman							1		1		1																
	21	clinical	76	81	mixture of first-line resistance profiles	gWT	5	14				36		15	9	2																
25) Farhat 2019	9	clinical	6	6	Mostly MDR	gWT												1			2				3							
	10		222	222		gWT									100		43		32	1	14	2			2					3	25	
11) Heifets 1999	10		1	10	H37Rv									9			1															
17) de Steenwinkel 2012, de Steenwinkel	15		1	2	H37Rv ATCC27294										2																	
	15	clinical	11	22										1	4		16	1														
23) Pholwat 2011	19		1	1	H37Rv						1																					
	19	clinical	13	13	Mostly MDR	gWT					3		4	4	1									1								
14) Kusunoki 1995	13	clinical	65	65									24	14	4				1								2		2		2	16
10) van Klingeren 2007	9	clinical	7956	7956	Mostly RIF-S									1234	5766		844	3			7				7	95						
22) Marubini 2016	18	clinical	9	9		gWT								7		2																
12) Moghazeh 1996	11	clinical	1	1	Pan-S	gWT											1															
15) Miotto 2018	12		1	1	H37Rv														1													
	12	clinical	7	7		gWT													7													
18) Cavusoglu 2004	8		1	1	H37Rv ATCC27294														1													

The green line denotes the current WHO and CLSI CC for RIF DST on 7H10 (1 mg/L). Notable limitations: studies RF19 and RF20, RF25 and RF11, RF25 and RF10, and RF13 and RF15 were conducted in the same laboratory, and RF21 had a systematic error with testing.

3.A.2.2 RIF MICs for mutated isolates on 7H10

rpoB 450 (531) mutants

Eleven studies reported 731 MICs for isolates with *rpoB* S450 (S531) codon mutations tested on 7H10 (Table 36). To err on the side of caution, Mvelase *et al*. was excluded from all calculations in this report as the S450 MICs reported in the study were systematically lower than those in all other studies for

both 7H10 and MGIT (Table 45). Excluding Mvelase *et al*. left 727 acceptable MICs. Farhat *et al*. also showed a wide MIC range for S450 mutants, suggesting a higher random error rate for testing or that the phenotypic effect of this mutation may depend on the genetic background. However, because the majority of MICs in this study were high, Farhat *et al*. was not excluded. Based on the current RIF CC, 20 (3%, 95% CI 2-4%) of the 727 mutated isolates tested phenotypically RIF-S. If the current CC was lowered one dilution to 0.5 mg/L, only 15 (2%, 95% CI 1-3%) MICs would be classified as RIF-S. The corresponding percentage given an ATU at 0.5 mg/L would be 1% (95% CI 1-3%).

Table 36. RIF MICs for *rpoB* S450 (S531) mutants on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Genotypic results	0.12	0.25	0.5	1	1.25	2	4	5	8	10	16	20	32	50	64	128	256	320
13) Rancoita 2018	12		10	20	*rpoB* S450L (S531L)				4		6	2			8								
19) Schön 2013	16		46	46	*rpoB* S450L (S531L)									1		3		6		19	11	6	
	16		1	1	*rpoB* S450W (S531W)																	1	
22) Marubini 2016	18		2	2	*rpoB* S450L (S531L)													2					
	18	clinical	1	1	*rpoB* S450W (S531W)													1					
	18		1	1	*rpoB* S450Q (S531Q)													1					
	18		1	1	*rpoB* S450P (S531P)	1																	
23) Pholwat 2011	19	clinical	18	18	*rpoB* S450L (S531L)															18			
24) Mvelase 2019	20	clinical	3	3	*rpoB* S450L (S531L)		1		1		1												
	20		1	1	*rpoB* S450P (S531P)		1																
26) Gygli 2019	21	clinical	43	45	*rpoB* S450L (S531L)							1						1		2	15	18	8
12) Moghazeh 1996	11	clinical	4	4	*rpoB* S450L (S531L)															4			
	11		1	1	*rpoB* S450W (S531W)															1			
16) Park 2017	14	mouse	5	5	*rpoB* S450L (S531L)												5						
	14		1	1	*rpoB* S450W (S531W)												1						
25) Farhat 2019	10		12	12	*rpoB* S450F (S531F)															12			
	10		442	442	*rpoB* S450L (S531L)		8	4	1	3	4		1	6					15	400			
	10	clinical	4	4	*rpoB* S450M (S450M)			1												3			
	10		5	5	*rpoB* S450Q (S531Q)															5			
	10		7	7	*rpoB* S450W (S531W)	1														6			
	9	clinical	80	80	*rpoB* S450L (S531L)					2			2	76									
	9		5	5	*rpoB* S450W (S531W)									5									
15) Miotto 2018	12	clinical	2	2	*rpoB* S450F (S531F)													2					
	12	clinical	1	1	*rpoB* S450L (S531L)													1					
18) Cavusoglu 2004	8	clinical	19	19	*rpoB* S450L (S531L)													2			1	1	15
	8	clinical	4	4	*rpoB* S450W (S531W)																		4

The green line denotes the current WHO and CLSI CC for RIF DST on 7H10 (1 mg/L). Notable limitations: studies RF13 and RF15 were conducted in the same laboratory and study RF24 had systematically lower MIC distributions for all mutants tested and was, therefore, excluded from calculations in this report.

rpoB borderline RRDR mutants

Eleven studies reported 112 MICs for *rpoB* borderline RRDR mutants tested on 7H10 (Table 37). Excluding the study by Mvelase *et al*., 21 (24%, 95% CI 16-35%) of 86 total mutated isolates tested phenotypically RIF-S at the current CC. If the CC was lowered one dilution to 0.5 mg/L, 13 (15%, 95% CI 8-24%) of the 86 mutants would still test phenotypically RIF-S on 7H10. This could be reduced to 6% (95% CI 2-13%) with an ATU at 0.5 mg/L.

Table 37. RIF MICs for *rpoB* borderline RRDR mutants on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Genotypic results	0.12	0.25	0.5	1	2	3	4	5	8	10	16	32	50	64	128
19) Schön 2013	16		1	1	*rpoB* L430P (L511P)	1														
22) Marubini 2016	18	clinical	1	1	*rpoB* L430P (L511P)		1													
23) Pholwat 2011	19	clinical	1	1	*rpoB* L430P (L511P)							1								
24) Mvelase 2019	20	clinical	7	7	*rpoB* L430P (L511P)			2	4	1										
25) Farhat 2019	10	clinical	3	3	*rpoB* L430P (L511P)		1	1	1											
19) Schön 2013	16		3	3	*rpoB* D435Y (D516Y)		1	1		2										
22) Marubini 2016	18	clinical	2	2	*rpoB* D435Y (D516Y)			1				1								
24) Mvelase 2019	20	clinical	11	11	*rpoB* D435Y (D516Y)		1	3	4	3										
25) Farhat 2019	10	clinical	11	11	*rpoB* D435Y (D516Y)			1	2	4	2			2						
9) van Ingen 2011	9	clinical	4	7	*rpoB* D435Y (D516Y)			1	1	5										
15) Miotto 2018	12	clinical	3	3	*rpoB* D435Y (D516Y)											3				
25) Farhat 2019	9	clinical	3	3	*rpoB* D435Y (D516Y)					1				2						
18) Cavusoglu 2004	8	clinical	2	2	*rpoB* D435Y (D516Y)												2			
19) Schön 2013	16		1	1	*rpoB* H445L (H526L)									1						
24) Mvelase 2019	20	clinical	6	6	*rpoB* H445L (H526L)			2	1	3										
26) Gygli 2019	21	clinical	1	1	*rpoB* H445L (H526L)				1											
25) Farhat 2019	10	clinical	13	13	*rpoB* H445L (H526L)			1	1	2			1	5	1				2	
12) Moghazeh 1996	11	clinical	2	2	*rpoB* H445L (H526L)									2						
25) Farhat 2019	9	clinical	1	1	*rpoB* H445L (H526L)								1							
22) Marubini 2016	18	clinical	1	1	*rpoB* H445N (H526N)	1														
25) Farhat 2019	10	clinical	3	3	*rpoB* H445N (H526N)		1	1			1									
12) Moghazeh 1996	11	clinical	1	1	*rpoB* H445N (H526N)											1				
15) Miotto 2018	12	clinical	1	1	*rpoB* H445N (H526N)							1								
24) Mvelase 2019	20	clinical	2	2	*rpoB* H445S (H526S)		1			1										
25) Farhat 2019	10	clinical	1	1	*rpoB* H445S (H526S)					1										
15) Miotto 2018	12	clinical	5	5	*rpoB* H445S (H526S)								2		1		2			
19) Schön 2013	16		1	1	*rpoB* L452P (L533P)									1						
22) Marubini 2016	18	clinical	1	1	*rpoB* L452P (L533P)												1			
26) Gygli 2019	21	clinical	2	3	*rpoB* L452P (L533P)				1	1										1
25) Farhat 2019	10	clinical	10	10	*rpoB* L452P (L533P)			1	1	2	1		1	1				1	2	
12) Moghazeh 1996	11	clinical	1	1	*rpoB* L452P (L533P)														1	
16) Park 2017	14	mouse	1	1	*rpoB* L452P (L533P)							1								
15) Miotto 2018	12	clinical	1	1	*rpoB* L452P (L533P)							1								
18) Cavusoglu 2004	8	clinical	1	1	*rpoB* L452P (L533P)					1										

The green line denotes the current WHO and CLSI CC for RIF DST on 7H10 (1 mg/L). Notable limitations: studies RF9 and RF25 were conducted in the same laboratory, and study RF24 had systematically lower MIC distributions for all mutants tested and was, therefore, excluded from calculations in this report.

Other *rpoB* RRDR mutants

Eleven studies reported 339 MICs for isolates that harbored *rpoB* RRDR mutations other than S450 (S531) and the borderline mutations tested on 7H10 (Table 38). Excluding Mvelase *et al*., only 3 (1%, 95% CI 0-3%) of 300 isolates would test phenotypically RIF-S at the current CC or if the CC were lowered one dilution to 0.5 mg/L. An ATU at 0.5 mg/L would eliminate this misclassification altogether.

Table 38. RIF MICs for other *rpoB* RRDR mutants on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Genotypic results	0.12	0.25	0.5	1	1.25	2	3	4	5	8	10	16	20	32	50	64	128	256	320
24) Mvelase 2019	20	clinical	1	1	*rpoB* S428T (S509T)			1																
23) Pholwat 2011	19	clinical	1	1	*rpoB* S431N (S512N)																	1		
15) Miotto 2018	12	clinical	1	1	*rpoB* S431R (S512R)														1					
25) Farhat 2019	10	clinical	2	2	*rpoB* Q432E (Q513E)																	2		
7) Cavusoglu 2006	8	clinical	1	1	*rpoB* Q432E (Q513E)																	1		
25) Farhat 2019	10	clinical	3	3	*rpoB* Q432K (Q513K)																	3		
12) Moghazeh 1996	11	clinical	1	1	*rpoB* Q432K (Q513K)												1							
12) Moghazeh 1996	11	clinical	1	1	*rpoB* Q432L (Q513L)												1							
15) Miotto 2018	12	clinical	3	3	*rpoB* Q432L (Q513L)															3				
24) Mvelase 2019	20	clinical	21	21	*rpoB* Q432P (Q513P)		3	11	3		4													
25) Farhat 2019	10	clinical	2	2	*rpoB* Q432P (Q513P)																	2		
25) Farhat 2019	9	clinical	1	1	*rpoB* Q432P (Q513P)										1									
18) Cavusoglu 2004	8	clinical	1	1	*rpoB* Q432P (Q513P)																			1
25) Farhat 2019	10	clinical	1	1	*rpoB* D435F (D516F)										1									
19) Schön 2013	16		9	9	*rpoB* D435V (D516V)								2		2		1		3		1			
23) Pholwat 2011	19	clinical	2	2	*rpoB* D435V (D516V)												1				1			
24) Mvelase 2019	20	clinical	16	16	*rpoB* D435V (D516V)	1	1	3	7		3			1										
26) Gygli 2019	21	clinical	18	20	*rpoB* D435V (D516V)									1	3		10		4		1	1		
25) Farhat 2019	10	clinical	130	130	*rpoB* D435V (D516V)			3		1	2	3		2	4	4				38	73			
25) Farhat 2019	9	clinical	9	9	*rpoB* D435V (D516V)								3		6									
18) Cavusoglu 2004	8	clinical	1	1	*rpoB* D435V (D516V)										1									
25) Farhat 2019	10	clinical	3	3	*rpoB* S441L (S522L)															3				
16) Park 2017	14	mouse	1	1	*rpoB* S441L (S522L)													1						
18) Cavusoglu 2004	8	clinical	2	2	*rpoB* S441W (S522W)																	1	1	
25) Farhat 2019	10	clinical	7	7	*rpoB* H445C (H526C)						1			1							4	1		
15) Miotto 2018	12	clinical	2	2	*rpoB* H445C (H526C)														2					
25) Farhat 2019	9	clinical	2	2	*rpoB* H445C (H526C)							2												
18) Cavusoglu 2004	8	clinical	1	1	*rpoB* H445C (H526C)						1													
19) Schön 2013	16		3	3	*rpoB* H445D (H526D)																	1	2	
26) Gygli 2019	21	clinical	7	9	*rpoB* H445D (H526D)																		5	4
12) Moghazeh 1996	11	clinical	2	2	*rpoB* H445D (H526D)																2			
25) Farhat 2019	10	clinical	7	7	*rpoB* H445D (H526D)							1								1	5			
25) Farhat 2019	9	clinical	4	4	*rpoB* H445D (H526D)										4									
18) Cavusoglu 2004	8	clinical	1	1	*rpoB* H445D (H526D)																	1		
25) Farhat 2019	10	clinical	1	1	*rpoB* H445G (H526G)																1			
25) Farhat 2019	9	clinical	1	1	*rpoB* H445G (H526G)									1										
25) Farhat 2019	10	clinical	1	1	*rpoB* H445P (H526P)																1			
15) Miotto 2018	12	clinical	1	1	*rpoB* H445Q (H526Q)													1						
22) Marubini 2016	18	clinical	1	1	*rpoB* H445R (H526R)													1						
24) Mvelase 2019	20	clinical	1	1	*rpoB* H445R (H526R)			1																
25) Farhat 2019	10	clinical	10	10	*rpoB* H445R (H526R)												1			1	8			
16) Park 2017	14	mouse	2	2	*rpoB* H445R (H526R)													2						
25) Farhat 2019	9	clinical	2	2	*rpoB* H445R (H526R)										2									
18) Cavusoglu 2004	8	clinical	4	4	*rpoB* H445R (H526R)																		4	
19) Schön 2013	16		3	3	*rpoB* H445Y (H526Y)																	1	2	
23) Pholwat 2011	19	clinical	2	2	*rpoB* H445Y (H526Y)																2			
26) Gygli 2019	21	clinical	5	6	*rpoB* H445Y (H526Y)																		3	3
12) Moghazeh 1996	11	clinical	4	4	*rpoB* H445Y (H526Y)																4			
25) Farhat 2019	10	clinical	16	16	*rpoB* H445Y (H526Y)															1	15			
16) Park 2017	14	mouse	4	4	*rpoB* H445Y (H526Y)													4						
25) Farhat 2019	9	clinical	5	5	*rpoB* H445Y (H526Y)										5									
18) Cavusoglu 2004	8	clinical	2	2	*rpoB* H445Y (H526Y)																		2	
25) Farhat 2019	10	clinical	2	2	*rpoB* R448K (R529K)																2			

The green line denotes the current WHO and CLSI CC for RIF DST on 7H10 (1 mg/L). Notable limitations: studies RF7 and RF18 were conducted in the same laboratory and study RF24 had systematically lower MIC distributions for all mutants tested and was, therefore, excluded from calculations in this report.

rpoB mutants outside the RRDR

Only 2 studies were identified that presented MIC data for isolates with *rpoB* mutations outside of the RRDR on 7H10 (Table 39). All 14 mutants had MICs above the RIF CC on 7H10, except for the one P454L (P535L) mutation, which would potentially be identified as false-resistant by the Nipro NTM MDR-TB2 and Xpert Ultra assays, assuming that this mutation is neutral. An ATU at 0.5 mg/L would not change these results.

Table 39. RIF MICs for *rpoB* mutants outside the RRDR on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	0.25	0.5	1	1.25	2	3	4	5	8	16	32	50	64	128	256
26) Gygli 2019	21	clinical	1	*rpoB* V170F (V146F)															1
25) Farhat 2019	9	clinical	1	*rpoB* V170F (V146F)				1											
	10	clinical	5	*rpoB* V170F (V146F)													5		
	10	clinical	1	*rpoB* P454L (P535L)	1														
	10	clinical	6	*rpoB* I491F (I572F)					2	1		1	1		1				

The green line denotes the current WHO and CLSI CC for RIF DST on 7H10 (1 mg/L).

3.A.2.3 Conclusion for RIF CC for 7H10

The experts were in agreement that the ECOFF for 7H10 corresponds to 0.5 mg/L (Table 35). Only 0.4% (95% CI 0.3-0.6%) of isolates currently considered to be RIF-S would be potentially misclassified as RIF-R at this concentration. However, this estimate was almost entirely driven by MIC data from Farhat *et al.*, for which many results were not in keeping with other well-established evidence. Excluding this one study reduces the potential rate of misclassification of RIF-S isolates to just 0.1% (95% 0.0-0.1%), whilst reducing the rate of misclassification of borderline RRDR mutations from 24% (95% CI 16-35%) to 15% (95% CI 8-24%), as shown in Table 40. An ATU at 0.5 mg/L would further reduce the rate of misclassification to 6% (95% CI 2-13%). Consequently, the CC was lowered to **0.5 mg/L** but an ATU was not endorsed (see Section 3.3 for more details).

