

Drug Susceptibility
Testing Program

MARCH 2025



Model Performance Evaluation Program Report of Results, March 2025

# Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for March 2025 Panel

#### **Purpose**

To present results of the U.S. Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP) for Mycobacterium tuberculosis complex (MTBC) drug susceptibility testing panel sent to participants in March 2025.

### **Report Content**

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Model Performance Evaluation Program (MPEP) | Tuberculosis (TB) | CDC

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Note on Accessibility: Find descriptions and explanations of figures in Appendix 1: Accessible Explanation of Figures on page 34.

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# **Technical Notes**

The following information pertains to all tables and figures for the 2025 MTBC isolates A, B, C, D, and E included in this report.

- The source of data in all tables and figures is the March 2025 MPEP MTBC DST panel.
- First-line and second-line drugs have been separated into individual tables for each isolate. Streptomycin is classified as a second-line drug for this report.
- Separate tables for molecular testing are included.
- Mutations of the rpoB gene are noted with the M. tuberculosis numbering system.
- Laboratories that use more than one DST method are encouraged to test isolates with each of
  the available methods and equivalent critical concentrations. Some laboratories have provided
  results for multiple DST methods. Consequently, the number of results for some drugs may be
  greater than the number of participating laboratories. This report contains all results reported
  by participating laboratories.
- The Sensititre® system allows determination of a MIC for each drug in the panel. Laboratories using this method may establish breakpoints individually, for some drugs, to provide a categorical interpretation of S or R.
- For participant result tables that have drug-method totals equal to 0, results were not received.
- Although data were collected for rifapentine, delamanid, and pretomanid, no laboratories reported growth-based testing for these drugs. Therefore, these drugs were not included in growth-based tables of participants' results.
- Due to a 2024 manufacturer recall, BD MGIT™ PZA susceptibility test kits were not widely available during the March 2025 MPEP panel testing timeframe. The number of reported results for this method and drug was lower than typically observed, reflecting laboratories' inability to perform testing.

# Abbreviations and Acronyms

Acronym	Definition
AMK	amikacin
AP	agar proportion — performed on Middlebrook 7H10 or 7H11
BDQ	Bedaquiline
CAP	capreomycin
CDC	U.S. Centers for Disease Control and Prevention
CIP	ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CYS	cycloserine
DNA	deoxyribonucleic acid
DST	drug susceptibility testing
EMB	ethambutol
ETA	ethionamide
FQ	fluoroquinolone
INH	isoniazid
KAN	kanamycin
LVX	levofloxacin
MDR	multidrug-resistant
MGIT™	BACTEC™ MGIT™ – Mycobacteria Growth Indicator Tube
MIC	minimum inhibitory concentration
MOX	moxifloxacin
MPEP	Model Performance Evaluation Program
MTBC	Mycobacterium tuberculosis complex
PAS	p-aminosalicylic acid
PZA	pyrazinamide
OFL	ofloxacin
R	resistant
RBT	rifabutin
RIF	rifampin
RNA	ribonucleic acid
S	susceptible
Sensititre®	Thermo Scientific Sensititre® MYCOTB AST or customized plate
STR	streptomycin
ТВ	tuberculosis
VersaTREK™	Thermo Scientific VersaTREK™ Myco susceptibility
XDR	extensively drug-resistant

# Introduction: Overview of MPEP Final Report

The Model Performance Evaluation Program (MPEP) is an educational, self-assessment tool in which five isolates of *M. tuberculosis* complex (MTBC) are sent to participating laboratories biannually for staff to monitor their ability to determine drug resistance among the isolates. It is not a formal, graded proficiency testing program. The associated report includes results for a subset of laboratories performing drug susceptibility testing (DST) for MTBC in the United States. MPEP is a voluntary program, and this report reflects data received from participating laboratories. This aggregate report is prepared in a format that will allow comparison of DST results with those obtained by other participants using the same methods and drugs, for each isolate. We encourage circulation of this report to personnel who are either involved with DST or reporting and interpreting results for MTBC.

CDC is neither recommending nor endorsing testing practices reported by participants. For standards, participants should refer to consensus documents published by the Clinical and Laboratory Standards Institute (CLSI), "M24: Susceptibility Testing of Mycobacteria, *Nocardia* spp., and Other Aerobic Actinomycetes" and "M24S: Performance Standards for Susceptibility Testing of Mycobacteria, *Nocardia* spp., and Other Aerobic Actinomycetes" [1, 2]. Additionally, the World Health Organization (WHO) published two technical reports investigating critical concentrations, by method, for anti-tuberculosis drugs [3, 4].

# **Expected Drug Susceptibility Testing Results**

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in March 2025 are shown in the tables below. Although CDC recommends broth-based methods for routine first-line DST of MTBC isolates, the results obtained by the reference agar proportion method (except for pyrazinamide, in which MGIT™ was performed, when possible) are shown in Table 1. Molecular results obtained by whole genome sequencing are listed in Table 2.

Table 1. Expected Growth-based Results for March 2025 Panel

Isolate	RIF	INH	EMB	PZA	FQ	Second-line Drug Resistances:
2025A	S	S	S	No Result†	R	
2025B	S	R (low-level*)	S	S	S	ETA§
2025C	S	S	R§	S	S	STR <sup>§</sup>
2025D	S	S	S	S	S	AMK, KAN, CAP
2025E	R <sup>§</sup>	S	S	S	S	

Note: RIF=rifampin, INH=isoniazid, EMB=ethambutol, PZA=pyrazinamide, FQ=fluoroquinolones, ETA=ethionamide, STR=streptomycin, AMK=amikacin, KAN=kanamycin, CAP=capreomycin, S=susceptible, R=resistant. Growth-based DST results were obtained by indirect agar proportion method, except for pyrazinamide, for which MGIT™ was performed.

Table 2. Expected Molecular Results (Mutations Detected in Loci Associated with Resistance) for March 2025 Panel

			ı	ı		
Isolate	rpoB*	inhA	embB	gyrA	rrs	mmpR
2025A†				Asp94Gly		
2025B		C-15T				
2025C <sup>§</sup>			Gln497Arg			
2025D					A1401G	Asp141fs <sup>¶</sup>
2025E	His445Leu					

Note: Empty cell=No mutation detected. Molecular results were obtained by whole genome sequencing. High confidence mutations were not detected in these loci: katG, fabG1, pncA, ethA, eis, and tlyA.

<sup>\*</sup> Resistant at 0.2 μg/ml by agar proportion. See Equivalent Critical Concentration table on page 8 for more information.

<sup>&</sup>lt;sup>†</sup> No result available as growth-based testing for pyrazinamide was not performed for this isolate. See Expected Molecular Result table below.

<sup>&</sup>lt;sup>6</sup> 80% consensus for a single categorical result across all methods reported for this drug of susceptible or resistant was not achieved for these isolates among participating laboratories.

<sup>\*</sup> M. tuberculosis numbering system used [5, 6].

<sup>&</sup>lt;sup>†</sup> No mutation detected in *pncA*; consistent with likely PZA susceptible.

 $<sup>^{\</sup>S}$  Multiple mutations detected in katG for this isolate but none with a known role in isoniazid resistance.

<sup>¶</sup>Asp141fs mutation detected in *mmpR* with unknown significance.

# **Equivalent Critical Concentrations**

(Concentrations listed as µg/ml)

# **Agar Proportion**

First-line Drugs	7H10 agar	7H11 agar
Isoniazid	0.2 and 1.0*	0.2 and 1.0*
Rifampin	0.5†	1.0
Ethambutol	5.0	7.5
Pvrazinamide	Not recommended	Not recommended

NOTE: Critical concentrations as indicated in CLSI M24 document, unless otherwise stated [1].

 $<sup>^\</sup>dagger$ WHO recommended critical concentration of 0.5 µg/ml differs from CLSI critical concentration of 1.0 µg/ml for RIF [1, 4].

