Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Model Performance Evaluation Program

Report of Results, August 2023

Centers for Disease Control and Prevention
National Center for Injury Prevention and Control

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 August 2023.

REPORT CONTENT

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Note on Accessibility: Find descriptions and explanations of figures in Appendix 1: Accessible Explanation of Figures on page 41.

Contents

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" [1-3]. Additionally, the World Health Organization (WHO) published two technical reports investigating critical concentrations, by method, for anti-tuberculosis drugs [4, 5].

Abbreviations and Acronyms

Acronym	Definition
AMK	Amikacin
AP	Agar proportion — performed on Middlebrook 7H10 or 7H11
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
TB	Tuberculosis
VersaTREK™	Thermo Scientific VersaTREK™ Myco susceptibility
XDR	Extensively drug-resistant

Expected Drug Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in August 2023 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) are shown in Table 1. Molecular results obtained by whole genome sequencing are listed in Table 2.

1 TABLE 1. EXPECTED GROWTH-BASED RESULTS FOR AUGUST 2023 PANEL

Isolate	RIF	INH	EMB	PZA	Second-line Drug Resistances:
2023F	S	R (low-level*)	S	S	STR, OFL, CIP
2023G	R†	S	S	S	
2023H	S	R (low-level*)†	S	S	ETA
2023I	R◊	S	S	S	
2023J	S	S	S	S	

Note—S=susceptible, R=resistant.

* Resistant at 0.2 µg/ml by agar proportion. See Equivalent Critical Concentration table on page 9 for more information.

† 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.

◊ > 80% consensus reported as susceptible across all methods among participating laboratories, although expected result was resistant.

Expected Drug Susceptibility Testing Results

2 TABLE 2. EXPECTED MOLECULAR RESULTS (MUTATIONS DETECTED IN LOCI ASSOCIATED WITH RESISTANCE) FOR AUGUST 2023 PANEL

Isolate	*rpoB**	*katG*	*fabG1*	*gyrA*	*rpsL*
2023F		Asp94Asn◊		Asp94Gly	Lys43Arg
2023G	His445Leu				
2023H	Arg447Arg†		Leu203Leu		
2023I	Asp435Tyr				
2023J					

Note—Empty cell=No mutation detected. High confidence mutations were not detected in these loci: *inhA*, *pncA*, *embB*, *ethA*, *eis*, *rrs*, and *tlyA*.

* *M. tuberculosis* numbering system used [6, 7]

◊ Effect of mutation is unknown.

† Mutation not associated with resistance [8]

Technical Notes

The following information pertains to all tables and figures for the 2023 MTBC isolates F, G, H, I, and J included in this report.

- The source of data in all tables and figures is the August 2023 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 was 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.

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	1.0†	1.0
Ethambutol	5.0	7.5
Pyrazinamide	Not recommended	Not recommended

NOTE—Critical concentrations as indicated in CLSI M24-A2 document [1]

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

† CLSI critical concentrations for RIF differ from revised WHO recommendation of 0.5 µg/ml published in 2021 [1, 9].

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¥
Kanamycin	5.0†	6.0¥
Ethionamide	5.0	10.0
Rifabutin	0.5	0.5
p-Aminosalicylic acid	2.0¥	8.0¥
Rifapentine	Not determined*	Not determined*
Bedaquiline	Not determined*	0.25‡
Linezolid	1.0‡	1.0‡
Clofazimine	Not determined*	Not determined*
Delamanid	Not determined*	0.016‡
Pretomanid	Not determined*	Not determined*

NOTE—Critical concentrations as indicated in CLSI M24-A2 document [1]

* Breakpoints for establishing susceptibility have not been determined.

† CLSI critical concentrations differ from revised WHO recommendations published in 2018 [1, 4].

- For AMK, the WHO recommended critical concentration for 7H10 agar is 2.0 µg/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.

¥ WHO has withdrawn the recommended critical concentrations for CAP and KAN for 7H11 agar and PAS for 7H10 and 7H11 [4].

‡ Critical concentrations as indicated in WHO 2018 Technical Report on critical concentrations [4].

BROTH BASED MEDIA

First-line Drugs	MGIT™	VersaTREK™
Isoniazid	0.1 (and 0.4*)	0.1 (and 0.4*)
Rifampin	1.0†	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

* The higher concentration of INH and EMB should be tested after resistance at the critical concentration is detected [2].

† CLSI critical concentrations for RIF differ from revised WHO recommendation of 0.5 µg/ml published in 2021 [9].