Table 40. Effect of changing the CC for RIF DST on 7H10 for detection of *rpoB* mutants.

Mutation type	Percentage of mutants classified RIF-S at		
	CC 1 mg/L	CC+ATU 1 mg/L or CC 0.5 mg/L	CC+ATU 0.5 mg/L
S450 (S531)	3% (95% CI 2-4%)	2% (95% CI 1-3%)	1% (95% CI 1-3%)
borderline RRDR	24% (95% CI 16-35%)	15% (95% CI 8-24%)	6% (95% CI 2-13%)
other RRDR	1% (95% CI 0-3%)	1% (95% CI 0-3%)	0% (95% CI 0-1%)
V170F (V146F)	0% (95% CI 0-41%)		
borderline I491F (I572F)	0% (95% CI 0-46%)		

The newly adopted CC is underlined.

3.A.3 RIF MIC data on 7H11

3.A.3.1 RIF MICs for pWT isolates on 7H11

Ten studies were identified that reported RIF MIC data for the pWT population on 7H11 (Table 41). Ten of these studies reported MICs for at least 10 pWT isolates, and the MIC distributions were largely untruncated. The mode of the distributions ranged from 0.25 up to 1 mg/L, the current CC. Modes that are identical to the ECOFF are unusual but based on the data from Heifets *et al*., this did not appear to result in an unusually high false-resistance rate.

Table 41. RIF MICs for pWT isolates on 7H11.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.03	0.06	0.12	0.25	0.5	1	2	4	5	8	10	16	32	40
27) Rastogi 2000	22		1	1	H37Rv					1										
	22		1	1	M. africanum ATCC 25420						1									
	22		1	1	M. bovis ATCC 19210						1									
	22		5	5	M. bovis BCG		2	1	1	1										
	22		6	6	Pan-S						4	2								
	22	clinical	4	4	M. africanum						2	2								
	22	clinical	4	4	M. bovis						3	1								
29) Coban 2013	24		1	1	H37Rv							1								
	24		1	1	M. tuberculosis ATCC 35822							1								
	24		1	1	M. tuberculosis ATCC 35820							1								
	24		1	1	M. tuberculosis ATCC 35837						1									
	24	clinical	40	40							1	7	14	3	3	1			1	10
31) Shishido 2007	26		1	15	BCG (Tokyo)						9	6								
35) Lee 1987	10	clinical	17	17	Pre-treatment					4	9	4								
37) Truffot-Pernot 1988	29	clinical	10	10	RIF-S						1	5	4							
33) Rodriguez 2003	28	clinical	16	16						3	7	5				1				
28) Fattorini 1999	23		1	1	H37Rv ATCC27294							1								
	23		1	1	M. tuberculosis ATCC 35838															1
	23	clinical	46	46	Mostly MDR					5	5	2	1					2	3	28
30) Rey-Jurado 2013 & Rey-Jurado 2012	25		1	2	H37Rv						1	1								
	25	clinical	10	20	INH-S					1	3	6	10							
	25	clinical	12	12	INH-R						6	6								
36) Heifets 1985	10	clinical	180	180	untreated							180								
32) Anthony 2005 &	27	lab	2	2	gWT									2						

The green line denotes the current WHO and CLSI CC for RIF DST on 7H11 (1 mg/L). Notable limitation: studies RF35 and RF36 were conducted in the same laboratory.

3.A.3.2 RIF MICs for mutated isolates on 7H11

rpoB 450 (531) mutants

Only 1 study reported RIF MICs for *rpoB* S450 (S531) codon mutants tested on 7H11 (Table 42). Based on the current RIF CC, all 5 mutants reported in this study (100%, 95% CI 48-100%) tested phenotypically RIF-R.

Table 42. RIF MICs for *rpoB* S450 (S531) mutants on 7H11.

Studies	Isolate origin	Unique isolates	Genotypic results	1	2	4	8	16	32	64	128	256
32) Anthony 2005 & Bergval 2007	lab	4	*rpoB* S450L (S531L)									4
	lab	1	*rpoB* S450W (S531W)									1

The green line denotes the current WHO and CLSI CC for RIF DST on 7H11 (1 mg/L).

rpoB borderline RRDR mutants

No studies presenting MIC data for *rpoB* borderline RRDR mutants on 7H11 were identified.

Other rpoB RRDR mutants

Only 1 study reported RIF MICs for *rpoB* RRDR mutants harboring mutations other than S450 (S531) and the borderline mutations on 7H11 (Table 43). Based on the current RIF CC, all 16 mutants reported in this study (100%, 95% CI 79-100%) tested phenotypically RIF-R.

Table 43. RIF MICs for other *rpoB* RRDR mutants on 7H11.

Studies	Isolate origin	Unique isolates	Genotypic results	1	2	4	8	16	32	64	128	256	512
	lab	1	*rpoB* Q432L (Q513L)									1	
	lab	1	*rpoB* S441L (S522L)						1				
32) Anthony 2005 & Bergval 2007	lab	4	*rpoB* H445D (H526D)								1	3	
	lab	5	*rpoB* H445R (H526R)								1	3	1
	lab	1	*rpoB* H445P (H526P)									1	
	lab	4	*rpoB* H445Y (H526Y)								1	3	
	lab	5	*rpoB* H445R (H526R)								1	3	1

The green line denotes the current WHO and CLSI CC for RIF DST on 7H11 (1 mg/L).

rpoB mutants outside the RRDR

No studies presenting MIC data for *rpoB* isolates with mutations outside the RRDR on 7H11 were identified.

3.A.3.3 Conclusion for RIF CC for 7H11

The quality and quantity of MIC data for pWT isolates for 7H11 (Table 41) were considerably worse than for 7H10 (Table 35). A change of the current CC of **1 mg/L** was not warranted based on these limited data. However, the fact that the CC for 7H10 had to be lowered from 1 mg/L to the ECOFF of 0.5 mg/L raises the possibility that 1 mg/L may be too high for 7H11 as well. Additional data are needed to clarify this question. The value of adopting an ATU could not be assessed given that no MICs for borderline resistance mutations were identified.

3.A.4 RIF MIC data in MGIT

3.A.4.1 RIF MICs for pWT isolates in MGIT

Twenty-one studies were identified that reported RIF MIC data for the pWT population by MGIT (Table 44). Thirteen of these studies reported MICs for at least 10 pWT isolates, although many of the MIC distributions were truncated, precluding an assessment of the shape of the distributions. Because genotypic information was not available for key datasets (e.g. Chigutsa *et al*. and Rockwood *et al*.), it was not clear whether 0.5 mg/L was the upper end of their respective pWT MIC distributions. It is therefore not clear whether 0.25 or 0.5 mg/L represents the tentative ECOFF. Lowering the CC to 0.5 mg/L would result in only one of 523 isolates (0.2%, 95% 0.0-1.1%) being misclassified as RIF-R. Notably, this isolate from Bernardelli *et al*. was isolated from a seal and might represent an animal variant of MTBC.

Table 44. RIF MICs for pWT isolates in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.016	0.022	0.03	0.04	0.06	0.08	0.1	0.12	0.25	0.5	1	2	4	5	8	10	16	20	50	100	120
55) Niward 2018	16		1	3	H37Rv ATCC27294									1	2												
	33		1	2	H37Rv ATCC27294						2																
	16	clinical	26	26	Pre-treatment	gWT	1		8		14			3													
58) Groenheit - unpublished	33		1	2	H37Rv						2																
	33	clinical	11	11		gWT			2		7			2													
48) Chigutsa 2015	41	clinical	54	54	Pre-treatment		1		5		12			26	7	3											
62) Colangeli 2018	46	clinical	107	107	Development cohort	gWT	3	7	24	20	28	19		6													
	46		24	24	Validation cohort	gWT	1	12	3	3	5																
44) Heyckendorf 2018	3		1	1	H37Rv ATCC27294									1													
	3		1	4	H37Rv ATCC27294									2	2												
	3	clinical	16	16		gWT			1		4			11													
46) Rockwood 2017 & Rockwood 2017	39		93	93		gWT by Xpert			12		35			33	10	3											
	39	clinical	21	21	pre-treatment (paired)				3		11			5	1	1											
	39		21	21	post-treatment (paired)				3		7			8	3												
41) Feuerriegel 2012	3		1	1	H37Rv ATCC27294										1												
57) Lin - unpublished	45		1	10	H37Rv						7			2	1												
	45		2	2		gWT								2													
63) Berrada 2016	45		1	1	H37Rv ATCC27294									1													
	45	clinical	6	6		gWT								6													
52) Bernardelli 2004	44		1	1	H37Rv ATCC27294											1											
	44		1	1	M. bovis BCG								1														
	44		1	1	M. bovis AN5								1														
	44	seals	7	7									3			3	1										
49) Jamieson 2014	42	clinical	1	1	mix of first-ine drug resistance	gWT																		1			
59) Mvelase 2019	20		1	9	H37Rv									1	7	1											
60) Torrea 2019	1		5	5	H37Rv									3	2												
	1	clinical	13	13		gWT								11	2												
61) Tessema 2017	3	clinical	18	18		gWT								12	1					5							
38) Kambli 2015	30		1	1	H37Ra										1												
	30	clinical	36	36		gWT									36												
50) Van Deun	1	clinical	5	5		gWT									5												
51) Sirgel 2013 &	43	clinical	26	26		gWT										26											
53) Machado 2018 & Machado (unpublished)	5		1	1	H37Rv ATCC27294												1										
	5	clinical	4	4	Pan-S and mono-INH-R	gWT											4										
54) Andres 2014 &	3	clinical	71	71		gWT											70								1		
56) Whitfield 2018	31		25	25		gWT											20				2					1	2
64) Gygli 2019	21		1	1	H37Rv ATCC27294												1										
	21		1	1	Erdman												1										
	21	clinical	22	22		gWT											22										

The green line denotes the current WHO and CLSI CC for RIF DST in MGIT (1 mg/L). Notable limitations: studies RF55 and RF58; studies RF44, RF41, RF61 and RF54; studies RF57 and RF63; and studies RF50 and RF60 were conducted in the same laboratory.

3.A.4.2 RIF MICs for mutated isolates in MGIT

rpoB 450 (531) mutants

Sixteen studies reported MICs for 796 isolates with *rpoB* S450 (S531) codon mutations tested by MGIT (Table 45). Excluding Mvelase *et al*., which also had unusual MICs for 7H10 (Table 36), and based on the current RIF CC, only 2 (0%, 95% CI 0-1%) of the 792 mutated isolates tested phenotypically RIF-S. If the current CC were lowered by one dilution to 0.5 mg/L, at least 1 (0%, 95% CI 0-1%) mutant would still test RIF-S by MGIT. An ATU in this case would have minimal effect (0% (95% CI 0-1%)).

Table 45. RIF MICs for *rpoB* S450 (S531) mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	0.12	0.25	0.5	1	2	4	5	8	10	20	32	40	50	64	80	100	128	160	320	640
43) Abanda 2018	37	clinical	1	*rpoB* S450W (S531W)									1											
44) Heyckendorf 2018	3	clinical	1	*rpoB* S450F (S531F)											1									
	3	clinical	12	*rpoB* S450L (S531L)											12									
	3	clinical	1	*rpoB* S450L (S531L)																	1			
49) Jamieson 2014	42	clinical	19	*rpoB* S450L (S531L)																6		11	2	
	42	clinical	3	*rpoB* S450W (S531W)																			3	
59) Mvelase 2019	21		3	*rpoB* S450L (S531L)	1		1		1															
	21		1	*rpoB* S450P (S531P)		1																		
60) Torrea 2019	1	clinical	5	*rpoB* S450L (S531L)					5															
61) Tessema 2017	3	clinical	324	*rpoB* S450L (S531L)											324									
	3	clinical	6	*rpoB* S450W (S531W)											6									
62) Berrada 2016	45	clinical	1	*rpoB* S450C (S531C)	1																			
	45	clinical	1	*rpoB* S450F (S531F)										1										
	45	clinical	3	*rpoB* S450W (S531W)										3										
	45	clinical	6	*rpoB* S450L (S531L)										6										
38) Kambli 2015	30	clinical	57	*rpoB* S450L (S531L)											57									
50) Van Deun	1	clinical	1	*rpoB* S450L (S531L)							1													
39) Cambau 2015	3, 5, 9, 12, 29, 31-34	clinical	1	*rpoB* S450W (S531W)											1									
	3, 5, 9, 12, 29, 31-34	clinical	1	*rpoB* S450P (S531P)											1									
	3, 5, 9, 12, 29, 31-34	clinical	1	*rpoB* S450M (S531M)											1									
	3, 5, 9, 12, 29, 31-34	clinical	101	*rpoB* S450L (S531L)						1				2	98									
47) El Maraachii 2015	40	clinical	14	*rpoB* S450L (S531L)							14													
	40	clinical	5	*rpoB* S450W (S531W)							5													
53) Machado 2018	5	clinical	31	*rpoB* S450L (S531L)											22				1			1	5	2
54) Andres 2014 & Andres (unpublished)	3	clinical	40	*rpoB* S450L (S531L)											40									
	3	clinical	1	*rpoB* S450W (S531W)											1									
56) Whitfield 2018	31		135	*rpoB* S450L (S531L)				1						2			14			26	92			
	31		4	*rpoB* S450W (S531W)																	4			
57) Lin - unpublished	45		1	*rpoB* S450Q (S531Q)								1												
64) Gygli 2019	21	clinical	16	*rpoB* S450L											16									

The green line denotes the current WHO and CLSI CC for RIF DST in MGIT (1 mg/L). Notable limitations: studies RF44, RF39, RF61 and RF54; studies RF57 and RF63; studies RF39 and RF56; studies RF53 and RF39; and studies RF50 and RF60 were conducted in the same laboratory and study RF59 had systematically lower MIC distributions for all mutants tested and was, therefore, excluded from calculations in this report.

rpoB borderline RRDR mutants

Seventeen studies were identified that reported RIF MICs for 181 isolates that harbored *rpoB* RRDR borderline mutations and were tested by MGIT (Table 46). Excluding the study by Mvelase *et al*. and based on the current RIF CC on MGIT, the majority, or 116 (74%, 95% CI 67-81%) of 156 mutated isolates were phenotypically RIF-S. If the CC was lowered one dilution to 0.5 mg/L, at least 83 (53%, 95% CI 45-61%) of the 156 mutants would still test phenotypically RIF-S by MGIT. If the CC was lowered two dilutions to 0.25 mg/L, at least 55 (35%, 95% CI 28-43%) mutants would still test phenotypically RIF-S by MGIT. An ATU at 0.25 mg/L would reduce this percentage to 11% (95% CI 6-17%).

Table 46. RIF MICs for *rpoB* borderline RRDR mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	0.12	0.25	0.5	1	2	2.5	4	5	8	10	20	50	100	120
40) Ho 2013	35	clinical	3	*rpoB* L430P (L511P)	1		1	1										
41) Feuerrigel 2012	3	clinical	1	*rpoB* L430P (L511P)				1										
43) Abanda 2018	37	clinical	2	*rpoB* L430P (L511P)		2												
49) Jamieson 2014	42	clinical	2	*rpoB* L430P (L511P)	2													
59) Mvelase 2019	21		7	*rpoB* L430P (L511P)				3	3	1								
60) Torrea 2019	1	clinical	10	*rpoB* L430P (L511P)	3	4		1	2									
61) Tessema 2017	3	clinical	5	*rpoB* L430P (L511P)	4	1												
63) Berrada 2016	45	clinical	3	*rpoB* L430P (L511P)			3											
50) Van Deun	1	clinical	3	*rpoB* L430P (L511P)			3											
39) Cambau 2015	3, 5, 9, 12, 29, 31-34	clinical	1	*rpoB* L430P (L511P)													1	
54) Andres 2014 &	3	clinical	1	*rpoB* L430P (L511P)													1	
56) Whitfield 2018	31		2	*rpoB* L430P (L511P)				2										
41) Feuerrigel 2012	3	clinical	3	*rpoB* D435Y (D516Y)		1	2											
42) Williamson 2012	36	clinical	1	*rpoB* D435Y (D516Y)		1												
43) Abanda 2018	37	clinical	4	*rpoB* D435Y (D516Y)										4				
44) Heyckendorf 2018	3	clinical	1	*rpoB* D435Y (D516Y)											1			
59) Mvelase 2019	20		11	*rpoB* D435Y (D516Y)		2	4	2	3									
60) Torrea 2019	1	clinical	9	*rpoB* D435Y (D516Y)	1	3	4	1										
61) Tessema 2017	3	clinical	2	*rpoB* D435Y (D516Y)			2											
63) Berrada 2016	45	clinical	4	*rpoB* D435Y (D516Y)		2	2											
38) Kambli 2015	30	clinical	3	*rpoB* D435Y (D516Y)			1					1		1				
50) Van Deun	1	clinical	4	*rpoB* D435Y (D516Y)		3	1											
51) Sirgel 2013 &	43	clinical	1	*rpoB* D435Y (D516Y)								1						
39) Cambau 2015	3, 5, 9, 12, 29, 31-34	clinical	1	*rpoB* D435Y (D516Y)													1	
47) El Maraachii 2015	40	clinical	2	*rpoB* D435Y (D516Y)				2										
56) Whitfield 2018	31		12	*rpoB* D435Y (D516Y)				10							2			
42) Williamson 2012	36	clinical	1	*rpoB* H445L (H526L)			1											
43) Abanda 2018	37	clinical	2	*rpoB* H445L (H526L)	1						1							
49) Jamieson 2014	42	clinical	2	*rpoB* H445L (H526L)			1		1									
59) Mvelase 2019	21		5	*rpoB* H445L (H526L)		1	2		2									
61) Tessema 2017	3	clinical	4	*rpoB* H445L (H526L)				2			2							
63) Berrada 2016	45	clinical	4	*rpoB* H445L (H526L)					2		1		1					
50) Van Deun	1	clinical	1	*rpoB* H445L (H526L)			1											
39) Cambau 2015	3, 5, 9, 12, 29, 31-34	clinical	3	*rpoB* H445L (H526L)							2						1	
47) El Maraachii 2015	40	clinical	2	*rpoB* H445L (H526L)				1	1									
64) Gygli 2019	21	clinical	1	*rpoB* H445L (H526L)				1										
56) Whitfield 2018	31		3	*rpoB* H445L (H526L)				2						1				
43) Abanda 2018	37	clinical	1	*rpoB* H445N (H526N)										1				
49) Jamieson 2014	42	clinical	1	*rpoB* H445N (H526N)		1												
61) Tessema 2017	3	clinical	6	*rpoB* H445N (H526N)	1	2										3		
63) Berrada 2016	45	clinical	3	*rpoB* H445N (H526N)	2	1												
38) Kambli 2015	30	clinical	3	*rpoB* H445N (H526N)		2	1											
56) Whitfield 2018	31		4	*rpoB* H445N (H526N)				3										1
59) Mvelase 2019	21		2	*rpoB* H445S (H526S)		1		1										
63) Berrada 2016	45	clinical	3	*rpoB* H445S (H526S)		1	1	1										
50) Van Deun	1	clinical	1	*rpoB* H445S (H526S)		1												
40) Ho 2013	35	clinical	1	*rpoB* L452P (L533P)				1										
41) Feuerrigel 2012	3	clinical	1	*rpoB* L452P (L533P)				1										
43) Abanda 2018	37	clinical	1	*rpoB* L452P (L533P)										1				
44) Heyckendorf 2018	3	clinical	1	*rpoB* L452P (L533P)							1							
60) Torrea 2019	1	clinical	8	*rpoB* L452P (L533P)	2	5	1											
61) Tessema 2017	3	clinical	6	*rpoB* L452P (L533P)			6											
63) Berrada 2016	45	clinical	2	*rpoB* L452P (L533P)			2											
38) Kambli 2015	30	clinical	4	*rpoB* L452P (L533P)					1		3							
50) Van Deun	1	clinical	2	*rpoB* L452P (L533P)		1	1											
64) Gygli 2019	21	clinical	1	*rpoB* L452P (L533P)				1										
56) Whitfield 2018	31		4	*rpoB* L452P (L533P)				3							1			