Second-line Drugs	7H10 agar	7H11 agar
Streptomycin	2.0	2.0
Levofloxacin	1.0	Not determined
Moxifloxacin	0.5	0.5
Amikacin	4.0*	Not determined
Capreomycin	10.0*	10.0 <sup>†</sup>
Kanamycin	5.0*	6.0 <sup>†</sup>
Ethionamide	5.0	10.0
Rifabutin	0.5	0.5
p-Aminosalicylic acid	2.0 <sup>†</sup>	8.0 <sup>†</sup>
Rifapentine	Not determined®	Not determined <sup>¶</sup>
Bedaquiline	Not determined®	0.25 <sup>§</sup>
Linezolid	1.0 <sup>§</sup>	1.0 <sup>§</sup>
Clofazimine	Not determined®	Not determined®
Delamanid	Not determined®	0.016 <sup>§</sup>
Pretomanid	Not determined®	Not determined®

NOTE: Critical concentrations as indicated in CLSI M24 document [1].

- For AMK, the WHO recommended critical concentration for 7H10 agar is 2.0  $\mu g/ml$ .
- For CAP, the WHO recommended critical concentration for 7H10 agar is 4.0 µg/ml and 'Not determined' for 7H11 agar.
- For KAN, the WHO recommended critical concentration for 7H10 agar is 4.0 μg/ml.

<sup>\*</sup>The higher concentration of INH should be tested as second-line drug after resistance at the critical concentration is detected [1].

<sup>\*</sup>CLSI critical concentrations differ from revised WHO recommendations published in 2018 [1, 3].

<sup>&</sup>lt;sup>†</sup>WHO has withdrawn the recommended critical concentrations for CAP and KAN for 7H11 agar and PAS for 7H10 and 7H11 [3].

<sup>§</sup>Critical concentrations as indicated in WHO 2018 Technical Report on critical concentrations [3].

<sup>&</sup>lt;sup>¶</sup>Critical concentrations for establishing susceptibility have not been determined.

#### **Broth-Based Media**

First-line Drugs	MGIT™	VersaTREK™
Isoniazid	0.1 (and 0.4*)	0.1 (and 0.4*)
Rifampin	0.5†	1.0
Ethambutol	5.0	5.0 (and 8.0*)
Pyrazinamide	100.0	300.0

NOTE: Critical concentrations as indicated in applicable manufacturer package inserts, unless otherwise stated.

<sup>&</sup>lt;sup>†</sup>WHO recommended critical concentration of 0.5 μg/ml differs from CLSI critical concentration of 1.0 μg/ml for RIF [4].

Second-line Drug	MGIT™
Streptomycin	1.0 (and 4.0*)
Levofloxacin	1.0†
Moxifloxacin	0.25
Amikacin	1.0
Capreomycin	2.5
Kanamycin	2.5
Ethionamide	5.0
Cycloserine	16.0 <sup>§</sup>
p-Aminosalicylic acid	Not recommended†
Rifapentine	Not determined
Bedaquiline	1.0
Linezolid	1.0
Clofazimine	1.0
Delamanid	0.06
Pretomanid	0.5 and 2.0 <sup>¶</sup>

NOTE: Critical concentrations as indicated in WHO 2018 Technical Report on critical concentrations unless noted otherwise [3]. Data for second-line critical concentrations not available for VersaTREK™.

- For LVX, the CLSI recommended critical concentration for MGIT™ is 1.5 μg/ml.
- For PAS, the CLSI recommended critical concentration for MGIT™ is 4.0 μg/ml.

 $^{5}$ Critical concentration as indicated in WHO 2024 Module 3: Diagnosis—Rapid diagnostics for tuberculosis detection (Third Edition) [7].  $^{9}$ Per WHO 2024 Module 3: Diagnosis—Rapid diagnostics for tuberculosis detection (Third Edition), no growth at 0.5  $\mu$ g/ml is susceptible; growth at 0.5  $\mu$ g/ml and no growth at 2.0  $\mu$ g/ml is susceptible, but with a comment on uncertainty; growth at 2.0  $\mu$ g/ml is resistant [7].

<sup>\*</sup>The higher concentration of INH and EMB should be tested after resistance at the critical concentration is detected [1].

<sup>\*</sup>Critical concentration as indicated in applicable manufacturer package insert. The higher concentration of STR should be tested after resistance at the critical concentration is detected.

<sup>&</sup>lt;sup>†</sup>WHO critical concentrations differ from CLSI M24S recommendations published in 2023 [2, 3].

# Descriptive Information about Participant Laboratories

# **Primary Classification**

This report contains DST results submitted to CDC by panel participants at 56 laboratories in 31 states, all of whom have participated in previous MPEP panels.

Participants were asked to indicate the primary classification of their laboratory (Figure 1).

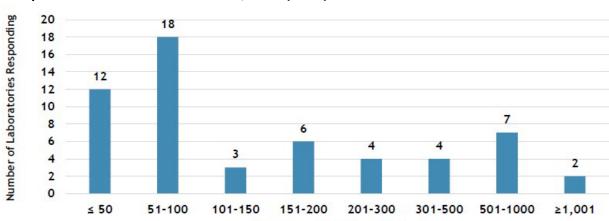
Federal government laboratory,
3, 5%
Independent/Reference
laboratory, 4, 7%
Hospital laboratory, 5, 9%

Figure 1. Primary Classification of Participating Laboratories, March 2025

# **Annual Number of MTBC Drug Susceptibility Tests Performed**

The number of MTBC isolates tested for drug susceptibility by the 56 participants in 2024 (excluding isolates used for quality control) is shown in Figure 2. In 2024, the counts ranged from 0 to 2,190 tests. Participants at 12 (21%) laboratories reported testing 50 or fewer DST isolates per year. Laboratories with low MTBC DST volumes are encouraged to consider referral of testing because of concerns about maintaining testing proficiency [8].

Public health laboratory, 44, 79%



Number of Isolates Tested

Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year, 2024 (n=56)

# MTBC Drug Susceptibility Test Methods Performed by Participants

The DST methods that were performed by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Of participating laboratories, 32 (57%) reported results for only one method, 18 (32%) reported two methods, 5 (9%) reported three susceptibility methods, and 1 (2%) reported four susceptibility methods. Fifty-two (93%) participating laboratories indicated use of MGIT™.

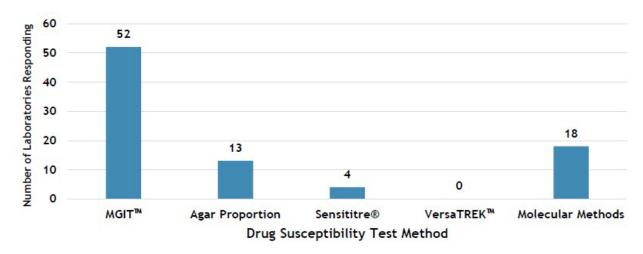


Figure 3. MTBC Drug Susceptibility Test Methods Performed (n=87 responses)

Molecular methods reported by participants are shown in Figure 4. The methods performed most frequently were targeted DNA sequencing (33%) and whole genome sequencing (33%).

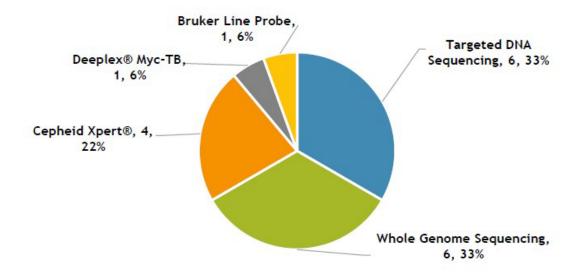


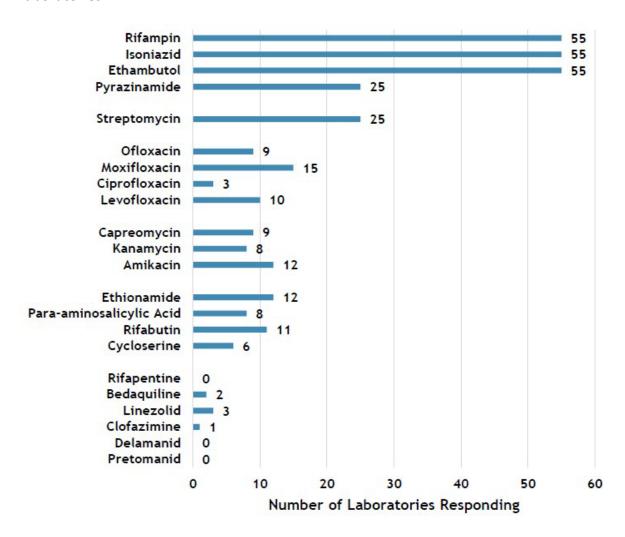
Figure 4. Molecular Method Reported (n=18 responses)

# **Antituberculosis Drugs Tested by Participants**

The number of participating laboratories that reported testing each antituberculosis drug in the March 2025 panel is presented in Figure 5. Although 56 laboratories participated in the March 2025 MPEP panel, only 55 laboratories provided growth-based testing results; one laboratory only reported results for molecular testing.