The green line denotes the current WHO and CLSI CC for RIF DST in MGIT (1 mg/L). Notable limitations: studies RF44, RF39, RF41, RF61 and RF54; studies RF39 and RF56; studies RF61 and RF39; and studies RF50 and RF60 were conducted in the same laboratory and study RF59 had systematically lower MIC distributions for all mutants tested and was, therefore, excluded from calculations in this report.

Other *rpoB* RRDR mutants

Fifteen studies were identified that reported RIF MICs for 402 isolates with *rpoB* RRDR mutations other than S450 (S531) and the borderline mutations (Table 47). Excluding Mvelase *et al*. and based on the current RIF CC on MGIT, 11 (3%, 95% CI 2-5%) of 364 isolates had MICs below the current CC. If the CC were set at 0.5 mg/L, 5 (1%, 95% CI 0-3%) of these isolates would be classified as RIF-S. If the CC were set at 0.25 mg/L, only 2 (1%, 95% CI 0-2%) isolates would be classified as RIF-S, which would be the same with an ATU at 0.25 mg/L.

Table 47. RIF MICs for other *rpoB* RRDR mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	0.12	0.25	0.5	1	2	4	5	8	10	15	20	24	50	80	100	160	320
47) El Maraachii 2015	40	clinical	1	*rpoB* S428R (S509R)							1										
59) Mvelase 2019	20		1	*rpoB* S428T (S509T)				1													
63) Berrada 2016	45	clinical	2	*rpoB* Q432E (Q513E)									2								
61) Tessema 2017	3	clinical	2	*rpoB* Q432K (Q513K)												2					
63) Berrada 2016	45	clinical	3	*rpoB* Q432K (Q513K)									3								
47) El Maraachii 2015	40	clinical	4	*rpoB* Q432K (Q513K)							4										
54) Andres 2014 &	3	clinical	1	*rpoB* Q432K (Q513K)												1					
56) Whitfield 2018	31		3	*rpoB* Q432K (Q513K)													1		2		
63) Berrada 2016	45	clinical	1	*rpoB* Q432L (Q513L)									1								
56) Whitfield 2018	31		2	*rpoB* Q432L (Q513L)															2		
43) Abanda 2018	37	clinical	2	*rpoB* Q432P (Q513P)									2								
59) Mvelase 2019	20		21	*rpoB* Q432P (Q513P)		5	10	5	1												
61) Tessema 2017	3	clinical	2	*rpoB* Q432P (Q513P)												2					
63) Berrada 2016	45	clinical	2	*rpoB* Q432P (Q513P)									2								
56) Whitfield 2018	31		2	*rpoB* Q432P (Q513P)																1	1
38) Kambli 2015	30	clinical	1	*rpoB* D435A (D516A)									1								
56) Whitfield 2018	31		1	*rpoB* D435A (D516A)				1													
63) Berrada 2016	45	clinical	1	*rpoB* D435F (D516F)					1												
38) Kambli 2015	30	clinical	1	*rpoB* D435F (D516F)									1								
51) Sirgel 2013 &	43	clinical	4	*rpoB* D435S (D516S)							2	2									
44) Heyckendorf 2018	3	clinical	1	*rpoB* D435V (D516V)				1													
49) Jamieson 2014	42	clinical	3	*rpoB* D435V (D516V)											1		2				
61) Tessema 2017	3	clinical	26	*rpoB* D435V (D516V)			2								12	12					
59) Mvelase 2019	21		15	*rpoB* D435V (D516V)	1	2	3	2	6	1											
63) Berrada 2016	45	clinical	18	*rpoB* D435V (D516V)								3	15								
38) Kambli 2015	30	clinical	3	*rpoB* D435V (D516V)									3								
51) Sirgel 2013 &	43	clinical	29	*rpoB* D435V (D516V)							4		15	10							
39) Cambau 2015	3, 5, 9, 12, 29, 31-34	clinical	12	*rpoB* D435V (D516V)						2					9	1					
47) El Maraachii 2015	40	clinical	15	*rpoB* D435V (D516V)							15										
53) Machado 2018 and	5	clinical	2	*rpoB* D435V (D516V)											2						
54) Andres 2014 &	3	clinical	1	*rpoB* D435V (D516V)											1						
64) Gygli 2019	21	clinical	2	*rpoB* D435V (D516V)											2						
56) Whitfield 2018	31		79	*rpoB* D435V (D516V)				4					14	32	18		9		1	1	
57) Lin - unpublished	45		1	*rpoB* N438K (N519K)							1										
49) Jamieson 2014	42	clinical	3	*rpoB* S441L (S522L)											3						
61) Tessema 2017	3	clinical	1	*rpoB* S441L (S522L)											1						
63) Berrada 2016	45	clinical	1	*rpoB* S441L (S522L)					1												
39) Cambau 2015	3, 5, 9, 12, 29, 31-34	clinical	1	*rpoB* S441L (S522L)												1					
47) El Maraachii 2015	40	clinical	1	*rpoB* S441L (S522L)							1										
56) Whitfield 2018	31		1	*rpoB* S441L (S522L)																1	
57) Lin - unpublished	45		1	*rpoB* S441T (S522T)	1																
63) Berrada 2016	45	clinical	1	*rpoB* H445A (H526A)					1												
39) Cambau 2015	3, 5, 9, 12, 29, 31-34	clinical	1	*rpoB* H445A (H526A)												1					
57) Lin - unpublished	45		1	*rpoB* H445A (H526A)				1													
63) Berrada 2016	45	clinical	2	*rpoB* H445C (H526C)					1			1									
38) Kambli 2015	30	clinical	1	*rpoB* H445C (H526C)					1												
49) Jamieson 2014	42	clinical	1	*rpoB* H445D (H526D)															1		
61) Tessema 2017	3	clinical	10	*rpoB* H445D (H526D)												10					
63) Berrada 2016	45	clinical	3	*rpoB* H445D (H526D)									3								
38) Kambli 2015	30	clinical	7	*rpoB* H445D (H526D)												7					
39) Cambau 2015	3, 5, 9, 12, 29, 31-34	clinical	6	*rpoB* H445D (H526D)												6					
47) El Maraachii 2015	40	clinical	2	*rpoB* H445D (H526D)							2										
53) Machado 2018 and	5	clinical	1	*rpoB* H445D (H526D)																	1
54) Andres 2014 &	3	clinical	2	*rpoB* H445D (H526D)												2					
64) Gygli 2019	21	clinical	5	*rpoB* H445D (H526D)												5					
56) Whitfield 2018	31		18	*rpoB* H445D (H526D)															1	17	
63) Berrada 2016	45	clinical	1	*rpoB* H445G (H526G)					1												
61) Tessema 2017	3	clinical	1	*rpoB* H445P (H526P)												1					
57) Lin - unpublished	45		1	*rpoB* H445P (H526P)							1										
57) Lin - unpublished	45		1	*rpoB* H445Q (H526Q)	1																
43) Abanda 2018	37	clinical	3	*rpoB* H445R (H526R)									3								
59) Mvelase 2019	20		1	*rpoB* H445R (H526R)			1														
61) Tessema 2017	3	clinical	5	*rpoB* H445R (H526R)												5					
63) Berrada 2016	45	clinical	2	*rpoB* H445R (H526R)									2								
38) Kambli 2015	30	clinical	1	*rpoB* H445R (H526R)												1					
47) El Maraachii 2015	40	clinical	2	*rpoB* H445R (H526R)							2										
56) Whitfield 2018	31		3	*rpoB* H445R (H526R)													1			2	
49) Jamieson 2014	42	clinical	2	*rpoB* H445Y (H526Y)															1		1
61) Tessema 2017	3	clinical	15	*rpoB* H445Y (H526Y)												15					
63) Berrada 2016	45	clinical	1	*rpoB* H445Y (H526Y)									1								
38) Kambli 2015	30	clinical	3	*rpoB* H445Y (H526Y)												3					
39) Cambau 2015	3, 5, 9, 12, 29, 31-34	clinical	4	*rpoB* H445Y (H526Y)											1	3					
47) El Maraachii 2015	40	clinical	3	*rpoB* H445Y (H526Y)							3										
53) Machado 2018 and	5	clinical	3	*rpoB* H445Y (H526Y)											1	1					1
64) Gygli 2019	21	clinical	2	*rpoB* H445Y (H526Y)												2					
56) Whitfield 2018	31		13	*rpoB* H445Y (H526Y)															1	12	
54) Andres 2014 &	3	clinical	1	*rpoB* R448K (R529K)												1					

The green line denotes the current WHO and CLSI CC for RIF DST in MGIT (1 mg/L). Notable limitations: studies RF44, RF39, RF61 and RF54; studies RF39 and RF56; studies RF53 and RF39; and studies RF57 and RF63 were conducted in the same laboratory and study RF58 had systematically lower RIF MICs for mutants than reported for other studies and study RF59 had systematically lower MIC distributions for all mutants tested and was, therefore, excluded from calculations in this report.

rpoB mutants outside the RRDR

Fourteen isolates from 6 studies harbored *rpoB* mutations outside the RRDR (Table 48). The most frequently reported mutation, *rpoB* I491F (I572F), had an MIC distribution with an upper end that

overlapped the CC for MGIT, whereas another mutation that was reported in multiple studies, *rpoB* V170F (V146F), had MICs at least two dilutions higher than the current RIF CC for MGIT. At the current CC (i.e. 1 mg/L), all 10 (100%, 95% CI 69-100%) I491F (I572F) mutants would be classified as RIF-S. If the RIF CC were set at 0.5 mg/L, at least 8 (80%, 95% CI 44-97%) I491F (I572F) mutants would be classified as RIF-S. If the RIF CC were set at 0.25 mg/L, at least 7 (70%, 95% CI 35-93%) I491F (I572F) mutants would be classified as RIF-S. With an ATU at 0.25 mg/L, it would be at least 7% (95% CI 0-34%).

Table 48. RIF MICs for *rpoB* mutants outside the RRDR in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	0.12	0.25	0.5	1	2	4	8	20	50
49) Jamieson 2014	42	clinical	1	*rpoB* V170F (V146F)									1
39) Cambau 2015	3, 5, 9, 12, 29, 31-34	clinical	1	*rpoB* V170F (V146F)									1
54) Andres 2014 & Andres	3	clinical	1	*rpoB* V170F (V146F)									1
57) Lin - unpublished	45	clinical	1	*rpoB* V170F (V146F)								1	
60) Torrea 2019	1	clinical	8	*rpoB* I491F (I572F)	1	5	1	1					
50) Van Deun (unpublished)	1	clinical	1	*rpoB* I491F (I572F)		1							
57) Lin - unpublished	45	clinical	1	*rpoB* I491F (I572F)				1					

The green line denotes the current WHO and CLSI CC for RIF DST in MGIT (1 mg/L). Notable limitations: studies RF50 and RF60; studies RF39 and RF54 were conducted in the same laboratory.

3.A.4.3 Conclusion for RIF CC for MGIT

The TEG agreed that 1 mg/L is higher than the ECOFF for MGIT (Table 44). The CC was consequently lowered to **0.5 mg/L** given that only a single isolate (i.e. 0.2%, 95% CI 0.0-1.1%) would be misclassified as RIF-R at this concentration. Notably, this change can be adopted easily in routine clinical practice by reconstituting the lyophilised RIF from the BD SIRE kit in 8 mL, instead of 4 mL, of sterile distilled/deionised water. [85] This off-label concentration for MGIT would reduce the rate of misclassification of borderline RRDR mutations from 74% (95% CI 67-81%) to 53% (95% CI 45-61%), which could be minimized further by adopting an ATU and/or lowering the CC by one more dilution (Table 49). However, there was a consensus that more MIC data for pWT isolates, ideally from studies meeting EUCAST standards, are needed urgently to investigate whether the CC can be lowered to 0.25 mg/L without resulting in high rate of misclassification of susceptible isolates. [86] An ATU was not adopted, in line with the decision for other media (see Section 3.3 for more details).

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[85] BD. BBL™ MGIT™ AST SIRE System for the antimycobacterial susceptibility testing of *Mycobacterium tuberculosis.* 88-2041-1JAA(04). 2019-09. (https://www.bd.com/resource.aspx?IDX=18298, accessed 13 September 2020).

[86] Schön T, Köser CU, Werngren J, *et al*. What is the role of the EUCAST reference method for MIC testing of the *Mycobacterium tuberculosis* complex? *Clin Microbiol Infect*. 2020;26(11):1453-1455. doi:10.1016/j.cmi.2020.07.037.

Table 49. Effect of changing the CC for RIF DST on MGIT for detection of *rpoB* mutants.

Mutation type	Percentage of mutants classified RIF-S at			
	CC 1 mg/L	CC+ATU 1 mg/L or CC 0.5 mg/L	CC+ATU 0.5 mg/L or CC 0.25 mg/L	CC+ATU 0.25 mg/L
S450 (S531)	0% (95% CI 0-1%)	0% (95% CI 0-1%)	0% (95% CI 0-1%)	0% (95% CI 0-1%)
borderline RRDR	74% (95% CI 67-81%)	53% (95% CI 45-61%)	35% (95% CI 28-43%)	11% (95% CI 6-17%)
other RRDR	3% (95% CI 2-5%)	1% (95% CI 0-3%)	1% (95% CI 0-2%)	1% (95% CI 0-2%)
V170F (V146F)	0% (95% CI 0-60%)			
borderline I491F (I572F)	100% (95% CI 69-100%)	80% (95% CI 44-97%)	70% (95% CI 35-93%)	7% (95% CI 0-34%)

The newly adopted CC is underlined.

3.A.5 References for RIF MIC studies

1. Vincent V, Rigouts L, Nduwamahoro E, *et al*. The TDR Tuberculosis Strain Bank: a resource for basic science, tool development and diagnostic services. *Int J Tuberc Lung Dis*. 2012;16(1):24-31. doi:10.5588/ijtld.11.0223.
2. Schönfeld N, Bergmann T, Vesenbeckh S, *et al*. Minimal inhibitory concentrations of first-line drugs of multidrug-resistant tuberculosis isolates. *Lung India.* 2012;29(4):309-12. doi:10.4103/0970-2113.102794.
3. Andres S, Hillemann D, Rüsch-Gerdes S, Richter E. Occurrence of *rpoB* mutations in isoniazid-resistant but rifampin-susceptible *Mycobacterium tuberculosis* isolates from Germany. *Antimicrob Agents Chemother*. 2014;58(1):590-592. doi:10.1128/AAC.01752-13.
4. Fabry W, Schmid EN, Ansorg R. Comparison of the E test and a proportion dilution method for susceptibility testing of *Mycobacterium tuberculosis*. *Zentralbl Bakteriol*. 1995;282(4):394-401. doi:10.1016/s0934-8840(11)80710-3.
5. (a) Rigouts L, Gumusboga M, de Rijk WB, *et al*. Rifampin resistance missed in automated liquid culture system for *Mycobacterium tuberculosis* isolates with specific *rpoB* mutations. *J Clin Microbiol*. 2013;51(8):2641-2645. doi:10.1128/JCM.02741-12.
   (b) Van Deun A, Barrera L, Bastian I, *et al*. *Mycobacterium tuberculosis* strains with highly discordant rifampin susceptibility test results. *J Clin Microbiol*. 2009;47(11):3501-3506. doi:10.1128/JCM.01209-09
   (c) Van Deun A. Personal communication. 2018.
6. Jagielski T, Bakuła Z, Brzostek A, *et al*. Characterization of mutations conferring resistance to rifampin in *Mycobacterium tuberculosis* clinical strains. *Antimicrob Agents Chemother*. 2018;62(10):e01093-18. doi:10.1128/AAC.01093-18.
7. Cavusoglu C, Turhan A, Akinci P, Soyler I. Evaluation of the Genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* isolates. *J Clin Microbiol*. 2006;44(7):2338-2342. doi:10.1128/JCM.00425-06.
8. Schönfeld N, Bergmann T, Vesenbeckh S, *et al*. Minimal inhibitory concentrations of first-line drugs of multidrug-resistant tuberculosis isolates. *Lung India.* 2012;29(4):309-12. doi:10.4103/0970-2113.102794.
9. van Ingen J, Simons S, de Zwaan R, *et al*. Comparative study on genotypic and phenotypic second-line drug resistance testing of *Mycobacterium tuberculosis* complex isolates. *J Clin Microbiol*. 2010;48(8):2749-2753. doi:10.1128/JCM.00652-10.
10. van Klingeren B, Dessens-Kroon M, van der Laan T, Kremer K, van Soolingen D. Drug susceptibility testing of *Mycobacterium tuberculosis* complex by use of a high-throughput, reproducible, absolute concentration method. *J Clin Microbiol*. 2007;45(8):2662-2668. doi:10.1128/JCM.00244-07.
11. Heifets L, Sanchez T, Vanderkolk J, Pham V. Development of rifapentine susceptibility tests for *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 1999;43(1):25-28. doi:10.1128/AAC.43.1.25.
12. Moghazeh SL, Pan X, Arain T, *et al*. Comparative antimycobacterial activities of rifampin, rifapentine, and KRM-1648 against a collection of rifampin-resistant *Mycobacterium tuberculosis* isolates with known *rpoB* mutations. *Antimicrob Agents Chemother*. 1996;40(11):2655-2657. doi:10.1128/AAC.40.11.2655.
13. Rancoita PM V., Cugnata F, Gibertoni Cruz AL, *et al*. Validating a 14-drug microtiter plate containing bedaquiline and delamanid for large-scale research susceptibility testing of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2018;62(9):e00344-18. doi:10.1128/AAC.00344-18.
14. Kusunoki S, Takemura K, Takagi K, *et al*. A new genetic assay for rifampicin susceptibility of *Mycobacterium tuberculosis*. *Kansenshogaku Zasshi*. 1995;69(12):1342-1347. doi:10.11150/kansenshogakuzasshi1970.69.1342.
15. Miotto P, Cabibbe AM, Borroni E, Degano M, Cirillo DM. Role of disputed mutations in the *rpoB* gene in interpretation of automated liquid MGIT culture results for rifampin susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2018;56(5):e01599-17. doi:10.1128/JCM.01599-17.
16. Park S-W, Tasneen R, Converse PJ, Nuermberger EL. Immunodeficiency and intermittent dosing promote acquired rifamycin monoresistance in murine tuberculosis. *Antimicrob Agents Chemother*. 2017;61(11):e01502-17. doi:10.1128/AAC.01502-17.
17. (a) de Steenwinkel JEM, ten Kate MT, de Knegt GJ, *et al*. Consequences of noncompliance for therapy efficacy and emergence of resistance in murine tuberculosis caused by the Beijing genotype of

Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2012;56(9):4937-4944. doi:10.1128/AAC.00124-12.

(b) de Steenwinkel JEM, ten Kate MT, de Knegt GJ, *et al*. Drug susceptibility of *Mycobacterium tuberculosis* Beijing genotype and association with MDR TB. *Emerg Infect Dis*. 2012;18(4):660-663. doi:10.3201/eid1804.110912.

(c) Bax HI, Bakker-Woudenberg IAJM, de Vogel CP, *et al*. The role of the time-kill kinetics assay as part of a preclinical modeling framework for assessing the activity of anti-tuberculosis drugs. *Tuberculosis*. 2017;105:80-85. doi:10.1016/j.tube.2017.04.010.

18. Cavusoglu C, Karaca-Derici Y, Bilgic A. *In-vitro* activity of rifabutin against rifampicin-resistant *Mycobacterium tuberculosis* isolates with known *rpoB* mutations. *Clin Microbiol Infect*. 2004;10(7):662-665. doi:10.1111/j.1469-0691.2004.00917.x.

19. Schön T, Juréen P, Chryssanthou E, *et al*. Rifampicin-resistant and rifabutin-susceptible *Mycobacterium tuberculosis* strains: a breakpoint artefact? *J Antimicrob Chemother*. 2013;68(9):2074-2077. doi:10.1093/jac/dkt150.

20. Schön T, Juréen P, Giske CG, *et al*. Evaluation of wild-type MIC distributions as a tool for determination of clinical breakpoints for *Mycobacterium tuberculosis*. *J Antimicrob Chemother*. 2009;64(4):786-793. doi:10.1093/jac/dkp262.

21. Garcia A-B, Palacios JJ, Ruiz M-J, *et al*. Strong *in vitro* activities of two new rifabutin analogs against multidrug-resistant *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2010;54(12):5363-5365. doi:10.1128/AAC.00149-10.

22. Marubini E. Evaluation of *Mycobacterium tuberculosis* clinical isolates with discordant rifampicin genotypic and phenotypic susceptibility testing results from Pretoria, South Africa. 2016. https://repository.up.ac.za/bitstream/handle/2263/56939/Marubini_Evaluation_2016.pdf.

23. Pholwat S, Heysell S, Stroup S, Foongladda S, Houpt E. Rapid first- and second-line drug susceptibility assay for *Mycobacterium tuberculosis* isolates by use of quantitative PCR. *J Clin Microbiol*. 2011;49(1):69-75. doi:10.1128/JCM.01500-10.

24. Mvelase NR, Pillay M, Sibanda W, *et al*. *rpoB* mutations causing discordant rifampicin susceptibility in *Mycobacterium tuberculosis*: Retrospective analysis of prevalence, phenotypic, genotypic, and treatment outcomes. *Open Forum Infect Dis*. 2019;6(4):ofz065. doi:10.1093/ofid/ofz065.

25. Farhat MR, Freschi L, Calderon R, *et al*. GWAS for quantitative resistance phenotypes in *Mycobacterium tuberculosis* reveals resistance genes and regulatory regions. *Nat Commun*. 2019;10(1):2128. doi:10.1038/s41467-019-10110-6.

26. Gygli SM, Keller PM, Ballif M, *et al*. Whole-genome sequencing for drug resistance profile prediction in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2019;63(4):e02175-18. doi:10.1128/AAC.02175-18.

27. Rastogi N, Goh KS, Berchel M, Bryskier A. Activity of rifapentine and its metabolite 25-O-desacetylrifapentine compared with rifampicin and rifabutin against *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis* and *M. bovis* BCG. *J Antimicrob Chemother*. 2000;46(4):565-570. doi:10.1093/jac/46.4.565.

28. Fattorini L, Iona E, Ricc ML, *et al*. Activity of 16 antimicrobial agents against drug-resistant strains of *Mycobacterium tuberculosis*. *Microb Drug Resist*. 1999;5(4):265-270. doi:10.1089/mdr.1999.5.265.

29. Coban AY. Blood agar validation for susceptibility testing of isoniazid, rifampicin, ethambutol, and streptomycin to *Mycobacterium tuberculosis* isolates. *PLoS One*. 2013;8(2):e55370. doi:10.1371/journal.pone.0055370.

30. (a) Rey-Jurado E, Tudó G, de la Bellacasa JP, Espasa M, González-Martín J. *In vitro* effect of three-drug combinations of antituberculous agents against multidrug-resistant *Mycobacterium tuberculosis* isolates. *Int J Antimicrob Agents*. 2013;41(3):278-280. doi:10.1016/j.ijantimicag.2012.11.011.

(b) Rey-Jurado E, Tudó G, Martínez JA, González-Martín J. Synergistic effect of two combinations of antituberculous drugs against *Mycobacterium tuberculosis*. *Tuberculosis*. 2012;92(3):260-263. doi:10.1016/j.tube.2012.01.005.

31. Shishido Y, Mitarai S, Otomo K, *et al*. Anti-tuberculosis drug susceptibility testing of *Mycobacterium bovis* BCG Tokyo strain. *Int J Tuberc Lung Dis*. 2007;11(12):1334-1338.

32. (a) Anthony RM, Schuitema AR, Bergval IL, *et al*. Acquisition of rifabutin resistance by a rifampicin resistant mutant of *Mycobacterium tuberculosis* involves an unusual spectrum of mutations and elevated frequency. *Ann Clin Microbiol Antimicrob*. 2005;4(1):9. doi:10.1186/1476-0711-4-9.

(b) Bergval IL, Klatser PR, Schuitema ARJ, Oskam L, Anthony RM. Specific mutations in the *Mycobacterium tuberculosis rpoB* gene are associated with increased *dnaE2* expression. *FEMS Microbiol Lett*. 2007;275(2):338-343. doi:10.1111/j.1574-6968.2007.00905.x.

33. Rodriguez Díaz JC, Ruiz M, López M, Royo G. Synergic activity of fluoroquinolones and linezolid against *Mycobacterium tuberculosis*. *Int J Antimicrob Agents*. 2003;21(4):354-356. doi:10.1016/s0924-8579(02)00387-4.

34. Pretet S, Lebeaut A, Parrot R *et al*. Combined chemotherapy including rifabutin for rifampicin and isoniazid resistant pulmonary tuberculosis. G.E.T.I.M. (Group for the Study and Treatment of Resistant Mycobacterial Infection). *Eur Respir J*. 1992;5(6):680-4.

35. Lee C-N, Heifets LB. Determination of minimal inhibitory concentrations of antituberculosis drugs by radiometric and conventional methods. *Am Rev Respir Dis*. 1987;136(2):349-352. doi:10.1164/ajrccm/136.2.349.

36. Heifets L, Sanchez T, Vanderkolk J, Pham V. Development of rifapentine susceptibility tests for *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 1999;43(1):25-28. doi:10.1128/AAC.43.1.25.

37. Truffot-Pernot C, Giroir AM, Grosset J. A study of the minimal inhibitory concentration of rifabutin (ansamycin LM 427) for *Mycobacterium tuberculosis*, *Mycobacterium xenopi* and *Mycobacterium avium-intracellulaire*. *Rev Mal Respir*. 1988;5(4):401-406.

38. Kambli P, Ajbani K, Sadani M, *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.

39. Cambau E, Viveiros M, Machado D, *et al*. Revisiting susceptibility testing in MDR-TB by a standardized quantitative phenotypic assessment in a European multicentre study. *J Antimicrob Chemother*. 2015;70(3):686-696. doi:10.1093/jac/dku438.

40. Ho J, Jelfs P, Sintchenko V. Phenotypically occult multidrug-resistant *Mycobacterium tuberculosis*: dilemmas in diagnosis and treatment. *J Antimicrob Chemother*. 2013;68(12):2915-2920. doi:10.1093/jac/dkt284.

41. Feuerriegel S, Oberhauser B, George AG, *et al*. Sequence analysis for detection of first-line drug resistance in *Mycobacterium tuberculosis* strains from a high-incidence setting. *BMC Microbiol*. 2012;12:90. doi:10.1186/1471-2180-12-90.

42. Williamson DA, Roberts SA, Bower JE, *et al*. Clinical failures associated with *rpoB* mutations in phenotypically occult multidrug-resistant *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis*. 2012;16(2):216-220. doi:10.5588/ijtld.11.0178.

43. Abanda NN, Djieugoué JY, Khadka VS, *et al*. Absence of hybridization with the wild-type and mutant *rpoB* probes in the Genotype MTBDR*plus* assay detects 'disputed' rifampicin mutations. *Clin Microbiol Infect*. 2018;24(7):781.e1-781.e3. doi:10.1016/j.cmi.2017.11.021.

44. Heyckendorf J, Andres S, Köser CU, *et al*. What is resistance? Impact of phenotypic versus molecular drug resistance testing on therapy for multi- and extensively drug-resistant Tuberculosis. *Antimicrob Agents Chemother*. 2017;62(2):e01550-17. doi:10.1128/AAC.01550-17.

45. Sanker P, Ambika AP, Santhosh VT, *et al*. Are WHO approved nucleic acid amplification tests causing large-scale "false identification" of rifampicin-resistant tuberculosis?: Programmatic experience from south india. *Int J Mycobacteriol*. 2017;6(1):21-26. doi:10.4103/2212-5531.201900.

46. (a) Rockwood N, Sirgel F, Streicher E, Warren R, Meintjes G, Wilkinson RJ. Low frequency of acquired isoniazid and rifampicin resistance in rifampicin-susceptible pulmonary tuberculosis in a setting of high HIV-1 infection and tuberculosis coprevalence. *J Infect Dis*. 2017;216(6):632-640. doi:10.1093/infdis/jix337.
(b) Rockwood N, Pasipanodya JG, Denti P, *et al*. Concentration-dependent antagonism and culture conversion in pulmonary tuberculosis. *Clin Infect Dis*. 2017;64(10):1350-1359. doi:10.1093/cid/cix158.

47. El Maraachli W, Slater M, Berrada ZL, *et al*. Predicting differential rifamycin resistance in clinical *Mycobacterium tuberculosis* isolates by specific *rpoB* mutations. *Int J Tuberc Lung Dis*. 2015;19(10):1222-1226. doi:10.5588/ijtld.14.0936.

48. Chigutsa E, Pasipanodya JG, Visser ME, *et al*. Impact of nonlinear interactions of pharmacokinetics and MICs on sputum bacillary kill rates as a marker of sterilizing effect in tuberculosis. *Antimicrob Agents Chemother*. 2015;59(1):38-45. doi:10.1128/AAC.03931-14.

49. Jamieson FB, Guthrie JL, Neemuchwala A, *et al*. Profiling of *rpoB* mutations and MICs for rifampin and rifabutin in *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2014;52(6):2157-2162. doi:10.1128/JCM.00691-14.

50. Van Deun A. Personal communication. 2018.

51. (a) Sirgel FA, Warren RM, Böttger EC, *et al*. The rationale for using rifabutin in the treatment of MDR and XDR tuberculosis outbreaks. *PLoS One*. 2013;8(3):e59414. doi:10.1371/journal.pone.0059414.
(b) Sirgel FA, Warren RM, Böttger EC, *et al*. Correction: The rationale for using rifabutin in the treatment of MDR and XDR tuberculosis outbreaks. *PLoS One*. 2015. doi:10.1371/journal.pone.0134190.

52. Bernardelli A, Morcillo N, Loureiro J, Quse V, Davenport S. *In vitro* susceptibility testing of *Mycobacterium tuberculosis* complex strains isolated from seals to antituberculosis drugs. *Biomedica*. 2004;24 Supp 1:85-91.

53. (a) Machado D, Perdigão J, Portugal I, *et al*. Efflux activity differentially modulates the levels of isoniazid and rifampicin resistance among multidrug resistant and monoresistant *Mycobacterium tuberculosis* strains. *Antibiotics*. 2018;7(1):18. doi:10.3390/antibiotics7010018.
(b) Machado D, Viveiros M. Personal Communication. 2018.

54. (a) Andres S, Hillemann D, Rüsch-Gerdes S, Richter E. Occurrence of *rpoB* mutations in isoniazid-resistant but rifampin-susceptible *Mycobacterium tuberculosis* isolates from Germany. *Antimicrob Agents Chemother*. 2014;58(1):590-592. doi:10.1128/AAC.01752-13.
(b) Andres S. Personal communication. 2018.

55. Niward K, Davies Forsman L, Bruchfeld J, *et al*. Distribution of plasma concentrations of first-line anti-TB drugs and individual MICs: a prospective cohort study in a low endemic setting. *J Antimicrob Chemother*. 2018;73(10):2838-2845. doi:10.1093/jac/dky268.

56. Whitfield MG, Warren RM, Mathys V, *et al*. The potential use of rifabutin for treatment of patients diagnosed with rifampicin-resistant tuberculosis. *J Antimicrob Chemother*. 2018;73(10):2667-2674. doi:10.1093/jac/dky248.

57. Lin G. Personal communication. 2018.

58. Groenheit R. Personal communication. 2018.

59. Mvelase NR, Pillay M, Sibanda W, *et al*. *rpoB* mutations causing discordant rifampicin susceptibility in *Mycobacterium tuberculosis*: Retrospective analysis of prevalence, phenotypic, genotypic, and treatment outcomes. *Open Forum Infect Dis*. 2019;6(4):ofz065. doi:10.1093/ofid/ofz065.

60. Torrea G, Ng K, Van Deun A. Variable ability of rapid tests to detect *Mycobacterium tuberculosis rpoB* mutations conferring phenotypically occult rifampicin resistance. *Sci Rep*. 2019;9(1):11826. doi:10.1038/s41598-019-48401-z.

61. Tessema B, Nabeta P, Valli E, *et al*. FIND Tuberculosis Strain Bank: A resource for researchers and developers working on tests to detect *Mycobacterium tuberculosis* and related drug resistance. *J Clin Microbiol*. 2017;55(4):1066-1073. doi:10.1128/JCM.01662-16.