CLSI recommends testing a full panel of first-line drugs (rifampin, isoniazid, pyrazinamide, and ethambutol) [1] because it represents a combination of tests that provides the clinician with comprehensive information related to the 6- or 9-month, four-drug RIPE TB treatment regimen used for many patients. Laboratories should consider the addition of fluoroquinolones to their testing panel as CDC recommends susceptibility testing for fluoroquinolones (e.g., moxifloxacin) with use of the alternate 4-month rifapentine-moxifloxacin treatment regimen; rifampin may be used as a proxy for rifapentine [9].

Figure 5. Antituberculosis Drugs Tested by Growth-based Method by Participating Laboratories



# Isolate 2025A

# **Expected Results:**

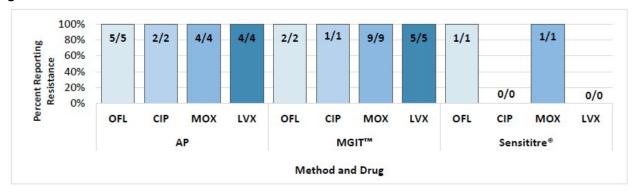
Drug	Growth-based*	Molecular*
RIF	S	rpoB wild-type
INH	S	katG, inhA, & fabG1 wild-type
EMB	S	embB wild-type
PZA	No Result⁺	<i>pncA</i> wild-type <sup>†</sup>
FQ	R	gyrA Asp94Gly; gyrB wild-type

Note: RIF=rifampin, INH=isoniazid, EMB=ethambutol, PZA=pyrazinamide, FQ=fluoroquinolones, S=susceptible, R=resistant.

#### Fluoroquinolones

DNA sequencing of *gyrA* in Isolate 2025A detected a A>G point mutation in *gyrA* resulting in wild-type aspartic acid being replaced with glycine at codon 94 (Asp94Gly). The Asp94Gly mutation has been associated with fluoroquinolone resistance [3, 18, 19]. Sequencing of the *gyrB* locus for this isolate revealed no mutations (i.e., wild-type sequence).

Figure 6. Isolate 2025A: Percent of laboratories reporting OFL, CIP, MOX, and LVX resistance, by growth-based method



Note: OFL=ofloxacin, CIP=ciprofloxacin, MOX=moxifloxacin, LVX=levofloxacin, FQ=fluoroquinolones, AP=agar proportion. Laboratories performing Sensititre® reported FQ MIC values for OFL as 16  $\mu$ g/ml (n=2), MOX as 4  $\mu$ g/ml (n=1) and 8  $\mu$ g/ml (n=2), and LVX as 8  $\mu$ g/ml (n=1); two laboratories reported 'No Interpretation' and are not included in the above figure.

For internal comparison purposes, isolate 2025A was previously sent as MPEP 2024H. Similar OFL, CIP, MOX, and LVX results were reported across all methods for MPEP 2025A and MPEP 2024H.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2025A are listed in Tables 3–9.

<sup>\*</sup>Growth-based expected results performed by agar proportion. Molecular expected results determined by whole genome sequencing.

<sup>&</sup>lt;sup>†</sup>No growth-based result available as growth-based testing for PZA was not performed for this isolate. No mutation detected in *pncA*; consistent with likely PZA susceptible.

Table 3. Isolate 2025A—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	10	0	10
Isoniazid—Low	9	0	9
Isoniazid—High	9	0	9
Ethambutol	9	0	9

Table 4. Isolate 2025A—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	51	0	51
Isoniazid—Low	51	0	51
Isoniazid—High	18	0	18
Ethambutol	51	0	51
Pyrazinamide	23	2	25

Table 5. Isolate 2025A—Participant Results for First-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	3	0	3*
Isoniazid—High	2	0	2*
Ethambutol	4	0	4

<sup>\*</sup>One additional laboratory reported 'Susceptible' for INH as a comment.

Table 6. Isolate 2025A—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	8	0	8
Ofloxacin	0	5	5
Ciprofloxacin	0	2	2
Moxifloxacin	0	4	4
Levofloxacin	0	4	4
Amikacin	7	0	7
Kanamycin	5	0	5
Capreomycin	6	0	6
Ethionamide	8	0	8
Rifabutin	5	0	5
Cycloserine	4	0	4
p-Aminosalicylic acid	5	0	5
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0

Table 7. Isolate 2025A—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	17	0	17
Ofloxacin	0	2	2
Ciprofloxacin	0	1	1
Moxifloxacin	0	9	9
Levofloxacin	0	5	5
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	2	0	2
Ethionamide	2	0	2
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Bedaquiline	1	0	1
Linezolid	2	0	2
Clofazimine	1	0	1

Table 8. Isolate 2025A—Participant Results for Second-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Streptomycin	2	0	2*
Ofloxacin	0	1	1*
Ciprofloxacin	0	0	0
Moxifloxacin	0	1	1*†
Levofloxacin	0	0	<b>0</b> <sup>†</sup>
Amikacin	2	0	2*
Kanamycin	1	0	1*
Capreomycin	1	0	1
Ethionamide	1	0	1*
Rifabutin	2	0	2*
Cycloserine	0	0	0* <sup>†</sup>
p-Aminosalicylic acid	2	0	2*
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0

<sup>\*</sup>One additional laboratory reported 'No Interpretation' for STR, OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre®.

<sup>&</sup>lt;sup>†</sup>One additional laboratory reported 'No Interpretation' for MOX, LVX, and CYC by Sensititre®.

Table 9. Isolate 2025A—Participant Results for Molecular Testing

Drug	Mutation Not Detected	Mutation Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	15	1*	16
Isoniazid	12	0	12
Ethambutol	10	0	10
Pyrazinamide	10	0	10
Streptomycin	5	0	5
Fluoroquinolones (Ofloxacin, Ciprofloxacin, Moxifloxacin, Levofloxacin)	1	10 <sup>†</sup>	11
Amikacin	7	0	7
Kanamycin	7	0	7
Capreomycin	6	0	6
Ethionamide	5	0	5
Cycloserine	1	0	1
p-Aminosalicylic acid	1	0	1
Bedaquiline	6	0	6
Linezolid	4	<b>1</b> §	5
Clofazimine	6	0	6
Delamanid	2	0	2
Pretomanid	2	0	2

<sup>\*</sup>One laboratory noted the detection of *rpoB* Leu449Gln mutation.

 $<sup>^{\</sup>dagger}\mbox{Nine}$  laboratories specifically noted the detection of  $\emph{gyrA}$  Asp94Gly mutation.

<sup>§</sup>One laboratory noted the detection of *rrl* C344T mutation.