62. Colangeli R, Jedrey H, Kim S, *et al*. Bacterial factors that predict relapse after tuberculosis therapy. *N Engl J Med*. 2018;379(9):823-833. doi:10.1056/NEJMoa1715849.

63. Berrada ZL, Lin S-YG, Rodwell TC, *et al*. Rifabutin and rifampin resistance levels and associated *rpoB* mutations in clinical isolates of *Mycobacterium tuberculosis* complex. *Diagn Microbiol Infect Dis*. 2016;85(2):177-181. doi:10.1016/j.diagmicrobio.2016.01.019.

64. Gygli SM, Keller PM, Ballif M, *et al*. Whole-genome sequencing for drug resistance profile prediction in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2019;63(4):e02175-18. doi:10.1128/AAC.02175-18.

3.B.1 RFB MIC data on LJ

3.B.1.1 RFB MICs for pWT isolates on LJ
No studies were identified that featured RFB MIC data for pWT isolates on LJ medium.

3.B.1.2 RFB MICs for mutated isolates on LJ
No studies were identified that featured RFB MIC data for mutated isolates on LJ medium.

3.B.1.3 Conclusion for RFB CC for LJ
Owing to a lack of data not even a tentative ECOFF could be defined for this medium. Even if sufficient MIC evidence had been available, a CC would not have been endorsed as RFB is not currently recommended for TB treatment by WHO. Refer to Section 3.3 for a strategy for RFB DST.

3.B.2 RFB MIC data on 7H10

3.B.2.1 RFB MICs for pWT isolates on 7H10

Seven studies were identified that reported RFB MIC data for the pWT population on 7H10 (Table 50). Five of these studies featured MIC data for more than 10 pWT isolates. Two of these studies had data truncations that precluded an assessment of the shape of the MIC distributions, including García *et al*. with unusually low MICs for pWT isolates, as previously seen for RIF (Table 35). The study was consequently excluded. The modes of the remaining studies, two of which were conducted in the same laboratory, ranged from $\leq$0.015 to 0.03 mg/L. 0.125 or 0.06 mg/L represent the tentative ECOFF.

Table 50. RFB MICs for pWT isolates on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.004	0.008	0.015	0.03	0.04	0.06	0.1	0.12	0.2	0.25	0.3	0.5	0.7	1	2	2.5	3	4	5	8	10	16	25	50
3) Schön 2013	3		1	2	H37Rv ATCC25618		1	1																						
	3		1	1	BTB 08-049				1																					
	3		22	22	Mostly RIF-R	gWT	1	7	9	2						1					1							1		
5) Schön 2011	3		1	4	H37Rv ATCC27294				1	2	1																			
	3		1	1	BTB 08-049					1																				
	3	clinical	99	99						8	41	25		23		1								1						
1) Rancoita 2018	1		1	2	H37Rv					2																				
	1		17	17		gWT		7		5			5																	
9) Gygli 2019	7		1	3	H37Rv ATCC27294						1	1		1																
	7		1	3	Erdman						1	1		1																
	7	clinical	76	80	mixture of first-line resistance profiles	gWT				6	24	28		21		1														
6) García 2010	5	reference	16	48					30																		15			3
	5	clinical	63	189					138																		45			6
2) Cavusoglu 2004	2		1	1	H37Rv ATCC27294							1																		
7) van Ingen 2010	4		1	1	H37Rv													1												

The blue line denotes the current CLSI CC for RFB DST on 7H10 (0.5 mg/L). Notable limitations: studies RB3 and RB5 were conducted in the same laboratory, and study RB6 had unusually low MICs for pWT isolates.

3.B.2.2 RFB MICs for mutated isolates on 7H10

rpoB 450 (531) mutants

Five studies were identified that reported 593 RFB MICs for isolates with *rpoB* S450 (S531) mutations on 7H10 (Table 51). The modes of the three untruncated distributions were 2-4 mg/L. If 0.125 mg/L was selected as the RFB CC for 7H10 only 23 isolates (4%, 95% CI 2-6%) would test RFB-S. At the CLSI CC (i.e. 0.5 mg/L), 41 (7%, 95% CI 5-9%) isolates would be classified as RFB-S. The effect of adopting 0.06 mg/L as a CC with or without an ATU could not be explored, as the lowest concentration tested by Farhat *et al*. was 0.125 mg/L. It is notable that the MIC distribution for S450 mutants in this study was unusually broad, which may suggest phenotypic differences depending on the genetic background.

Table 51. RFB MICs for *rpoB* S450 (S531) mutants on 7H10.

Studies	Lab	Unique isolates	Total MICs	Genotypic results	0.06	0.12	0.25	0.5	0.75	0.83	1	1.5	2	2.5	4	5	8	16
1) Rancoita 2018	1	10	10	*rpoB* S450L (S531L)				2			2		4		2			
2) Cavusoglu 2004	2	19	19	*rpoB* S450L (S531L)							1			1		1	16	
	2	4	4	*rpoB* S450W (S531W)													4	
3) Schön 2013	3	46	46	*rpoB* S450L (S531L)	1		3				9		17		12		3	1
	3	1	1	*rpoB* S450W (S531W)				1										
9) Gygli 2019	7	43	45	*rpoB* S450L (S531L)										3	28		13	1
8) Farhat 2019	6	12	12	*rpoB* S450F (S531F)											12			
	6	440	440	*rpoB* S450L (S531L)		19	3	8	5	10	11	36		75	256			
	6	4	4	*rpoB* S450M (S531M)		1									3			
	6	5	5	*rpoB* S450Q (S531Q)		1		1					1		1			
	6	7	7	*rpoB* S450W (S531W)		1								1	5			

The blue line denotes the current CLSI CC for RFB DST on 7H10 (0.5 mg/L).

rpoB borderline RRDR mutants

Four studies were identified that reported RFB MICs for isolates with *rpoB* borderline mutations tested on 7H10 (Table 52). The vast majority of MICs reported for these isolates were between 0.02-0.5 mg/L.

At the CLSI CC (i.e. 0.5 mg/L), 56 (92%, 95% CI 82-97%) isolates would be classified as RFB-S. If a CC were set at 0.125 mg/L, at least 41 of 61 (67%, 95% CI 54-79%) isolates would be classified as RFB-S. The consequence of a lower CC or an ATU could not be assessed given the severe truncations of most MICs.

Table 52. RFB MICs for *rpoB* borderline RRDR mutants on 7H10.

Studies	Isolate origin	Unique isolates	Total MICs	Genotypic results	0.02	0.03	0.06	0.12	0.2	0.25	0.5	0.75	0.83	1	1.5	2	2.5	3	4
3) Schön 2013		1	1	*rpoB* L430P (L511P)	1														
8) Farhat 2019	clinical	3	3	*rpoB* L430P (L511P)				3											
3) Schön 2013		3	3	*rpoB* D435Y (D516Y)		1	1			1									
2) Cavusoglu 2004		2	2	*rpoB* D435Y (D516Y)							2								
8) Farhat 2019	clinical	11	11	*rpoB* D435Y (D516Y)				11											
4) van Ingen 2011	clinical	4	7	*rpoB* D435Y (D516Y)					4		3								
3) Schön 2013		1	1	*rpoB* H445L (H526L)				1											
9) Gygli 2019	clinical	1	1	*rpoB* H445L (H526L)				1											
8) Farhat 2019	clinical	13	13	*rpoB* H445L (H526L)				12		1									
8) Farhat 2019	clinical	3	3	*rpoB* H445N (H526N)				2				1							
8) Farhat 2019	clinical	1	1	*rpoB* H445S (H526S)				1											
3) Schön 2013		1	1	*rpoB* L452P (L533P)			1												
9) Gygli 2019	clinical	2	3	*rpoB* L452P (L533P)						1					1				1
2) Cavusoglu 2004		1	1	*rpoB* L452P (L533P)							1								
8) Farhat 2019	clinical	10	10	*rpoB* L452P (L533P)				6			1							2	

The blue line denotes the current CLSI CC for RFB DST on 7H10 (0.5 mg/L).

Other *rpoB* RRDR mutants

Four studies were identified that reported 246 RFB MICs for isolates with *rpoB* mutations other than S450 (S531) and the borderline mutations on 7H10 (Table 53). The D435V (D516V) mutants had low MICs, overall, with the majority, or 129 of 159 (81% 95% CI 74-87%) having MICs lower than 0.125 mg/L. At the CLSI CC (i.e. 0.5 mg/L), 147 (60%, 95% CI 53-66%) isolates would be classified as RFB-S. If a CC were set at 0.125 mg/L, 95 (39%, 95% CI 33-45%) of the total isolates would be classified as RFB-S. Given that most MICs at 0.125 mg/L were truncated, the effect of an ATU at this concentration or a CC at 0.06 mg/L (with or without an ATU) could not be calculated. However, a CC and ATU at 0.06 mg/L would eliminate any mutants testing RFB-S for untruncated datasets.

Table 53. RFB MICs for other *rpoB* RRDR mutants on 7H10.

Studies	Unique isolates	Total MICs	Genotypic results	0.06	0.12	0.25	0.5	0.6	0.7	0.75	0.83	1	1.5	2	2.5	4	5	8	16	32
8) Farhat 2019	2	2	*rpoB* Q432E (Q513E)										1		1					
8) Farhat 2019	1	1	*rpoB* D435F (Q513F)		1															
8) Farhat 2019	3	3	*rpoB* Q432K (Q513K)													3				
2) Cavusoglu 2004	1	1	*rpoB* Q432P (Q513P)															1		
8) Farhat 2019	2	2	*rpoB* Q432P (Q513P)			1				1										
2) Cavusoglu 2004	1	1	*rpoB* D435V (D516V)				1													
3) Schön 2013	9	9	*rpoB* D435V (D516V)	2	4	3														
9) Gygli 2019	18	20	*rpoB* D435V (D516V)		5	7	3					3	1			1				
8) Farhat 2019	129	129	*rpoB* D435V (D516V)	70	28	6		8		5		1		2		9				
8) Farhat 2019	3	3	*rpoB* S441L (S522L)	2	1															
2) Cavusoglu 2004	2	2	*rpoB* S441W (S522W)													2				
2) Cavusoglu 2004	1	1	*rpoB* H445C (H526C)	1																
8) Farhat 2019	7	7	*rpoB* H445C (H526C)		7															
2) Cavusoglu 2004	1	1	*rpoB* H445D (H526D)													1				
3) Schön 2013	3	3	*rpoB* H445D (H526D)												1	1	1			
9) Gygli 2019	7	9	*rpoB* H445D (H526D)												1	3	4	1		
8) Farhat 2019	7	7	*rpoB* H445D (H526D)		1										6					
8) Farhat 2019	1	1	*rpoB* H445G (H526G)	1																
8) Farhat 2019	1	1	*rpoB* H445P (H526P)												1					
2) Cavusoglu 2004	4	4	*rpoB* H445R (H526R)													4				
8) Farhat 2019	10	10	*rpoB* H445R (H526R)												10					
2) Cavusoglu 2004	2	2	*rpoB* H445Y (H526Y)													2				
3) Schön 2013	3	3	*rpoB* H445Y (H526Y)													1	2			
9) Gygli 2019	5	6	*rpoB* H445Y (H526Y)													2	3	1		
8) Farhat 2019	16	16	*rpoB* H445Y (H526Y)	1											15					
8) Farhat 2019	2	2	*rpoB* R448K (R529K)		2															

The blue line denotes the current CLSI CC for RFB DST on 7H10 (0.5 mg/L).

rpoB mutants outside the RRDR

Only 2 studies featured RFB MIC data for isolates with *rpoB* mutations outside of the RRDR on 7H10 medium (Table 54). 67% (95% CI, 22-86%) of I491F (I572F) mutants would test susceptible at the CLSI CC of 0.5 mg/L. Lowering the CC to 0.125 mg/L would reduce this to 50% (95% CI, 12-88%). Lower concentrations could not be explored.

Table 54. RFB MICs for *rpoB* mutants outside the RRDR on 7H10.

Studies	Unique isolates	Genotypic results	0.12	0.25	0.5	0.6	0.75	0.83	1	1.5	2	2.5	3
9) Gygli 2019	1	*rpoB* V170F (V146F)									1		
8) Farhat 2019	5	*rpoB* V170F (V146F)				1				2		2	
	6	*rpoB* I491F (I572F)	3	1		1							1

The blue line denotes the current CLSI CC for RFB DST on 7H10 (0.5 mg/L).

3.B.2.3 Conclusion for RFB CC for 7H10

The three well-designed studies by Gygli *et al*. and Schön *et al*. (Table 50) provided a good insight into the pWT MIC distribution, but was not clear whether 0.125 mg/L or 0.06 mg/L was the likely tentative ECOFF. Notably, these results were in line with a study by Heifets *et al*. that did not meet the inclusion criteria for this review because it was not clear precisely which concentrations were tested.[87] The effect of adopting an ATU at 0.125 mg/L or a CC at 0.06 mg/L (with or without an ATU) could not be calculated with confidence because insufficiently low concentrations were tested for most mutants. However, neither 0.125 nor 0.06 mg/L were adopted as CCs given that WHO does not currently recommend RFB for TB treatment by WHO. Refer to Section 3.3 for a strategy for RFB DST.

Table 55. Effect of adopting a CC for RFB DST on 7H10 for detection of *rpoB* mutants.

Mutation type	Percentage of mutants classified RFB-S at	
	<u>CC 0.5 mg/L</u>	CC 0.125 mg/L
S450 (S531)	7% (95% CI 5-9%)	4% (95% CI 2-6%)
borderline RRDR	92% (95% CI 82-97%)	67% (95% CI 54-79%)
other RRDR	60% (95% CI 53-66%)	39% (95% CI 33-45%)
V170F (V146F)	0% (95% CI 0-46%)	
borderline I491F (I572F)	67% (95% CI 22-86%)	50% (95% CI 12-88%)

The current CLSI CC is underlined.

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[87] Heifets LB, Lindholm-Levy PJ, Iseman MD. Rifabutine: minimal inhibitory and bactericidal concentrations for *Mycobacterium tuberculosis*. *Am Rev Respir Dis*. 1988;137(3):719-721. doi:10.1164/ajrccm/137.3.719.

3.B.3 RFB MIC data on 7H11

3.B.3.1 RFB MICs for pWT isolates on 7H11

Three studies were identified that reported RFB MIC data for the pWT population by 7H11 (Table 56). Only one study reported MICs for at least 10 pWT isolates. The RFB MIC distributions were ≤0.15 to 0.25 mg/L.

Table 56. RFB MICs for pWT isolates on 7H11.

Studies	Isolate origin	Unique isolates	Type of isolates	Genotypic results	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4
		1	H37Rv						1				
		1	M. africanum ATCC 25420							1			
		1	M. bovis ATCC 19210							1			
10) Rastogi 2000		5	M. bovis BCG		2		3						
		6	Pan-S				2	4					
	clinical	4	M. africanum						1	3			
		4	M. bovis							4			
13) Truffot-Pernot 198	clinical	10	RIF-S			7	3						
11) Anthony 2005 &	lab	2	gWT								2		

The blue line denotes the current CLSI CC for RFB DST on 7H10 (0.5 mg/L).

3.B.3.2 RFB MICs for mutated isolates on 7H11

rpoB 450 (531) mutants

Only 1 study was identified that reported RFB MICs for isolates with *rpoB* S450 (S531) codon mutants on 7H11 (Table 57). The five isolates tested in this study all had RFB MICs >32 mg/L, with a mode of 128 mg/L.

Table 57. RFB MICs for *rpoB* S450 (S531) mutants on 7H11.

Studies	Isolate origin	Unique isolates	Genotypic results	0.5	1	2	4	8	16	32	64	128	160
11) Anthony 2005 &	lab	4	*rpoB* S450L (S531L)								1	2	1
Bergval 2007	lab	1	*rpoB* S450W (S531W)									1	

The blue line denotes the current CLSI CC for RFB DST on 7H10 (0.5 mg/L).

rpoB borderline RRDR mutants

No studies were identified that featured RFB MIC data for isolates with *rpoB* borderline RRDR mutations on 7H11 medium.

Other rpoB RRDR mutants

Only 1 study was identified that reported RFB MICs for isolates with *rpoB* RRDR mutations other than the borderline mutations and S450 (S531) codon mutations (Table 58). The 16 MICs reported for these isolates were all >32 mg/L, with a mode of 128 mg/L, except one S522L mutant with an MIC ≤1 mg/L.

Table 58. RFB MICs for other *rpoB* RRDR mutants on 7H11.

Studies	Isolate origin	Unique isolates	Genotypic results	0.5	1	2	4	8	16	32	64	128	160
	lab	1	*rpoB* Q432L (Q513L)										1
	lab	1	*rpoB* S441L (S522L)	1									
11) Anthony 2005 &	lab	4	*rpoB* H445D (H526D)									3	1
Bergval 2007	lab	5	*rpoB* H445R (H526R)									5	
	lab	1	*rpoB* H445P (H526P)										1
	lab	4	*rpoB* H445Y (H526Y)								1	2	1

The blue line denotes the current CLSI CC for RFB DST on 7H10 (0.5 mg/L).

rpoB mutants outside the RRDR

No studies were identified that featured RFB MIC data for isolates with *rpoB* mutations outside of the RRDR on 7H11 medium.

3.B.3.3 Conclusion for RFB CC for 7H11

Given a lack of genetic data and MIC data truncations, the three studies identified by this review contained insufficient data for pWT isolates to define even a tentative ECOFF for RFB CC for 7H11. Even if sufficient MIC evidence had been available, a CC would not have been endorsed as RFB is not currently recommended for TB treatment by WHO. Refer to Section 3.3 for a strategy for RFB DST.