## Isolate 2025B

# **Expected Results:**

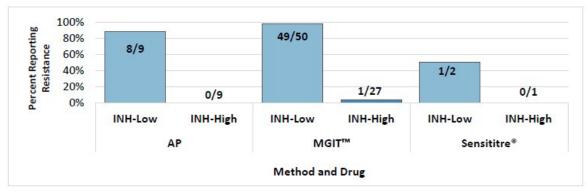
Drug	Growth-based*	Molecular*
· ·		
RIF	S	<i>rpoB</i> wild-type
INH	R (low-level) <sup>†</sup>	inhA C-15T; katG & fabG1 wild-type
EMB	S	embB wild-type
PZA	S	pncA wild-type
FQ	S	gyrA & gyrB wild-type
ETA	R <sup>§</sup>	inhA C-15T; ethA wild-type

Note: RIF=rifampin, INH=isoniazid, EMB=ethambutol, PZA=pyrazinamide, FQ=fluoroquinolones, ETA=ethionamide, S=susceptible, R=resistant.

#### Isoniazid

DNA sequence analysis of *inhA*, *katG*, and *fabG1* of Isolate 2025B revealed a C>T point mutation at nucleotide position -15 of the *inhA* promoter region; *katG* and *fabG1* were wild-type (i.e., no mutations were detected). The *inhA* C-15T mutation is known to cause low-level isoniazid resistance.

Figure 7. Isolate 2025B: Percent of laboratories reporting INH-Low and INH-High resistance, by growth-based method



Note: INH-Low=isoniazid-low concentration, INH-High=isoniazid-high concentration, AP=agar proportion. Laboratories performing Sensititre® reported INH MIC values as  $0.12 \, \mu g/ml$  (n=1) and  $0.25 \, \mu g/ml$  (n=2); one laboratory reported INH as 'Resistant' in a comment and is not included in the above figure.

#### **Ethionamide**

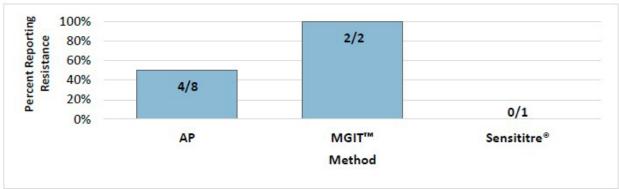
Resistance to ethionamide is commonly due to mutations in the *ethA* gene or mutations in *fabG1* or *inhA* resulting in cross-resistance with isoniazid. DNA sequencing analysis revealed the *inhA* C-15T mutation; *ethA* was wild-type (i.e., no mutations were detected).

<sup>\*</sup>Growth-based expected results performed by agar proportion, except for PZA which was performed by MGIT™. Molecular expected results determined by whole genome sequencing.

<sup>&</sup>lt;sup>†</sup>Resistant at 0.2 μg/ml by agar proportion. See Equivalent Critical Concentration table on page 8 for more information.

<sup>§80%</sup> consensus for a single categorical result across all methods reported for this drug of susceptible or resistant was not achieved for this isolate among participating laboratories.

Figure 8. Isolate 2025B: Percent of laboratories reporting ETA resistance, by growth-based method



Note: ETA=ethionamide, AP=agar proportion. Laboratories performing Sensititre® reported ETA MIC values as 2.5  $\mu$ g/ml (n=1) and 5  $\mu$ g/ml (n=1); one laboratory reported 'No Interpretation' and is not included in the figure above.

For internal comparison purposes, isolate 2025B was previously sent as MPEP 2024A. Similar INH and ETA results were reported across all methods for MPEP 2025B and MPEP 2024A.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2025B are listed in Tables 10–16.

Table 10. Isolate 2025B—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total*
Rifampin	10	0	10
Isoniazid—Low	1	8	9
Isoniazid—High	9	0	9
Ethambutol	9	0	9

<sup>\*</sup>One additional laboratory reported 'Contaminated/No Growth' for RIF, INH-Low, INH-High, and EMB by AP.

Table 11. Isolate 2025B—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total*
Rifampin	50	0	50
Isoniazid—Low	1	49	50
Isoniazid—High	26	1	27
Ethambutol	50	0	50
Pyrazinamide	23	1	24

<sup>\*</sup>One additional laboratory reported 'No Interpretation' for RIF, INH-Low, INH-High, EMB, and PZA by MGIT™.

Table 12. Isolate 2025B—Participant Results for First-Line DST by Sensititre®

	•	•	
Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	1	1	2*
Isoniazid—High	1	0	1*
Ethambutol	4	0	4

<sup>\*</sup>One additional laboratory reported 'Resistant' for INH as a comment.

Table 13. Isolate 2025B—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	8	0	8*
Ofloxacin	5	0	5
Ciprofloxacin	2	0	2
Moxifloxacin	4	0	4
Levofloxacin	4	0	4
Amikacin	6	0	6
Kanamycin	4	0	4
Capreomycin	5	0	5
Ethionamide	4	4	8
Rifabutin	5	0	5
Cycloserine	4	0	4
p-Aminosalicylic acid	5	0	5
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	0	0	0

<sup>\*</sup>One additional laboratory reported 'Contaminated/No Growth' for STR by AP.

Table 14. Isolate 2025B—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	17	0	17*
Ofloxacin	2	0	2
Ciprofloxacin	1	0	1
Moxifloxacin	10	0	10
Levofloxacin	5	0	5
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	2	0	2
Ethionamide	0	2	2
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Bedaquiline	1	0	1
Linezolid	2	0	2
Clofazimine	1	0	1

<sup>\*</sup>One additional laboratory reported 'No Interpretation' for STR by MGIT  $^{\!\scriptscriptstyle\mathsf{IM}}$  .

Table 15. Isolate 2025B—Participant Results for Second-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Streptomycin	1	0	1*
Ofloxacin	1	0	1*
Ciprofloxacin	0	0	0
Moxifloxacin	1	0	<b>1</b> * <sup>†</sup>
Levofloxacin	0	0	<b>0</b> <sup>†</sup>
Amikacin	2	0	2*
Kanamycin	1	0	1*
Capreomycin	1	0	1
Ethionamide	1	0	1*
Rifabutin	2	0	2*
Cycloserine	1	0	1*
p-Aminosalicylic acid	2	0	2*
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0

<sup>\*</sup>One additional laboratory reported 'No Interpretation' for STR, OFL, MOX, AMK, KAN, ETA, RBT, CYS, and PAS by Sensititre®.

Table 16. Isolate 2025B—Participant Results for Molecular Testing

Drug	Mutation Not Detected	Mutation Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	16	0	16
Isoniazid	0	12* <sup>†</sup>	12
Ethambutol	7	<b>3</b> §	10
Pyrazinamide	10	0	10
Streptomycin	6	0	6
Fluoroquinolones (Ofloxacin, Ciprofloxacin, Moxifloxacin, Levofloxacin)	10	119	11
Amikacin	7	0	7
Kanamycin	7	0	7
Capreomycin	6	0	6
Ethionamide	2	3**	5
Cycloserine	1	0	1
p-Aminosalicylic acid	1	0	1
Bedaquiline	6	0	6
Linezolid	5	1**	6
Clofazimine	6	0	6
Delamanid	2	0	2
Pretomanid	2	0	2

<sup>\*</sup>Eleven laboratories noted the detection of inhA C-15T mutation.

<sup>&</sup>lt;sup>†</sup>One additional laboratory reported 'No Interpretation' for MOX and LVX by Sensititre®.

<sup>&</sup>lt;sup>†</sup>Three laboratories also noted the detection of *katG* mutations either not associated with resistance or of unknown significance.

<sup>§</sup>Five laboratories noted the detection of *embB* Glu378Asp mutation not associated with resistance, although only three laboratories reported mutation detected.

<sup>¶</sup>One laboratory noted the detection of a *gyrA* mutation not associated with resistance.

<sup>\*\*</sup>Five laboratories noted the detection of *inhA* C-15T mutation for ethionamide, although only three laboratories reported mutation detected

<sup>&</sup>lt;sup>††</sup>One laboratory noted the detection of *rrl* G982A mutation with uncertain significance.