3.B.4 RFB MIC data in MGIT

3.B.4.1 RFB MICs for pWT isolates in MGIT

Nine studies were identified that reported RFB MIC data for the pWT population by MGIT (Table 59). Five of these studies reported MICs for at least 10 pWT isolates, though the majority of MIC distributions were truncated, and so the shape of the distributions could not be determined in most cases. Nevertheless, the results suggested a tentative ECOFF of 0.125 mg/L.

Table 59. RFB MICs for pWT isolates in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.015	0.03	0.06	0.1	0.12	0.25	0.4	0.5	1	2	4
15) Heyckendorf 2018	17		1	1	H37Rv ATCC27294	-					1						
16) Jamieson 2014	18	clinical	1	1	mixture of first-line resistance profiles	gWT									1		
17) Sirgel 2013 &	19	clinical	26	26		gWT			3		23						
18) Sharma 2011	20		36	36	mixture of first-line resistance profiles							19	2			15	
19) Rüsch-Gerdes 2006	17, 21-22		10	30	Pan-S							30					
	17, 21-22		21	63	mixture of first-line resistance profiles							8	4			51	
20) Whitfield 2018	11		3	3		gWT								2			1
21) Lin - unpublished	23		1	10	H37Rv				4	5	1						
	23		2	2		gWT					2						
23) Berrada 2016	23	clinical	44	44		gWT					39	5					
24) Gygli 2019	7		1	1	H37Rv ATCC27294						1						
	7		1	1	Erdman						1						
	7	clinical	22	22		gWT					22						

The blue line denotes the current CLSI CC for RFB DST in MGIT (0.5 mg/L). Notable limitations: studies RB21 and RB23; and studies RB15 and RB19 were conducted in the same laboratory.

3.B.4.2 RFB MICs for mutated isolates in MGIT

rpoB 450 (531) mutants

Eight studies were identified that reported RFB MICs for 218 isolates with *rpoB* S450 (S531) codon mutants by MGIT (Table 60). All MICs reported for these isolates were >1 mg/L except for 2 (1%, 95% CI 0-3%) *rpoB* S450L (S531L) mutants with MICs <0.25 mg/L from two different studies. If a CC were set at 0.125 mg/L, it is likely that no isolates would be classified as RFB-S.

Table 60. RFB MICs for *rpoB* S450 (S531) mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	0.1	0.12	0.25	0.4	0.5	1	2	4	5	8	10	15
14) Cambau 2015	1, 4, 10-16	clinical	1	rpoB S450P (S531P)								1				
	1, 4, 10-16	clinical	1	rpoB S450M (S531M)								1				
	1, 4, 10-16	clinical	89	rpoB S450L (S531L)							19	70				
22) Machado	12	clinical	27	rpoB S450L (S531L)							9	18				
24) Gygli 2019	7	clinical	16	rpoB S450L (S531L)							10	6				
15) Heyckendorf 2018	17	clinical	1	rpoB S450F (S531F)												1
	17	clinical	12	rpoB S450L (S531L)											12	
23) Berrada 2016	23	clinical	1	rpoB S450C (S531C)											1	
	23	clinical	1	rpoB S450F (S531F)											1	
	23	clinical	4	rpoB S450W (S531W)								1		2	1	
	23	clinical	34	rpoB S450L (S531L)							7	25	2			
20) Whitfield 2018	11		8	rpoB S450L (S531L)			1			4		3				
16) Jamieson 2014	18	clinical	19	rpoB S450L (S531L)		1							18			
	18	clinical	3	rpoB S450W (S531W)									3			
21) Lin - unpublished	23		1	rpoB S450Q (S531Q)											1	

The blue line denotes the current CLSI CC for RFB DST in MGIT (0.5 mg/L). Notable limitations: studies RB14 and RB22; studies RB14 and RB20; and studies RB23 and RB21 were conducted in the same laboratory.

rpoB borderline RRDR mutants

Seven studies were identified that reported RFB MICs for isolates with *rpoB* borderline mutations tested by MGIT (Table 61). The MICs for these 42 isolates ranged from <0.06 to >2 mg/L. At the CLSI CC (i.e. 0.5 mg/L), 38 (90%, 95% CI 77-97%) isolates would be classified as RFB-S. If a CC were set at

0.125 mg/L, at least 26 (62%, 95% CI 46-76%) isolates would be classified as RFB-S. An ATU at 0.125 mg/L could reduce this to 33% (95% CI 20-50%).

Table 61. RFB MICs for *rpoB* borderline RRDR mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	0.06	0.1	0.12	0.25	0.4	0.5	1	2	5
23) Berrada 2016	23	clinical	3	*rpoB* L430P (L511P)	3								
14) Cambau 2015	1, 4, 10-16	clinical	1	*rpoB* L430P (L511P)									1
20) Whitfield 2018	11		2	*rpoB* L430P (L511P)			2						
16) Jamieson 2014	18	clinical	2	*rpoB* L430P (L511P)				2					
15) Heyckendorf 2018	17	clinical	1	*rpoB* D435Y (D516Y)	1								
17) Sirgel 2013 &	19	clinical	1	*rpoB* D435Y (D516Y)			1						
23) Berrada 2016	23	clinical	4	*rpoB* D435Y (D516Y)	4								
14) Cambau 2015	1, 4, 10-16	clinical	1	*rpoB* D435Y (D516Y)					1				
20) Whitfield 2018	11		2	*rpoB* D435Y (D516Y)			2						
23) Berrada 2016	23	clinical	4	*rpoB* H445L (H526L)			2	1		1			
14) Cambau 2015	1, 4, 10-16	clinical	3	*rpoB* H445L (H526L)		1			1				1
24) Gygli 2019	7	clinical	1	*rpoB* H445L (H526L)		1							
20) Whitfield 2018	11		1	*rpoB* H445L (H526L)							1		
16) Jamieson 2014	18	clinical	2	*rpoB* H445L (H526L)						2			
23) Berrada 2016	23	clinical	3	*rpoB* H445N (H526N)	2		1						
20) Whitfield 2018	11		2	*rpoB* H445N (H526N)			2						
16) Jamieson 2014	18	clinical	1	*rpoB* H445N (H526N)				1					
23) Berrada 2016	23	clinical	3	*rpoB* H445S (H526S)	2		1						
15) Heyckendorf 2018	17	clinical	1	*rpoB* L452P (L533P)						1			
23) Berrada 2016	23	clinical	2	*rpoB* L452P (L533P)			1	1					
24) Gygli 2019	7	clinical	1	*rpoB* L452P (L533P)					1				
20) Whitfield 2018	11		1	*rpoB* L452P (L533P)							1		

The blue line denotes the current CLSI CC for RFB DST in MGIT (0.5 mg/L). Notable limitations: studies RB14 and RB20 were conducted in the same laboratory.

Other *rpoB* RRDR mutants

Nine studies were identified that reported RFB MICs for isolates with *rpoB* RRDR mutations other than the borderline mutations and S450 (S531) codon mutations (Table 62). The MICs reported for these 150 isolates ranged from <0.03 to >8 mg/L, with the modes of some distributions between 0.125 and 0.5 mg/L. At the CLSI CC (i.e. 0.5 mg/L), 93 (62%, 95% CI 54-70%) of these isolates would be classified as RFB-S. If a CC were set at 0.125 mg/L, at least 41 (27%, 95% CI 20-35%) isolates would be classified as RFB-S. An ATU at 0.125 mg/L might lower this substantially to 3% (95% CI 1-8%).

Table 62. RFB MICs for other *rpoB* RRDR mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	0.03	0.06	0.1	0.12	0.25	0.4	0.5	1	2	4	5	8	10
23) Berrada 2016	23	clinical	2	*rpoB* Q432E (Q513E)									2				
23) Berrada 2016	23	clinical	3	*rpoB* Q432K (Q513K)													3
20) Whitfield 2018	11		2	*rpoB* Q432K (Q513K)										2			
23) Berrada 2016	23	clinical	1	*rpoB* Q432L (Q513L)													1
20) Whitfield 2018	11		1	*rpoB* Q432L (Q513L)										1			
23) Berrada 2016	23	clinical	2	*rpoB* Q432P (Q513P)								1	1				
20) Whitfield 2018	11		1	*rpoB* Q432P (Q513P)							1						
20) Whitfield 2018	11		1	*rpoB* D435A (D516A)				1									
23) Berrada 2016	23	clinical	1	*rpoB* D435F (D516F)		1											
17) Sirgel 2013 &	19	clinical	4	*rpoB* D435S (D516S)				4									
17) Sirgel 2013 &	19	clinical	29	*rpoB* D435V (D516V)				20	9								
15) Heyckendorf 2018	17	clinical	1	*rpoB* D435V (D516V)				1									
23) Berrada 2016		clinical	18	*rpoB* D435V (D516V)				2	6		10						
24) Gygli 2019	7	clinical	2	*rpoB* D435V (D516V)						1				1			
14) Cambau 2015	1, 4, 10-16	clinical	10	*rpoB* D435V (D516V)			1				6		2	1			
22) Machado	12	clinical	2	*rpoB* D435V (D516V)							2						
20) Whitfield 2018	11		13	*rpoB* D435V (D516V)				3	9		1						
16) Jamieson 2014	18	clinical	3	*rpoB* D435V (D516V)					1		2						
21) Lin - unpublished	23		1	*rpoB* N438K (N519K)								1					
23) Berrada 2016	23	clinical	1	*rpoB* S441L (S522L)		1											
14) Cambau 2015	1, 4, 10-16	clinical	1	*rpoB* S441L (S522L)						1							
20) Whitfield 2018	11		1	*rpoB* S441L (S522L)				1									
16) Jamieson 2014	18	clinical	3	*rpoB* S441L (S522L)							3						
21) Lin - unpublished	23		1	*rpoB* S441T (S522T)													1
21) Lin - unpublished	23		1	*rpoB* H445A (H526A)		1											
23) Berrada 2016	23	clinical	1	*rpoB* H445A (H526A)				1									
14) Cambau 2015	1, 4, 10-16	clinical	1	*rpoB* H445A (H526A)									1				
23) Berrada 2016	23	clinical	2	*rpoB* H445C (H526C)				2									
23) Berrada 2016	23	clinical	3	*rpoB* H445D (H526D)												2	1
14) Cambau 2015	1, 4, 10-16	clinical	5	*rpoB* H445D (H526D)										5			
24) Gygli 2019	7	clinical	4	*rpoB* H445D (H526D)											4		
20) Whitfield 2018	11		4	*rpoB* H445D (H526D)										4			
16) Jamieson 2014	18	clinical	1	*rpoB* H445D (H526D)											1		
23) Berrada 2016	23	clinical	1	*rpoB* H445G (H526G)				1									
21) Lin - unpublished	23		1	*rpoB* H445P (H526P)	1												
21) Lin - unpublished	23		1	*rpoB* H445Q (H526Q)													1
23) Berrada 2016	23	clinical	5	*rpoB* H445R (H526R)												2	3
20) Whitfield 2018	11		2	*rpoB* H445R (H526R)										2			
23) Berrada 2016	23	clinical	4	*rpoB* H445Y (H526Y)												2	2
14) Cambau 2015	1, 4, 10-16	clinical	3	*rpoB* H445Y (H526Y)										3			
22) Machado	12	clinical	1	*rpoB* H445Y (H526Y)										1			
24) Gygli 2019	7	clinical	2	*rpoB* H445Y (H526Y)											2		
20) Whitfield 2018	11		2	*rpoB* H445Y (H526Y)										2			
16) Jamieson 2014	18	clinical	2	*rpoB* H445Y (H526Y)												2	

The blue line denotes the current CLSI CC for RFB DST in MGIT (0.5 mg/L). Notable limitations: studies RB14 and RB20; studies RB14 and RB22; and studies RB23 and RB21 were conducted in the same laboratory.

rpoB mutants outside the RRDR

Three studies were identified that reported RFB MICs for isolates with mutations outside the *rpoB* RRDR by MGIT (Table 63). The MICs for V170F (V146F) mutants were clearly elevated from the wildtype distribution, with MICs ≥1 mg/L. An ATU at 0.125 mg/L would eliminate the misclassification of the sole I491F (I572F) mutant.

Table 63. RFB MICs for *rpoB* mutants outside of RRDR in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	0.1	0.12	0.25	0.4	0.5	1	2	4
16) Jamieson 2014	17	clinical	1	*rpoB* V170F (V146F)						1		
14) Cambau 2015	1, 4, 9-15	clinical	1	*rpoB* V170F (V146F)							1	
21) Lin - unpublished	22		1	*rpoB* V170F (V146F)						1		
21) Lin - unpublished	22		1	*rpoB* I491F (I572F)		1						

The blue line denotes the current CLSI CC for RFB DST in MGIT (0.5 mg/L).

3.B.4.3 Conclusion for RFB CC for MGIT

Although 0.125 mg/L appeared to be the tentative ECOFF for RFB for this medium (Table 59), this observation was based on limited data and no CC was adopted given that RFB is not currently recommended for the treatment of TB by WHO. Refer to Section 3.3 for a strategy for RFB DST.

Table 64. Effect of adopting a CC for RFB DST on MGIT for detection of *rpoB* mutants.

Mutation type	Percentage of mutants classified RFB-S at		
	CC 0.5 mg/L	CC 0.125 mg/L	CC+ATU 0.125 mg/L
S450 (S531)	1% (95% CI 0-4%)	0% (95% CI 0-2%)	0% (95% CI 0-2%)
borderline RRDR	90% (95% CI 77-97%)	62% (95% CI 46-76%)	33% (95% CI 20-50%)
other RRDR	62% (95% CI 54-70%)	27% (95% CI 20-35%)	3% (95% CI, 1-8%)
borderline I491F (I572F)	not calculated[a]		

The current CLSI CC is underlined.

[a] Too few MICs.

3.B.5 References for RFB MIC studies

1. Rancoita PM V., Cugnata F, Gibertoni Cruz AL, *et al*. Validating a 14-drug microtiter plate containing bedaquiline and delamanid for large-scale research susceptibility testing of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2018;62(9):e00344-18. doi:10.1128/AAC.00344-18.

2. Cavusoglu C, Karaca-Derici Y, Bilgic A. *In-vitro* activity of rifabutin against rifampicin-resistant *Mycobacterium tuberculosis* isolates with known *rpoB* mutations. *Clin Microbiol Infect*. 2004;10(7):662-665. doi:10.1111/j.1469-0691.2004.00917.x.

3. Schön T, Juréen P, Chryssanthou E, *et al*. Rifampicin-resistant and rifabutin-susceptible *Mycobacterium tuberculosis* strains: a breakpoint artefact? *J Antimicrob Chemother*. 2013;68(9):2074-2077. doi:10.1093/jac/dkt150.

4. van Ingen J, Simons S, de Zwaan R, *et al*. Comparative study on genotypic and phenotypic second-line drug resistance testing of *Mycobacterium tuberculosis* complex Isolates. *J Clin Microbiol*. 2010;48(8):2749-2753. doi:10.1128/JCM.00652-10.

5. Schön T, Juréen P, Chryssanthou E, *et al*. Wild-type distributions of seven oral second-line drugs against *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis.* 2011;15(4):502-9. doi:10.5588/ijtld.10.0238.

6. Garcia A-B, Palacios JJ, Ruiz M-J, *et al*. Strong *in vitro* activities of two new rifabutin analogs against multidrug-resistant *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2010;54(12):5363-5365. doi:10.1128/AAC.00149-10.

7. van Ingen J, Simons S, de Zwaan R, *et al*. Comparative study on genotypic and phenotypic second-line drug resistance testing of *Mycobacterium tuberculosis* complex isolates. *J Clin Microbiol*. 2010;48(8):2749-2753. doi:10.1128/JCM.00652-10.

8. Farhat MR, Sixsmith J, Calderon R, Hicks ND, Fortune SM, Murray M. Rifampicin and rifabutin resistance in 1003 *Mycobacterium tuberculosis* clinical isolates. *J Antimicrob Chemother*. 2019;74(6):1477-1483. doi:10.1093/jac/dkz048.

9. Gygli SM, Keller PM, Ballif M, *et al*. Whole-genome sequencing for drug resistance profile prediction in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2019;63(4):e02175-18. doi:10.1128/AAC.02175-18.

10. Rastogi N, Goh KS, Berchel M, Bryskier A. Activity of rifapentine and its metabolite 25-O-desacetylrifapentine compared with rifampicin and rifabutin against *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis* and *M. bovis* BCG. *J Antimicrob Chemother*. 2000;46(4):565-570. doi:10.1093/jac/46.4.565.

11. (a) Anthony RM, Schuitema AR, Bergval IL, *et al*. Acquisition of rifabutin resistance by a rifampicin resistant mutant of *Mycobacterium tuberculosis* involves an unusual spectrum of mutations and elevated frequency. *Ann Clin Microbiol Antimicrob*. 2005;4(1):9. doi:10.1186/1476-0711-4-9.
    (b) Bergval IL, Klatser PR, Schuitema ARJ, Oskam L, Anthony RM. Specific mutations in the *Mycobacterium tuberculosis rpoB* gene are associated with increased *dnaE2* expression. *FEMS Microbiol Lett*. 2007;275(2):338-343. doi:10.1111/j.1574-6968.2007.00905.x.

12. Pretet S, Lebeaut A, Parrot R, *et al*. Combined chemotherapy including rifabutin for rifampicin and isoniazid resistant pulmonary tuberculosis. G.E.T.I.M. (Group for the Study and Treatment of Resistant Mycobacterial Infection). *Eur Respir J.* 1992;5(6):680-4.