# Isolate 2025C

#### **Expected Results:**

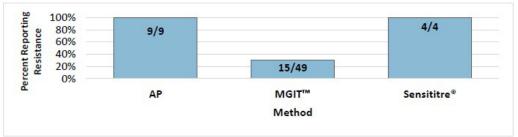
Drug	Growth-based*	Molecular*
RIF	S	rpoB wild-type
INH	S	katG, inhA, & fabG1 wild-type
ЕМВ	R <sup>†</sup>	embB Gln497Arg
PZA	S	pncA wild-type
FQ	S	gyrA & gyrB wild-type
STR	R <sup>†</sup>	rrs & rpsL wild-type

Note: RIF=rifampin, INH=isoniazid, EMB=ethambutol, PZA=pyrazinamide, FQ=fluoroquinolones, STR=streptomycin, S=susceptible, R=resistant.

#### **Ethambutol**

DNA sequence analysis of *embB* of Isolate 2025C revealed a A>G point mutation in the *embB* gene resulting in wild-type glutamine being replaced by arginine at codon 497 (Gln497Arg). The Gln497Arg mutation is associated with EMB resistance [10].

Figure 9. Isolate 2025C: Percent of laboratories reporting EMB resistance, by growth-based method

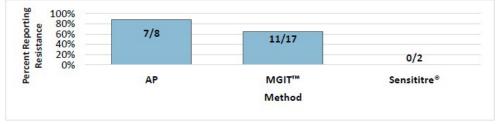


Note: EMB=ethambutol, AP=agar proportion. Laboratories performing Sensititre® reported EMB MIC values as 8  $\mu$ g/ml (n=2) and 16  $\mu$ g/ml (n=2).

#### Streptomycin

DNA sequencing analysis did not reveal a mutation in rrs or rpsL.

Figure 10. Isolate 2025C: Percent of laboratories reporting STR resistance, by growth-based method



Note: STR=streptomycin, AP=agar proportion. Laboratories performing Sensititre® reported STR MIC values as 1  $\mu$ g/ml (n=1) and 2  $\mu$ g/ml (n=2); one laboratory reported 'No Interpretation' and is not included in the above figure.

<sup>\*</sup>Growth-based expected results performed by agar proportion. Molecular expected results determined by whole genome sequencing.

<sup>&</sup>lt;sup>†</sup>80% consensus for a single categorical result across all methods reported for this drug of susceptible or resistant was not achieved for this isolate among participating laboratories.

For internal comparison purposes, isolate 2025C was previously sent as MPEP 2021B. Similar EMB results were reported across all methods for MPEP 2025C and MPEP 2021B. Similar STR results were reported for agar proportion and Sensititre®, but a decrease in reported resistance was noted for MGIT™ for MPEP 2025C compared to MPEP 2021B.

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2025C are listed in Tables 17–23.

Table 17. Isolate 2025C—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	10	0	10
Isoniazid—Low	8	1	9
Isoniazid—High	9	0	9
Ethambutol	0	9	9

Table 18. Isolate 2025C—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	50	0	50
Isoniazid—Low	49	1	50
Isoniazid—High	19	0	19
Ethambutol	34	15	49
Pyrazinamide	24	1	25

Table 19. Isolate 2025C—Participant Results for First-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	3	0	3*
Isoniazid—High	2	0	2*
Ethambutol	0	4	4

<sup>\*</sup>One additional laboratory reported 'Susceptible' for INH as a comment.

Table 20. Isolate 2025C—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	1	7	8
Ofloxacin	5	0	5
Ciprofloxacin	2	0	2
Moxifloxacin	4	0	4
Levofloxacin	4	0	4
Amikacin	7	0	7
Kanamycin	5	0	5
Capreomycin	7	0	7
Ethionamide	8	0	8
Rifabutin	5	0	5
Cycloserine	4	0	4
p-Aminosalicylic acid	5	0	5
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	0	0	0

Table 21. Isolate 2025C—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	6	11	17
Ofloxacin	2	0	2
Ciprofloxacin	1	0	1
Moxifloxacin	9	0	9
Levofloxacin	5	0	5
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	2	0	2
Ethionamide	2	0	2
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Bedaquiline	1	0	1
Linezolid	2	0	2
Clofazimine	1	0	1

Table 22. Isolate 2025C—Participant Results for Second-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Streptomycin	2	0	2*
Ofloxacin	1	0	1*
Ciprofloxacin	0	0	0
Moxifloxacin	1	0	1*†
Levofloxacin	1	0	1
Amikacin	2	0	2*
Kanamycin	1	0	1*
Capreomycin	1	0	1
Ethionamide	1	0	1*
Rifabutin	2	0	2*
Cycloserine	1	0	1*
p-Aminosalicylic acid	2	0	2*
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0

<sup>\*</sup>One additional laboratory reported 'No Interpretation' for STR, OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre®.

Table 23. Isolate 2025C—Participant Results for Molecular Testing

Table 23. Isolate 2025C—Participant Results for Molecular Testing				
Drug	Mutation Not Detected	Mutation Detected	Total	
Rifamycins (Rifampin, Rifabutin, Rifapentine)	15	1	16	
Isoniazid	5	7*	12	
Ethambutol	2	8 <sup>†</sup>	10	
Pyrazinamide	10	0	10	
Streptomycin	4	<b>1</b> §	5	
Fluoroquinolones (Ofloxacin, Ciprofloxacin, Moxifloxacin, Levofloxacin)	9	1 <sup>¶</sup>	10	
Amikacin	7	0	7	
Kanamycin	7	0	7	
Capreomycin	6	0	6	
Ethionamide	5	0	5	
Cycloserine	1	0	1	
p-Aminosalicylic acid	1	0	1	
Bedaquiline	6	0	6	
Linezolid	6	0	6	
Clofazimine	6	0	6	
Delamanid	1	1**	2	
Pretomanid	1	1**	2	

<sup>\*</sup>Seven laboratories noted the detection of multiple *katG* mutations with unknown significance.

<sup>&</sup>lt;sup>†</sup>One additional laboratory reported 'No Interpretation' for MOX by Sensititre®.

<sup>&</sup>lt;sup>†</sup> Eight laboratories noted the detection of *embB* Gln497Arg mutation; one of these laboratories also noted detection of an *embA* C-12T mutation of unknown significance.

<sup>§</sup> One laboratory noted the detection of a *gidB* mutation with unknown significance.

<sup>¶</sup>One laboratory noted the detection of a *gyrA* mutation not associated with resistance.

<sup>\*\*</sup>One laboratory noted the detection of an fgd1 mutation.

## Isolate 2025D

### **Expected Results:**

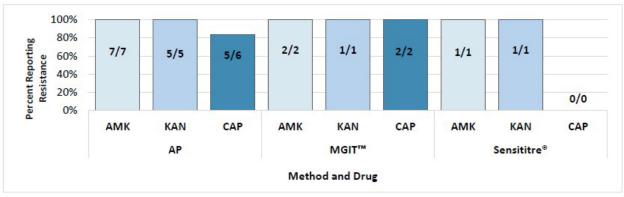
Drug	Growth-based*	Molecular*
RIF	S	rpoB wild-type
INH	S	katG, inhA, & fabG1 wild-type
EMB	S	embB wild-type
PZA	S	pncA wild-type
FQ	S	gyrA & gyrB wild-type
AMK, KAN, CAP	R	rrs A1401G; eis & tlyA wild-type
BDQ, CFZ	U	mmpR Asp141fs <sup>†</sup>

Note: RIF=rifampin, INH=isoniazid, EMB=ethambutol, PZA=pyrazinamide, FQ=fluoroquinolones, AMK=amikacin, KAN=kanamycin, CAP=capreomycin, BDQ=bedaquiline, CFZ=clofazimine, S=susceptible, R=resistant, U=unknown.

#### **Second-line Injectables**

DNA sequence analysis of *rrs* in Isolate 2025D revealed an A>G point mutation in codon 1401 (A1401G); *eis* and *tlyA* were wild-type (i.e., no mutations were detected). Isolates with an A1401G mutation have been shown to have resistance to amikacin, kanamycin, and capreomycin [11, 12].

Figure 11. Isolate 2025D: Percent of laboratories reporting AMK, KAN, and CAP resistance, by growth-based method



Note: AMK=amikacin, KAN=kanamycin, CAP=capreomycin, AP=agar proportion. Laboratories performing Sensititre® reported MIC values for AMK as 16  $\mu$ g/ml (n=1) and >16  $\mu$ g/ml (n=2), KAN as >40  $\mu$ g/ml (n=2), and CAP as 10  $\mu$ g/ml (n=1); two laboratories reported 'No Interpretation' and are not included in the above figure.