13. Truffot-Pernot C, Giroir AM, Grosset J. A study of the minimal inhibitory concentration of rifabutin (ansamycin LM 427) for *Mycobacterium tuberculosis*, *Mycobacterium xenopi* and *Mycobacterium avium-intracellulaire*. *Rev Mal Respir*. 1988;5(4):401-406.

14. Cambau E, Viveiros M, Machado D, *et al*. Revisiting susceptibility testing in MDR-TB by a standardized quantitative phenotypic assessment in a European multicentre study. *J Antimicrob Chemother*. 2015;70(3):686-696. doi:10.1093/jac/dku438.

15. Heyckendorf J, Andres S, Köser CU, *et al*. What is resistance? Impact of phenotypic versus molecular drug resistance testing on therapy for multi- and extensively drug-resistant tuberculosis. *Antimicrob Agents Chemother*. 2017;62(2):e01550-17. doi:10.1128/AAC.01550-17.

16. Jamieson FB, Guthrie JL, Neemuchwala A, *et al*. Profiling of *rpoB* mutations and MICs for rifampin and rifabutin in *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2014;52(6):2157-2162. doi:10.1128/JCM.00691-14.

17. Sharma M, Thibert L, Chedore P, *et al*. Canadian multicenter laboratory study for standardized second-line antimicrobial susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2011;49(12):4112-4116. doi:10.1128/JCM.05195-11.

18. Rüsch-Gerdes S, Pfyffer GE, Casal M, Chadwick M, Siddiqi S. Multicenter laboratory validation of the BACTEC MGIT 960 technique for testing susceptibilities of *Mycobacterium tuberculosis* to classical second-line drugs and newer antimicrobials. *J Clin Microbiol*. 2006;44(3):688-692. doi:10.1128/JCM.44.3.688-692.2006.

19. Whitfield MG, Warren RM, Mathys V, *et al*. The potential use of rifabutin for treatment of patients diagnosed with rifampicin-resistant tuberculosis. *J Antimicrob Chemother*. 2018;73(10):2667-2674. doi:10.1093/jac/dky248

20. Lin G. Personal communication. 2018.

21. Machado D, Perdigão J, Portugal I, *et al.* Efflux activity differentially modulates the levels of isoniazid and rifampicin resistance among multidrug resistant and monoresistant *Mycobacterium tuberculosis* strains. *Antibiotics*. 2018;7(1):18. doi:10.3390/antibiotics7010018.

22. Berrada ZL, Lin S-YG, Rodwell TC, *et al*. Rifabutin and rifampin resistance levels and associated *rpoB* mutations in clinical isolates of *Mycobacterium tuberculosis* complex. *Diagn Microbiol Infect Dis*. 2016;85(2):177-181. doi:10.1016/j.diagmicrobio.2016.01.019.

23. Gygli SM, Keller PM, Ballif M, *et al*. Whole-genome sequencing for drug resistance profile prediction in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2019;63(4):e02175-18. doi:10.1128/AAC.02175-18.

3.C.1 RPT MIC data on LJ

3.C.1.1 RPT MICs for pWT isolates on LJ

No studies were identified that featured RPT MIC data for pWT isolates on LJ medium.

3.C.1.2 RPT MICs for mutated isolates on LJ

No studies were identified that featured RPT MIC data for mutated isolates on LJ medium.

3.C.1.3 Conclusion for RPT CC for LJ

Given the lack of data, a CC for RPT could not be set for this medium. RIF should, instead, be used as a surrogate (Section 3.3).

3.C.2 RPT MIC data on 7H10

3.C.2.1 RPT MICs for pWT isolates on 7H10

Just two studies were identified with RPT MIC data for 7H10 (Table 65). Only one study reported MICs for at least 10 pWT isolates and the MIC distribution was severely truncated at the lower end, precluding an assessment of the shape of the distribution.

Table 65. RPT MICs for pWT isolates on 7H10.

Studies	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16
1) Heifets 1999	lab	1	10	H37Rv		7		3							
	lab	1	1	H37Rv		1									
	lab	1	1	Erdman		1									
	lab	1	1	Atencio		1									
	lab	5	5	Pan-S		3	1		1						
	clinical	85	128	mixture of first-line resistance profiles						93	2		1	4	28
2) Moghazeh 1996	clinical	1	1	Pan-S	gWT							1			

3.C.2.2 RPT MICs for mutated isolates on 7H10

rpoB 450 (531) mutants

Five clinical isolates from 1 study harbored *rpoB* S450 (S531) codon mutations (Table 66). All 5 isolates had MICs >32 mg/L.

Table 66. RPT MICs for *rpoB* S450 (S531) mutants on 7H10.

Studies	Isolate origin	Unique isolates	Genotypic results	0.25	0.5	1	2	4	8	16	32	64
2) Moghazeh 1996	clinical	4	*rpoB* S450L (S531L)									4
	clinical	1	*rpoB* S450W (S531W)									1

rpoB borderline RRDR mutants

Only 4 clinical isolates from 1 study harbored *rpoB* RRDR borderline mutations (Table 67). The isolates were only tested on 7H10, and RPT MICs ranged from 8->32 mg/L.

Table 67. RPT MICs for *rpoB* borderline RRDR mutants on 7H10.

Studies	Isolate origin	Unique isolates	Genotypic results	0.25	0.5	1	2	4	8	16	32	64
	clinical	1	*rpoB* H445N (H526N)							1		
2) Moghazeh 1996	clinical	2	*rpoB* H445L (H526L)						2			
	clinical	1	*rpoB* L452P (L533P)									1

Other rpoB RRDR mutants

Only 8 clinical isolates from 1 study harbored *rpoB* RRDR mutations other than S450 (S531) and the borderline mutations (Table 68). The isolates had RPT MICs that ranged from 16->32 mg/L.

Table 68. RPT MICs for other *rpoB* RRDR mutants on 7H10.

Studies	Isolate origin	Unique isolates	Genotypic results	RPT MIC [mg/L]								
				0.25	0.5	1	2	4	8	16	32	64
	clinical	1	*rpoB* Q432K (Q513K)							1		
2) Moghazeh 1996	clinical	1	*rpoB* Q432L (Q513L)							1		
	clinical	2	*rpoB* H445D (H526D)									2
	clinical	4	*rpoB* H445Y (H526Y)									4

rpoB mutants outside the RRDR

No studies were identified that featured RPT MIC data for isolates with *rpoB* mutations outside the RRDR.

3.C.2.3 Conclusion for RPT CC for 7H10

The available data (Table 65) were insufficient to set even a tentative CC for this medium. RIF should, instead, be used as a surrogate for RPT resistance (Section 3.3).

3.C.3 RPT MIC data on 7H11

3.C.3.1 RPT MICs for pWT isolates on 7H11

Just one study was identified that reported RPT MIC data for the pWT population by 7H11 (Table 69). Rastogi *et al.* (study RP3) reported an untruncated pWT MIC distribution of 0.06-0.5 mg/L for 14 *M. tuberculosis* complex isolates, with a mode of 0.125 mg/L. The authors also tested the H37Rv control strain, with an MIC of 0.25 mg/L, *M. africanum* ATCC 25420 with an MIC of 0.25 mg/L, and six *M. bovis* isolates, with MICs ≤0.015-0.125 mg/L.

Table 69. RPT MICs for pWT isolates on 7H11.

Studies	Isolate origin	Unique isolates	Type of isolates	RPT MIC [mg/L]						
				0.02	0.03	0.06	0.12	0.25	0.5	1
		1	H37Rv					1		
		1	M. africanum ATCC 25420					1		
		1	M. bovis ATCC 19210				1			
3) Rastogi 2000		5	M. bovis BCG	2	1	2				
		6	Pan-S			1	5			
	clinical	4	M. africanum				1	2	1	
		4	M. bovis					2	2	

3.C.3.2 RPT MICs for mutated isolates on 7H11

No studies were identified that featured RPT MIC data for mutated isolates on 7H11 medium.

3.C.3.3 Conclusion for RPT CC for 7H11

Given that only a single study was identified by this review (Table 69), a RPT CC could not be set for this medium. RIF should, instead, be used as a surrogate for RPT resistance (Section 3.3).

3.C.4 RPT MIC data in MGIT

3.C.4.1 RPT MICs for pWT isolates in MGIT
No studies were identified that featured RPT MIC data for pWT isolates tested by MGIT.

3.C.4.2 RPT MICs for mutated isolates in MGIT
No studies were identified that featured RPT MIC data for mutated isolates tested by MGIT.

3.C.4.3 Conclusion for RPT CC for MGIT
Given the lack of data, a CC for RPT could not be set for this medium. RIF should, instead, be used as a surrogate for RPT DST (Section 3.3).

3.C.5 References for RPT MIC studies

1. Heifets L, Sanchez T, Vanderkolk J, Pham V. Development of rifapentine susceptibility tests for *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 1999;43(1):25-28. doi:10.1128/AAC.43.1.25.
2. Moghazeh SL, Pan X, Arain T, *et al*. Comparative antimycobacterial activities of rifampin, rifapentine, and KRM-1648 against a collection of rifampin-resistant *Mycobacterium tuberculosis* isolates with known *rpoB* mutations. *Antimicrob Agents Chemother*. 1996;40(11):2655-2657. doi:10.1128/AAC.40.11.2655.
3. Rastogi N, Goh KS, Berchel M, Bryskier A. Activity of rifapentine and its metabolite 25-O-desacetylrifapentine compared with rifampicin and rifabutin against *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis* and *M. bovis* BCG. *J Antimicrob Chemother*. 2000;46(4):565-570. doi:10.1093/jac/46.4.565.

3.3 Rifamycin conclusions and comments

Rifampicin

The six RRDR and the I491F (I572F) borderline resistance mutations (Table 26) have been the focus of considerable debate in the literature, particularly in light of the role of L452P (L533P) in KwaZulu-Natal, South Africa, and in the clonal spread of I491F (I572F) in Eswatini.[88, 89] Specifically, there is uncertainty as to why these mutations are more likely to test susceptible by pDST and whether they are clinically relevant at the currently recommended RIF dose of 10 mg/kg (8-12 mg/kg) daily (the 600 mg daily cap was removed in 2017 to improve dosing in higher body-weight bands).[90,91]

This systematic review revealed that one reason for the discordant results for borderline resistance mutations was that the CCs for 7H10 and MGIT had been set too high for the pWT population, which was corrected by lowering these CCs to the ECOFFs of 0.5 mg/L. However, this only reduces rather than eliminates the discordance between genotype and phenotype, as borderline resistance mutations are typically associated with only modest MIC increases. Specifically, their MIC distributions usually overlap with the MIC distributions of pWT isolates, which means that the ECOFF for RIF intersects these MIC distributions, resulting in a poor reproducibility with categorical pDST (i.e. even if the same isolate is tested in the same laboratory, susceptibility results may vary due to the inevitable technical variation in pDST). This issue applies to all media, including the WHO-endorsed microscopic observation of drug susceptibility and the nitrate reductase assay, although the problem is particularly pronounced with MGIT, which may be due to the lower fitness of isolates with these mutations and the shorter incubation time of MGIT.[92]

Clinical outcome data for the seven borderline resistance mutations, particularly I491F (I572F), are limited and it is unclear whether these associations may be confounded. For example, some isolates with borderline resistance mutations have very high RIF MICs, which could be due to any of the following reasons. A sample might be mixed and also harbour a subpopulation with a HLR mutation, such as *rpoB* S450L (S531L), below the limit of detection of the gDST method employed.[93] Alternatively, secondary mutations in *rpoA*, *rpoB*, *rpoC*, or elsewhere in the genome may raise the MIC of a given

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[88] Brown TS, Challagundla L, Baugh EH, *et al*. Pre-detection history of extensively drug-resistant tuberculosis in KwaZulu-Natal, South Africa. *Proc Natl Acad Sci U S A*. 2019;116(46):23284-23291. doi:10.1073/pnas.1906636116.

[89] Sanchez-Padilla E, Merker M, Beckert P, *et al*. Detection of drug-resistant tuberculosis by Xpert MTB/RIF in Swaziland. *N Engl J Med*. 2015;372(12):1181-1182. doi:10.1056/NEJMc1413930.

[90] World Health Organization. Guidelines for treatment of drug-susceptible tuberculosis and patient care. 2017 update. Annex 6. Essential first-line antituberculosis drugs. (https://www.who.int/tb/publications/2017/tb_guidelines2017_annex6_en_v4.pdf, accessed 18 September 2020)

[91] World Health Organization. Technical report on the pharmacokinetics and pharmacodynamics (PK/PD) of medicines used in the treatment of drug-resistant tuberculosis. (http://apps.who.int/iris/bitstream/handle/10665/260440/WHO-CDS-TB-2018.6-eng.pdf, accessed 15 September 2018).

[92] Torrea G, Ng K, Van Deun A. Variable ability of rapid tests to detect *Mycobacterium tuberculosis rpoB* mutations conferring phenotypically occult rifampicin resistance. *Sci Rep*. 2019;9(1):11826. doi:10.1038/s41598-019-48401-z.

[93] Ng KCS, Supply P, Cobelens FGJ, *et al*. How well do routine molecular diagnostics detect rifampin heteroresistance in *Mycobacterium tuberculosis*? *J Clin Microbiol*. 2019;57(11):e00717-19. doi:10.1128/JCM.00717-19.

isolate well above the CC.[94,95,96] Nevertheless, the available evidence points towards worse treatment outcomes for at least some of these mutations.[97,98,99,100,101,102,103,104] In fact, the poor treatment outcomes attributed to INH mono-resistance are likely partly due to missed RIF resistance due to the presence of these borderline resistance mutations.[105]

In addition, the 10 mg/kg/day dose represents the minimal effective dose for RIF, which was chosen historically because of the limited supply and high cost of the drug and fear for dose-related adverse events.[106,107] This dose is likely insufficient for a proportion of patients infected with pWT isolates and, thus, risks the selection of RIF resistance, particularly in light of substantial PK variability.[108,109] Further, RIF displays profound concentration-dependent killing, which is not activated by 10 mg/kg/day.[110] As a result, several trials are evaluating higher doses.[111] Because RIF is likely not optimally dosed even for pWT isolates, any MIC increase caused by *rpoB* mutations must, by definition, increase the proportion of patients who are not optimally treated.

[94] Zaczek A, Brzostek A, Augustynowicz-Kopec E, Zwolska Z, Dziadek J. Genetic evaluation of relationship between mutations in *rpoB* and resistance of *Mycobacterium tuberculosis* to rifampin. *BMC Microbiol*. 2009;9:10. doi:10.1186/1471-2180-9-10.

[95] Meftahi N, Namouchi A, Mhenni B, *et al*. Evidence for the critical role of a secondary site *rpoB* mutation in the compensatory evolution and successful transmission of an MDR tuberculosis outbreak strain. *J Antimicrob Chemother*. 2016;71(2):324-332. doi:10.1093/jac/dkv345.

[96] Shea J, Halse TA, Kohlerschmidt D, *et al*. Low-level rifampin resistance and *rpoB* mutations in *Mycobacterium tuberculosis*: An analysis of whole-genome sequencing and drug susceptibility test data in New York. *J Clin Microbiol*. 2020 Online ahead of print. doi:10.1128/JCM.01885-20.

[97] Williamson DA, Roberts SA, Bower JE, *et al*. Clinical failures associated with *rpoB* mutations in phenotypically occult multidrug-resistant *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis*. 2012;16(2):216-220. doi:10.5588/ijtld.11.0178.

[98] Ho J, Jelfs P, Sintchencko V. Phenotypically occult multidrug-resistant *Mycobacterium tuberculosis*: dilemmas in diagnosis and treatment. *J Antimicrob Chemother*. 2013;68(12):2915-2920. doi:10.1093/jac/dkt284.

[99] Van Deun A, Aung KJ, Bola V, *et al*. Rifampin drug resistance tests for tuberculosis: challenging the gold standard. *J Clin Microbiol*. 2013;51(8):2633-2640. doi:10.1128/JCM.00553-13.

[100] Pang Y, Ruan YZ, Zhao J, *et al*. Diagnostic dilemma: treatment outcomes of tuberculosis patients with inconsistent rifampicin susceptibility. *Int J Tuberc Lung Dis*. 2014;18(3):357-362. doi:10.5588/ijtld.13.0459.

[101] Van Deun A, Aung KJ, Hossain A, et al. Disputed *rpoB* mutations can frequently cause important rifampicin resistance among new tuberculosis patients. *Int J Tuberc Lung Dis*. 2015;19(2):185-190. doi:10.5588/ijtld.14.0651.

[102] Shah N, Lin SYG, Barry PM, Cheng YN, *et al*. Clinical impact on tuberculosis treatment outcomes of discordance between molecular and growth-based assays for rifampin resistance, California 2003-2013. *Open Forum Infect Dis*. 2016;3(3):ofw150. doi:10.1093/ofid/ofw150.

[103] Hu P, Zhang H, Fleming J, *et al*. Retrospective analysis of false-positive and disputed rifampin resistance Xpert MTB/RIF assay results in clinical samples from a referral hospital in Hunan, China. *J Clin Microbiol*. 2019;57(4):e01707-18. doi:10.1128/JCM.01707-18.

[104] van Ingen J, Aarnoutse R, de Vries G, Boeree MJ, van Soolingen D. Low-level rifampicin-resistant *Mycobacterium tuberculosis* strains raise a new therapeutic challenge. *Int J Tuberc Lung Dis*. 2011;15(7):990-992. doi:10.5588/ijtld.10.0127.