For internal comparison purposes, isolate 2025D was previously sent as MPEP 2023E. Similar AMK, KAN, and CAP results were reported across all methods for MPEP 2025D and MPEP 2023E.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2025D are listed in Tables 24–30.

<sup>\*</sup>Growth-based expected results performed by agar proportion, except for PZA which was performed by MGIT™. Molecular expected results determined by whole genome sequencing.

<sup>&</sup>lt;sup>†</sup> Asp141fs mutation detected in *mmpR* with unknown significance.

Table 24. Isolate 2025D—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	10	0	10
Isoniazid—Low	9	0	9
Isoniazid—High	9	0	9
Ethambutol	9	0	9

Table 25. Isolate 2025D—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total*
Rifampin	49	0	49
Isoniazid—Low	49	0	49
Isoniazid—High	17	0	17
Ethambutol	49	0	49
Pyrazinamide	22	1	23

<sup>\*</sup>One additional laboratory reported 'No Interpretation' for RIF, INH-Low, INH-High, EMB, and PZA by MGIT™.

Table 26. Isolate 2025D—Participant Results for First-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	3	0	3*
Isoniazid—High	2	0	2*
Ethambutol	4	0	4

<sup>\*</sup>One additional laboratory reported 'Susceptible' for INH as a comment.

Table 27. Isolate 2025D—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	8	0	8
Ofloxacin	5	0	5
Ciprofloxacin	2	0	2
Moxifloxacin	4	0	4
Levofloxacin	4	0	4
Amikacin	0	7	7
Kanamycin	0	5	5
Capreomycin	1	5	6
Ethionamide	8	0	8
Rifabutin	5	0	5
Cycloserine	4	0	4
p-Aminosalicylic acid	5	0	5
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	0	0	0

Table 28. Isolate 2025D—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	16	0	16*
Ofloxacin	2	0	2
Ciprofloxacin	1	0	1
Moxifloxacin	9	0	9
Levofloxacin	5	0	5
Amikacin	0	2	2
Kanamycin	0	1	1
Capreomycin	0	2	2
Ethionamide	2	0	2
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Bedaquiline	0	1	1
Linezolid	2	0	2
Clofazimine	1	0	1

<sup>\*</sup>One additional laboratory reported 'No Interpretation' for STR by MGIT™.

Table 29. Isolate 2025D—Participant Results for Second-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Streptomycin	2	0	2*
Ofloxacin	1	0	1*
Ciprofloxacin	0	0	0
Moxifloxacin	1	0	1*†
Levofloxacin	1	0	1
Amikacin	0	1	1*†
Kanamycin	0	1	1*
Capreomycin	0	0	<b>0</b> <sup>†</sup>
Ethionamide	1	0	1*
Rifabutin	2	0	2*
Cycloserine	0	0	0* <sup>†</sup>
p-Aminosalicylic acid	2	0	2*
Bedaquiline	0	1	1
Linezolid	1	0	1
Clofazimine	0	0	0

<sup>\*</sup>One additional laboratory reported 'No Interpretation' for STR, OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre®.

<sup>&</sup>lt;sup>†</sup>One additional laboratory reported 'No Interpretation' for MOX, AMK, CAP, and CYC by Sensititre®.

Table 30. Isolate 2025D—Participant Results for Molecular Testing

Drug	Mutation Not Detected	Mutation Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	16	0	16
Isoniazid	12	0	12
Ethambutol	10	0	10
Pyrazinamide	10	0	10
Streptomycin	4	0	4
Fluoroquinolones (Ofloxacin, Ciprofloxacin, Moxifloxacin, Levofloxacin)	9	2*	11
Amikacin	0	8 <sup>†</sup>	8
Kanamycin	1	<b>7</b> <sup>†</sup>	8
Capreomycin	0	<b>7</b> <sup>†</sup>	7
Ethionamide	5	0	5
Cycloserine	1	0	1
p-Aminosalicylic acid	1	0	1
Bedaquiline	0	6 <sup>§</sup>	6
Linezolid	6	0	6
Clofazimine	1	5 <sup>¶</sup>	6
Delamanid	2	0**	2
Pretomanid	2	0**	2

<sup>\*</sup> One laboratory noted the detection of a *gyrA* Ala120Val mutation with unknown significance and another laboratory noted another *gyrA* mutation not associated with resistance.

<sup>&</sup>lt;sup>†</sup> Seven laboratories noted the detection of *rrs* A1401G mutation.

<sup>&</sup>lt;sup>9</sup> Six laboratories noted the detection of a Rv0678 frameshift mutation with unknown significance.

 $<sup>\</sup>P$  Five laboratories noted the detection of a Rv0678 frameshift mutation with unknown significance.

<sup>\*\*</sup> One laboratory noted the detection of an fgd1 mutation.

#### Isolate 2025E

# **Expected Results:**

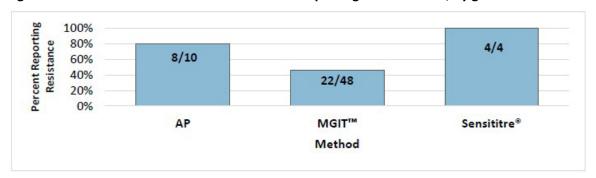
Drug	Growth-based*	Molecular*
RIF	R <sup>†</sup>	rpoB His445Leu
		,
INH	S	katG, inhA, & fabG1 wild-type
EMB	S	embB wild-type
PZA	S	pncA wild-type
FQ	S	gyrA & gyrB wild-type

Note: RIF=rifampin, INH=isoniazid, EMB=ethambutol, PZA=pyrazinamide, FQ=fluoroquinolones, ETA=ethionamide, S=susceptible, R=resistant.

#### Rifampin

DNA sequence analysis of *rpoB* in Isolate 2025E revealed a A>T point mutation in codon 445 resulting in wild-type histidine being replaced by leucine (His445Leu). Isolates with His445Leu mutations are associated with low-level rifampin resistance and can test as susceptible in growth-based assays [10, 13, 14]. Although this mutation is associated with low-level rifampin resistance, few participating laboratories performing MGIT™ growth-based DST detected resistance; this is likely due to the RIF critical concentration being too high and consideration should be given to evaluating a lower critical concentration [15-17].

Figure 12. Isolate 2025E: Percent of laboratories reporting RIF resistance, by growth-based method



Note: RIF=rifampin, AP=agar proportion. Laboratories performing Sensititre® reported RIF MIC values as 1  $\mu$ g/ml (n=1), 4  $\mu$ g/ml (n=2), and 16  $\mu$ g/ml (n=1).

For internal comparison purposes, isolate 2025E was previously sent as MPEP 2023G. Similar RIF results were reported for agar proportion and MGIT™, but an increase in reported resistance was noted for Sensititre® for MPEP 2025E compared to MPEP 2023G.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2025E are listed in Tables 31–37.

<sup>\*</sup>Growth-based expected results performed by agar proportion, except for PZA which was performed by MGIT™. Molecular expected results determined by whole genome sequencing.

<sup>&</sup>lt;sup>†</sup>80% consensus for a single categorical result across all methods reported for this drug of susceptible or resistant was not achieved for this isolate among participating laboratories.

Table 31. Isolate 2025E—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	2	8	10
Isoniazid—Low	9	0	9
Isoniazid—High	9	0	9
Ethambutol	9	0	9

Table 32. Isolate 2025E—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	26	22	48* <sup>†</sup>
Isoniazid—Low	49	0	49* <sup>†</sup>
Isoniazid—High	17	0	17*
Ethambutol	49	0	49* <sup>†</sup>
Pyrazinamide	23	1	24*

<sup>\*</sup>One additional laboratory reported 'No Interpretation' for RIF, INH-Low, INH-High, EMB, and PZA by MGIT™.

Table 33. Isolate 2025E—Participant Results for First-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Rifampin	0	4	4
Isoniazid—Low	3	0	3*
Isoniazid—High	2	0	2*
Ethambutol	3	0	<b>3</b> <sup>†</sup>

<sup>\*</sup>One additional laboratory reported 'Susceptible' for INH by Sensititre® as a comment.

Table 34. Isolate 2025E—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	7	1	8
Ofloxacin	5	0	5
Ciprofloxacin	2	0	2
Moxifloxacin	4	0	4
Levofloxacin	4	0	4
Amikacin	7	0	7
Kanamycin	5	0	5
Capreomycin	6	0	6
Ethionamide	8	0	8
Rifabutin	5	0	5
Cycloserine	4	0	4
p-Aminosalicylic acid	5	0	5
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	0	0	0

<sup>&</sup>lt;sup>†</sup>One additional laboratory reported 'No Interpretation' for RIF, INH-Low, and EMB by MGIT™.

<sup>&</sup>lt;sup>†</sup>One additional laboratory reported 'No Interpretation' for EMB by Sensititre®.

Table 35. Isolate 2025E—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	15	0	15*
Ofloxacin	2	0	2
Ciprofloxacin	1	0	1
Moxifloxacin	9	0	9
Levofloxacin	5	0	5
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	2	0	2
Ethionamide	2	0	2
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Bedaquiline	1	0	1
Linezolid	2	0	2
Clofazimine	1	0	1

<sup>\*</sup>Two additional laboratories reported 'No Interpretation' for STR by MGIT™.

Table 36. Isolate 2025E—Participant Results for Second-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Streptomycin	2	0	2*
Ofloxacin	1	0	1*
Ciprofloxacin	0	0	0
Moxifloxacin	1	0	1*†
Levofloxacin	1	0	1
Amikacin	2	0	2*
Kanamycin	1	0	1*
Capreomycin	1	0	1
Ethionamide	1	0	1*
Rifabutin	1	1	2*
Cycloserine	0	0	0* <sup>†</sup>
p-Aminosalicylic acid	2	0	2*
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0

<sup>\*</sup>One additional laboratory reported 'No Interpretation' for STR, OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre®.

<sup>&</sup>lt;sup>†</sup>One additional laboratory reported 'No Interpretation' for MOX and CYC by Sensititre®.

Table 37. Isolate 2025E—Participant Results for Molecular Testing

Drug	Mutation Not Detected	Mutation Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	0	16*	16
Isoniazid	12	0	12
Ethambutol	10	0	10
Pyrazinamide	10	0	10
Streptomycin	5	0	5
Fluoroquinolones (Ofloxacin, Ciprofloxacin, Moxifloxacin, Levofloxacin)	10	<b>1</b> <sup>†</sup>	11
Amikacin	7	0	7
Kanamycin	7	0	7
Capreomycin	6	0	6
Ethionamide	5	0	5
Cycloserine	1	0	1
p-Aminosalicylic acid	1	0	1
Bedaquiline	5	<b>1</b> §	6
Linezolid	6	0	6
Clofazimine	5	<b>1</b> §	6
Delamanid	2	0	2
Pretomanid	2	0	2

<sup>\*</sup>Eleven laboratories noted the detection of  $\it rpoB$  His445Leu mutation.

 $<sup>^{\</sup>dagger}$ One laboratory noted the detection of a  $\it gyrA$  mutation not associated with resistance.

 $<sup>{}^{\</sup>S}$  One laboratory noted the detection of  $\it mmpL5$  Ala147Thr mutation with unknown significance.

## References

- 1. CLSI, Susceptibility Testing of Mycobacteria, Nocardiae spp., and Other Aerobic Actinomycetes, in 3rdEd. CLSI Standard M24. 2018, Clinical and Laboratory Standards Institute: Wayne, PA.
- 2. CLSI, Performance Standards for Susceptibility Testing of Mycobacteria, Nocardia spp., and Other Aerobic Actinomycetes, in 2nd edition. CLSI supplement M24S. 2023, Clinical and Laboratory Standards Institute: Wayne, PA.
- 3. World Health Organization, *Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis.* 2018: Geneva.
- 4. World Health Organization, *Technical Report on critical concentrations for drug susceptibility testing of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine)*. 2021: Geneva.
- 5. Andre, E., et al., *Consensus numbering system for the rifampicin resistance-associated rpoB gene mutations in pathogenic mycobacteria*. Clin Microbiol Infect, 2017. **23**(3): p. 167-172.
- 6. Association of Public Health Laboratories, *Issues in Mycobacterium tuberculosis complex (MTBC)*Drug Susceptibility Testing: Rifampin (RIF), in APHL Issues in Brief: Infectious Diseases. 2019,
  Association of Public Health Laboratories: Washington, D.C.
- 7. World Health Organization, *Operational Handbook on Tuberculosis. Module 3: Diagnosis—Rapid Diagnostics for Tuberculosis Detection, Third Edition.* 2024, World Health Organization: Geneva.
- 8. Association of Public Health Laboratories, *TB Drug Susceptibility Testing Expert Panel Meeting Summary Report*. 2007, Association of Public Health Laboratories: Washington, D.C.
- 9. Carr W, K.E., Starks A, Goswami N, Allen L, Winston C., *Interim Guidance: 4-Month Rifapentine-Moxifloxacin Regimen for the Treatment of Drug-Susceptible Pulmonary Tuberculosis United States, 2022.* MMWR Morb Mortal Wkly Rep, 2022(71): p. 285–289.
- 10. World Health Organization, *Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance*. 2021, World Health Organization: Geneva.
- 11. Siu, G.K., et al., *Mutations outside the rifampicin resistance-determining region associated with rifampicin resistance in Mycobacterium tuberculosis.* J Antimicrob Chemother, 2011. **66**(4): p. 730-3.
- 12. Maus, C.E., B.B. Plikaytis, and T.M. Shinnick, *Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis.* Antimicrob Agents Chemother, 2005. **49**(8): p. 3192-7.
- 13. Van Deun, A., et al., *Mycobacterium tuberculosis strains with highly discordant rifampin susceptibility test results.* J Clin Microbiol, 2009. **47**(11): p. 3501-6.
- 14. Rigouts, L., et al., *Rifampin resistance missed in automated liquid culture system for Mycobacterium tuberculosis isolates with specific rpoB mutations.* J Clin Microbiol, 2013. **51**(8): p. 2641-5.
- 15. World Health Organization, *Technical report on critical concentrations for drug susceptibility testing of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine)*. 2021, Geneva: World Health Organization.
- 16. Köser, C.U., et al., On the Consequences of Poorly Defined Breakpoints for Rifampin Susceptibility Testing of Mycobacterium tuberculosis Complex. J Clin Microbiol, 2021. **59**(4).
- 17. Yu, H.J., et al., *Performance Evaluation of the BACTEC MGIT 960 System for Rifampin Drug-Susceptibility Testing of Mycobacterium tuberculosis Using the Current WHO Critical Concentration.* J Clin Microbiol, 2023. **61**(1): p. e0108622.

# Appendix 1: Accessible Explanations of Figures

**Figure 1**. The primary classification of the 56 laboratories participating in the March 2025 MPEP panel is shown in this pie chart. The largest slice represents 44 laboratories, or 79% of the 56 that have self-classified as a health department laboratory. The next major slice signifies 5 laboratories, or 9% of the 56 that self-classified as hospital laboratories. The remaining two slices of the pie chart represent 4, or 7% of the 56 that self-classified as independent laboratories; and 3, or 5% of the 56 that self-classified as federal government laboratories.

Figure 2. The annual volume of MTBC isolates tested for drug susceptibility by participating laboratories (N=56) in 2024 is displayed in this vertical bar graph. The vertical y—axis is the number of laboratories responding and ranges from 0 to 20 using increments of 2. Along the horizontal x-axis are eight vertical bars representing the number of isolates tested per year. From left to right, 12 laboratories tested less than or equal to 50 isolates per year; 18 laboratories tested between 51 to 100 isolates per year; 3 laboratories tested between 101 to 150 isolates per year; 6 laboratories tested between 151 to 200 isolates per year; 4 laboratories tested between 201 to 300 isolates per year; 4 laboratories tested between 301 to 500 isolates per year; 7 laboratories tested between 501 to 1000 isolates per year; and 2 laboratories tested greater than or equal to 1,001 isolates per year.