[105] Van Deun A, Decroo T, Kya Jai Maug A, *et al*. The perceived impact of isoniazid resistance on outcome of first-line rifampicin-throughout regimens is largely due to missed rifampicin resistance. *PLoS One*. 2020;15(5):e0233500. doi:10.1371/journal.pone.0233500.

[106] van Ingen J, Aarnoutse RE, Donald PR, et al. Why Do we use 600 mg of rifampicin in tuberculosis treatment? *Clin Infect Dis*. 2011;52(9):e194-e199. doi:10.1093/cid/cir184.

[107] Maug AKJ, Hossain MA, Gumusboga M, *et al*. First-line tuberculosis treatment with double-dose rifampicin is well tolerated. *Int J Tuberc Lung Dis*. 2020;24(5):499-505. doi:10.5588/ijtld.19.0063.

[108] Gumbo T, Louie A, Deziel MR, *et al*. Concentration-dependent *Mycobacterium tuberculosis* killing and prevention of resistance by rifampin. Antimicrob Agents Chemother. 2007;51(11):3781-3788. doi:10.1128/AAC.01533-06.

[109] Stott KE, Pertinez H, Sturkenboom MGG, *et al*. Pharmacokinetics of rifampicin in adult TB patients and healthy volunteers: a systematic review and meta-analysis. *J Antimicrob Chemother*. 2018;73(9):2305-2313. doi:10.1093/jac/dky152.

[110] Peloquin CA, Velásquez GE, Lecca L, *et al*. Pharmacokinetic evidence from the HIRIF trial to support increased doses of rifampin for tuberculosis. *Antimicrob Agents Chemother*. 2017;61(8):e00038-17. doi:10.1128/AAC.00038-17.

[111] Velásquez GE, Brooks MB, Coit JM, *et al*. Efficacy and safety of high-dose rifampin in pulmonary tuberculosis. A randomized controlled trial. *Am J Respir Crit Care Med*. 2018;198(5):657-666. doi:10.1164/rccm.201712-2524OC.

Given this direct and indirect evidence and to err on the side of caution, the TEG recommended that all seven mutations should be regarded as clinically relevant for the current dose of RIF (i.e. they should be treated with an MDR-TB regimen according to the least treatment guidelines and are, therefore, best referred to as "borderline resistance" mutations rather than "disputed", "discordant" or "occult" to underline their clinical importance and that they cannot be confirmed reliably using pDST).[112] This decision will be reassessed when more evidence regarding the clinician relevance of these mutations with the current dose becomes available. It was beyond the remit of this review to assess the role of therapeutic drug monitoring or whether a higher of RIF can overcome the MIC increase typically associated with these borderline resistance mutations.[98,106,113,114] However, these questions will become a priority should WHO endorse a higher dose of RIF in the future.

The TEG considered two approaches to minimize the misclassification of borderline resistance mutations by pDST. First, Torrea *et al*. found that when the incubation period of MGIT is extended, the MICs of isolates with borderline resistance mutations increased more than those of pWT isolates (i.e. the overlap between the MIC distributions is reduced, resulting in an improved ability to resolve both populations).[92] However, any pDST methodology change would require an extensive revalidation of MGIT prior to implementation (e.g. the CC may have to be adjusted), which means that this option would not be feasible in the short term. Second, several experts noted that adopting ATUs by testing two concentrations rather than one (i.e. the CC and the concentration below) would not be currently feasible in most settings.[115]

In light of these limitations of pDST, the TEG endorsed the following expert rules:
1. gDST is the only reliable way of detecting RIF resistance caused by borderline mutations, which means that countries that do not conduct routine gDST as the initial test using an appropriately validated method risk missing these likely clinically relevant mechanisms of resistance.
2. Because pDST is not a reliable confirmatory method, there is little value in conducting pDST for RIF if a borderline resistance mutation is specifically detected using gDST upfront. Therefore, pDST is not needed in these cases. If pDST is unavoidable, the detection of one of these mutations by sequencing or a mutant probe should overrule a susceptible pDST result at the CC after a review of all results to rule out obvious laboratory or clerical errors (i.e. if there is a concern regarding the positive predictive value of the genotypic result, gDST, ideally using an alternative method, is the

[112] World Health Organization. WHO consolidated guidelines on tuberculosis. Module 4: treatment – drug-resistant tuberculosis treatment (https://apps.who.int/iris/rest/bitstreams/1280998/retrieve, accessed 15 June 2020).

[113] Jeong DH, Kang YW, Kim JY, *et al*. Successful treatment with a high-dose rifampin-containing regimen for pulmonary tuberculosis with a disputed *rpoB* mutation. *Intern Med*. 2018;57(22):3281-3284. doi:10.2169/internalmedicine.9571-17.

[114] van den Elsen SHJ, Akkerman OW, Wessels M, *et al*. Dose optimisation of first-line tuberculosis drugs using therapeutic drug monitoring in saliva: feasible for rifampicin, not for isoniazid. *Eur Respir J*. 2020 Oct 22;56(4):2000803. doi:10.1183/13993003.00803-2020.

[115] European Committee on Antimicrobial Susceptibility Testing. Area of Technical Uncertainty (ATU) in antimicrobial susceptibility testing (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Area_of_Technical_Uncertainty_-_guidance_2019-1.pdf, accessed 15 February 2020).

appropriate confirmatory approach). [116,117] In other words, the genotype is designated as the reference standard for borderline resistance mutations.

3. Because additional *rpoB* mutations likely exist that are equally difficult to detect using pDST as the seven borderline resistance mutations defined in this review (Table 26) and have the same clinical consequences, the TEG reaffirmed the expert rule that any mutation in RRDR, apart from synonymous mutations, should be assumed to confer RIF resistance, as stated in the current technical manual for DST (i.e. even if these mutations are novel).[118] The possibility that some mutations in RRDR are neutral and do not affect the MIC at all and/or have no adverse clinical consequences was acknowledged, but to confirm this possibility conclusively, MIC testing would have to be carried out for multiple replicates of the mutant in question along with an on-scale quality control strain and measures would have to be taken to rule out other confounders (e.g. low-frequency resistance mutations missed by the initial gDST method).[94,119,120] This is not possible as part of routine clinical care but should be prioritised by reference laboratories for novel mutations and mutations that increase in frequency over time. Moreover, sufficiently powered studies would have to be conducted to investigate the clinical impact of such mutations. Depending on the outcomes of such studies, WHO will modify this expert rule (e.g. by excluding individual RRDR mutations[96]).

Finally, the TEG agreed that WHO should provide more practical guidance to implement these expert rules, and to explain the possible reasons for discordant DST results (e.g. because not all gDST assays interrogate the same parts of *rpoB* (Table 27), and to address the possibility of systematic errors due to synonymous mutations).[121] In this context, it was emphasized that laboratories ensure that they follow the latest interpretation guides for gDST assays. For instance, Hain recently updated under which conditions "WT" bands are interpreted as negative to minimize false-susceptible results due to the presence of borderline resistance mutations.[122] This change is already included in the ELI interpretation guide but has yet to be recognized by GLI.[116,123]

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[116] World Health Organization. Regional Office for Europe. Interpretation guide for GenoType MTBDR*plus* VER 2.0 and GenoType MTBDR*sl* VER 2.0. A technical guidance document developed by the European Laboratory Initiative. Version 1.0. (https://openwho.org/courses/multi-drug-resistant-tb, accessed 9 May 2020).

[117] Miotto P, Cabibbe AM, Borroni E, Degano M, Cirillo DM. Role of disputed mutations in the *rpoB* gene in interpretation of automated liquid MGIT culture results for rifampin susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2018;56(5):e01599-17. doi:10.1128/JCM.01599-17.

[118] World Health Organization. Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. (http://apps.who.int/iris/bitstream/handle/10665/275469/9789241514842-eng.pdf, accessed 17 November 2018).

[119] Jagielski T, Bakuła Z, Brzostek A, *et al*. Characterization of mutations conferring resistance to rifampin in *Mycobacterium tuberculosis* clinical strains. *Antimicrob Agents Chemother*. 2018;62(10):e01093-18. doi:10.1128/AAC.01093-18.

[120] Schön T, Matuschek E, Mohamed S, *et al*. Standards for MIC testing that apply to the majority of bacterial pathogens should also be enforced for *Mycobacterium tuberculosis* complex. *Clin Microbiol Infect*. 2019;25(4):403-405. doi:10.1016/j.cmi.2019.01.019.

[121] Omar SV, Hillemann D, Pandey S, *et al*. Systematic rifampicin resistance errors with Xpert® MTB/RIF Ultra: implications for regulation of genotypic assays. *Int J Tuberc Lung Dis*. 2020;24(12):1307-1311. doi:10.5588/ijtld.20.0396.

[122] Hain Lifescience. GenoType MTBDR*plus* VER 2.0. Instructions for use. IFU-304A-09. (https://www.hain-lifescience.de/include_datei/kundenmodule/packungsbeilage/download.php?id=2877, accessed 1 Nov 2020).

[123] Global Laboratory Initiative. Line probe assays for drug-resistant tuberculosis detection: interpretation and reporting guide for laboratory staff and clinicians; 2019 (http://www.stoptb.org/wg/gli/assets/documents/LPA_test_web_ready.pdf, accessed 22 May 2020).

Rifabutin

RFB is not currently recommended by WHO for the treatment of active or latent TB.[112,124] Therefore, the TEG did not set a CC for this rifamycin. Nevertheless, some researchers are of the opinion that RFB is effective for specific *rpoB* mutations that confer resistance to RIF.[125] The most important example is D435V (D516V), as this mutation can be differentiated from other *rpoB* mutations using the Hain and Nipro LPAs (Table 27).[126] This and other *rpoB* mutations appear to confer lower relative MIC increases for RFB than for RIF. Indeed, the mode of D435V (D516V) for RIF is approximately 20 times higher than the newly endorsed CC of 0.5 mg/L in MGIT (Table 47), whereas the mode of D435V (D516V) for RFB is identical to or only slightly above the tentative ECOFF of 0.125 mg/L (Table 62). Consequently, isolates with this mutation are still clearly pNWT and are only classified as RFB-S (i.e. treatable with RFB) because the CLSI CC of 0.5 mg/L is 4 times higher than the tentative ECOFF in MGIT of 0.125 mg/L.[127] The same applies for 7H10, where the CLSI CC of 0.5 mg/L is 4-8 times higher than the tentative RFB ECOFF (i.e. 0.06 or 0.125 mg/L).[128,129]

The rationale used by CLSI to set the CCs for RFB at 0.5 mg/L is unclear, but the work by Heifets *et al*. likely influenced this decision.[130] Heifets *et al*. had originally proposed 0.125 mg/L as the RFB CC for 7H10, 7H11 and the radiometric BACTEC™ 460 (BACTEC) method with 7H12 medium given that 17 RIF-S isolates had RFB MICs ≤0.06 mg/L (0.125 rather than 0.06 mg/L was chosen to buffer for potential methodological differences).[131,132] Moreover, the authors raised the possibility that pNWT isolates with only slight MIC increases might be treatable in light of the serum concentrations and concentrations in selected tissues achievable with RFB. However, the MICs that the authors considered to be associated with "moderately susceptible" isolates changed over time (i.e. they narrowed their original range of 0.25-0.5 mg/L for BACTEC, which they found to yield equivalent MICs to 7H10 and 7H11, to just 0.25 mg/L).[131,132] A subsequent study by Pfyffer *et al*., which is also cited by CLSI, only tested 0.5 mg/L as the lowest concentration for 7H10 and did not consider any *rpoB* sequencing information.[133] Therefore, this publication provided no insight into the shape of the pWT MIC distribution and the authors provided few details to justify their choice of 1 mg/L as the RFB CC for 7H10. Similar limitations apply to the subsequent study by Rüsch-Gerdes *et al*. that informed the

[124] World Health Organization. WHO consolidated guidelines on tuberculosis. Module 1: prevention – tuberculosis preventive treatment (https://apps.who.int/iris/rest/bitstreams/1270183/retrieve, accessed 24 May 2020).

[125] Domínguez J, Boettger EC, Cirillo D, *et al*. Clinical implications of molecular drug resistance testing for *Mycobacterium tuberculosis*: a TBNET/RESIST-TB consensus statement. *Int J Tuberc Lung Dis* 2016;20(1):24-42. doi:10.5588/ijtld.15.0221.

[126] Williams DL, Spring L, Collins L, *et al*. Contribution of *rpoB* mutations to development of rifamycin cross-resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 1998;42(7):1853-7. doi:10.1128/AAC.42.7.1853.

[127] Heyckendorf J, Andres S, Köser CU, *et al*. What is resistance? Impact of phenotypic versus molecular drug resistance testing on therapy for multi- and extensively drug-resistant tuberculosis. *Antimicrob Agents Chemother*. 2017;62(2):e01550-17. doi:10.1128/AAC.01550-17.

[128] Ängeby K, Juréen P, Kahlmeter G, Hoffner S, Schön T. Challenging a dogma: antimicrobial susceptibility testing breakpoints for *Mycobacterium tuberculosis*. *Bull World Health Organ*. 2012. doi:10.2471/blt.11.096644.

[129] Schön T, Juréen P, Chryssanthou E, *et al.* Rifampicin-resistant and rifabutin-susceptible *Mycobacterium tuberculosis* strains: a breakpoint artefact? *J Antimicrob Chemother*. 2013;68(9):2074-2077. doi:10.1093/jac/dkt150.

[130] Clinical & Laboratory Standards Institute. Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 3rd edition approved standard. CLSI Document M24; 2018.

[131] Heifets LB, Lindholm-Levy PJ, Iseman MD. Rifabutine: minimal inhibitory and bactericidal concentrations for *Mycobacterium tuberculosis*. *Am Rev Respir Dis*. 1988;137(3):719-721. doi:10.1164/ajrccm/137.3.719.

[132] Heifets LB. Drug susceptibility tests in the management of chemotherapy of tuberculosis. In Heifets LB, ed. *Drug susceptibility in the chemotherapy of mycobacterial infections*. CRC Press; 1991.

[133] Pfyffer GE, Bonato DA, Ebrahimzadeh A, *et al*. Multicenter laboratory validation of susceptibility testing of Mycobacterium tuberculosis against classical second-line and newer antimicrobial drugs by using the radiometric BACTEC 460 technique and the proportion method with solid media. *J Clin Microbiol*. 1999;37(10):3179-3186. doi:10.1128/JCM.37.10.3179-3186.1999.

MGIT CC of 0.5 mg/L (i.e. the lowest concentration tested was only 0.25 mg/L and no *rpoB* results were considered).[134] Thus, there appears to be no strong evidence to support the RFB CCs set by CLSI.

It is possible that a minority of *rpoB* mutations (e.g. some borderline RIF resistance mutations) have no clinically relevant effect for RFB. Yet, until sufficiently powered studies have been conducted to demonstrate this, the cautious approach would be to assume full cross-resistance with RIF. [135] Specifically, gDST and pDST results for RIF instead of RFB would maximize the detection of *rpoB* mutations (i.e. in the same way that TEG recommends RIF as a surrogate for RPT).

Finally, some experts noted that even if some *rpoB* mutations that were easily identifiable using gDST could be treated with RFB, pDST would still be needed to exclude unusually high RFB MICs (e.g. although the MICs for D435V (D516V) are typically below the current CLSI CC, isolates with considerably higher MICs exist (Table 53)). For this reason, trial NCT02236078, which was designed to investigate the potential utility of RFB and is due to complete at the end of 2020, relies on direct pDST to generate timely results.[136]

Rifapentine

RPT is currently recommended only for the preventative treatment of TB.[112,124] Given the lack of available MIC data for this antibiotic, the TEG recommended that complete cross-resistance with RIF should be assumed until sufficient data to the contrary are available (i.e. gDST and pDST results for RIF should be used as the surrogate for RPT).[126,137] This point will be reevaluated based on the findings of the phase 3 NCT02410772 trial, which investigates whether the treatment of drug-susceptible TB can be shortened by replacing RIF with high-dose RPT.[138]

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[134] Rüsch-Gerdes S, Pfyffer GE, Casal M, Chadwick M, Siddiqi S. Multicenter laboratory validation of the BACTEC MGIT 960 technique for testing susceptibilities of *Mycobacterium tuberculosis* to classical second-line drugs and newer antimicrobials. *J Clin Microbiol*. 2006;44(3):688-692. doi:10.1128/JCM.44.3.688-692.2006.

[135] Weiner M, Benator D, Burman W, *et al*. Association between acquired rifamycin resistance and the pharmacokinetics of rifabutin and isoniazid among patients with HIV and tuberculosis. *Clin Infect Dis*. 2005;40(10):1481-1491. doi:10.1086/429321.

[136] Click ES, Kurbatova EV, Alexander H, *et al*. Isoniazid and rifampin-resistance mutations associated with resistance to second-line drugs and with sputum culture conversion. *J Infect Dis*. 2020;221(12):2072-2082. doi:10.1093/infdis/jiaa042.

[137] Moghazeh SL, Pan X, Arain T, *et al*. Comparative antimycobacterial activities of rifampin, rifapentine, and KRM-1648 against a collection of rifampin-resistant *Mycobacterium tuberculosis* isolates with known *rpoB* mutations. *Antimicrob Agents Chemother*. 1996;40(11):2655-2657. doi:10.1128/AAC.40.11.2655.

[138] Dorman SE, Nahid P, Kurbatova EV, *et al*. High-dose rifapentine with or without moxifloxacin for shortening treatment of pulmonary tuberculosis: study protocol for TBTC Study 31/ACTG A5349 phase 3 clinical trial. *Contemp Clin Trials*. 2020;90:105938. doi:10.1016/j.cct.2020.105938.