Figure 3. The drug susceptibility testing methods performed by MPEP participants (N=87) are displayed in this vertical bar graph. The vertical y-axis is the number of laboratories reporting with ranges from 0 to 60, by increments of 10, and the horizontal x- axis lists the susceptibility testing methods. Each bar represents the number of reporting laboratories performing a particular drug susceptibility test method. From left to right: 52 performed MGIT™, 13 performed agar proportion, 4 performed Sensititre®, 0 performed VersaTREK™, and 18 performed molecular methods.

Figure 4. The molecular methods performed by MPEP participants (N=18) are displayed in this pie chart. The largest slice represents the 6 laboratories, or 33% of the 18 laboratories, that performed targeted DNA sequencing. The next four slices represent 6 laboratories, or 33% of the 18 laboratories, that performed whole genome sequencing; 4 laboratories, or 22% of the 18 laboratories, that performed the Cepheid Xpert® MTB/RIF assay; 1 laboratory, or 6% of the 18 laboratories, that performed the Deeplex Myc-TB assay; and 1 laboratory, or 6% of the 18 laboratories, that performed the Bruker line probe assay.

Figure 5. The antituberculosis drugs tested by growth-based method by MPEP participants are displayed in a horizontal bar graph. The vertical y -axis contains a list of each drug tested and the horizontal x-axis contains the number of laboratories with ranges from 0 to 60, by increments of 10. There are 22 horizontal bars with each bar representing the number of laboratories reporting a result for a particular drug for susceptibility testing. 55 laboratories tested rifampin; 55 laboratories tested isoniazid; 55 laboratories tested ethambutol; 25 laboratories tested pyrazinamide; 25 laboratories tested streptomycin; 9 laboratories tested ofloxacin; 15 laboratories tested moxifloxacin; 3 laboratories tested ciprofloxacin; 10 laboratories tested levofloxacin; 9 laboratories tested capreomycin; 8 laboratories tested kanamycin; 12 laboratories tested amikacin; 12 laboratories tested ethionamide; 8 laboratories tested p-Aminosalicylic acid; 11 laboratories tested rifabutin; 6 laboratories tested cycloserine; 0 laboratories tested rifapentine; 2 laboratories tested bedaquiline; 3 laboratories tested linezolid; 1 laboratory tested clofazimine; 0 laboratories tested delamanid; and 0 laboratories tested pretomanid.

Figure 6. The percent of laboratories reporting resistance to ofloxacin, ciprofloxacin, moxifloxacin, and levofloxacin, by growth-based method, for isolate 2025A is displayed in this vertical bar graph. The vertical y-axis is the percent of laboratories reporting resistance with ranges from 0% to 100%, by increments of 20, and the horizontal x-axis lists the method and drugs. Each bar represents the percent of laboratories reporting resistance. From left to right: laboratories performing agar proportion for OFL is 5 of 5 (100%) reporting resistance, CIP is 2 of 2 (100%) reporting resistance, MOX is 4 of 4 (100%) reporting resistance; laboratories performing MGIT™ for OFL is 2 of 2 (100%) reporting resistance, CIP is 1 of 1 (100%) reporting resistance, MOX is 9 of 9 (100%) reporting resistance, LVX is 5 of 5 (100%) reporting resistance; and laboratories performing Sensititre® for OFL is 1 of 1 (100%) reporting resistance, CIP is 0 of 0 (0%) reporting resistance, MOX is 1 of 1 (100%) reporting resistance, LVX is 0 of 0 (0%) reporting resistance.

Figure 7. The percent of laboratories reporting resistance to isoniazid (low and high concentrations), by growth-based method, for isolate 2025B is displayed in this vertical bar graph. The vertical y-axis is the percent of laboratories reporting resistance with ranges from 0% to 100%, by increments of 20, and the horizontal x-axis lists the method and drugs. Each bar represents the percent of laboratories reporting resistance. From left to right: laboratories performing agar proportion for INH-Low is 8 of 9 (89%) reporting resistance and INH-High is 0 of 9 (0%) reporting resistance; laboratories performing MGIT™ for INH-Low is 49 of 50 (98%) reporting resistance and INH-High is 1 of 27 (4%) reporting resistance; and laboratories performing Sensititre® for INH-Low is 1 of 2 (50%) reporting resistance and INH-High is 0 of 1 (0%) reporting resistance.

Figure 8. The percent of laboratories reporting resistance to ethionamide, by growth-based method, for isolate 2025B is displayed in this vertical bar graph. The vertical y-axis is the percent of laboratories reporting resistance with ranges from 0% to 100%, by increments of 20, and the horizontal x-axis lists the method. Each bar represents the percent of laboratories reporting resistance. From left to right: laboratories performing agar proportion for ethionamide is 4 of 8 (50%) reporting resistance; laboratories performing MGIT™ for ethionamide is 2 of 2 (100%) reporting resistance; and laboratories performing Sensititre® for ethionamide is 0 of 1 (0%) reporting resistance.

Figure 9. The percent of laboratories reporting resistance to ethambutol, by growth-based method, for 2025C is displayed in this vertical bar graph. The vertical y-axis is the percent of laboratories reporting resistance with ranges from 0% to 100%, by increments of 20, and the horizontal x-axis lists the method. Each bar represents the percent of laboratories reporting resistance. From left to right: laboratories performing agar proportion for ethambutol is 9 of 9 (100%) reporting resistance; laboratories performing MGIT™ for ethambutol is 15 of 49 (31%) reporting resistance; and laboratories performing Sensititre® for ethambutol is 4 of 4 (100%) reporting resistance.

Figure 10. The percent of laboratories reporting resistance to streptomycin, by growth-based method, for 2025C is displayed in this vertical bar graph. The vertical y-axis is the percent of laboratories reporting resistance with ranges from 0% to 100%, by increments of 20, and the horizontal x-axis lists the method. Each bar represents the percent of laboratories reporting resistance. From left to right: laboratories performing agar proportion for streptomycin is 7 of 8 (88%) reporting resistance; laboratories performing MGIT™ for streptomycin is 11 of 17 (65%) reporting resistance; and laboratories performing Sensititre® for streptomycin is 0 of 2 (0%) reporting resistance.

Figure 11. The percent of laboratories reporting resistance to amikacin, kanamycin, and capreomycin, by growth-based method, for isolate 2025D is displayed in this vertical bar graph. The vertical y-axis is the percent of laboratories reporting resistance with ranges from 0% to 100%, by increments of 20, and the horizontal x-axis lists the method and drugs. Each bar represents the percent of laboratories reporting resistance. From left to right: laboratories performing agar proportion for AMK is 7 of 7 (100%) reporting resistance, KAN is 5 of 5 (100%) reporting resistance, CAP is 5 of 6 (83%) reporting resistance; laboratories performing MGIT™ for AMK is 2 of 2 (100%) reporting resistance, KAN is 1 of 1 (100%) reporting resistance, CAP is 2 of 2 (100%) reporting resistance; and laboratories performing Sensititre® for AMK is 1 of 1 (100%) reporting resistance, CAP is 0 of 0 (0%) reporting resistance.

Figure 12. The percent of laboratories reporting resistance to rifampin, by growth-based method, for 2025E is displayed in this vertical bar graph. The vertical y-axis is the percent of laboratories reporting resistance with ranges from 0% to 100%, by increments of 20, and the horizontal x-axis lists the method. Each bar represents the percent of laboratories reporting resistance. From left to right: laboratories performing agar proportion for rifampin is 8 of 10 (80%) reporting resistance; laboratories performing MGIT™ for rifampin is 22 of 48 (46%) reporting resistance; and laboratories performing Sensititre® for rifampin is 4 of 4 (100%) reporting resistance.