Second-line Drugs	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
p-Aminosalicylic acid	Not recommended†
Rifapentine	Not determined
Bedaquiline	1.0
Linezolid	1.0
Clofazimine	1.0
Delamanid	0.06
Pretomanid	Not determined

NOTE—Critical concentrations as indicated in WHO 2018 Technical Report on critical concentrations unless noted otherwise [4]. Data for second-line critical concentrations not available for VersaTREK™.

* 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.

† WHO critical concentrations differ from CLSI M62 recommendations published in 2018 [3, 4].

- 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.

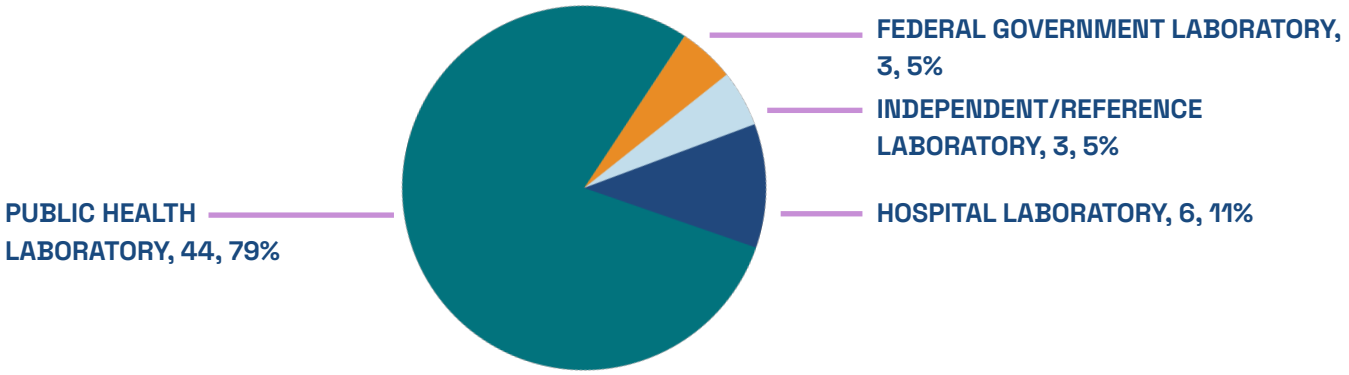
Descriptive Information about Participant Laboratories

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).

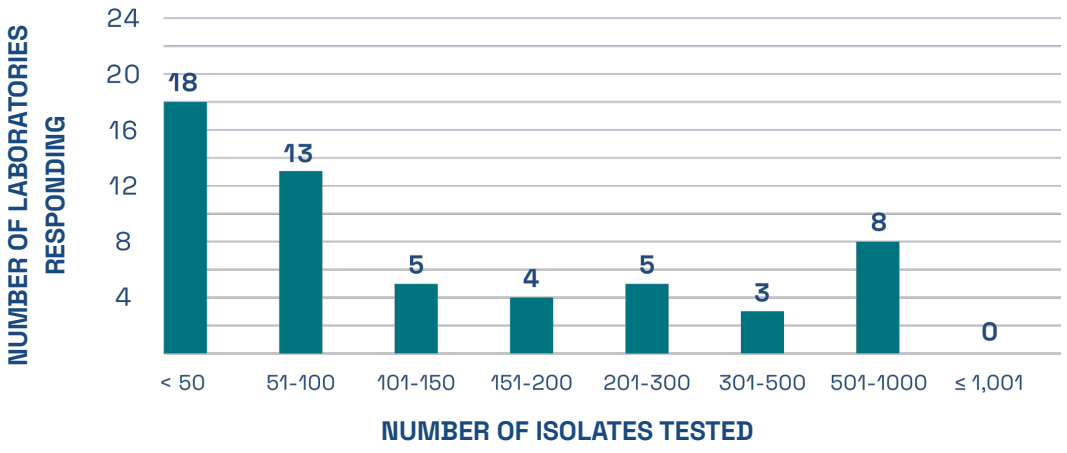
1 **FIGURE 1. PRIMARY CLASSIFICATION OF PARTICIPATING LABORATORIES, AUGUST 2023**



ANNUAL NUMBER OF MTBC DRUG SUSCEPTIBILITY TESTS PERFORMED

The number of MTBC isolates tested for drug susceptibility by the 56 participants in 2022 (excluding isolates used for quality control) is shown in Figure 2. In 2022, the counts ranged from 0 to 922 tests. Participants at 18 (32%) 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 proficiency [10].

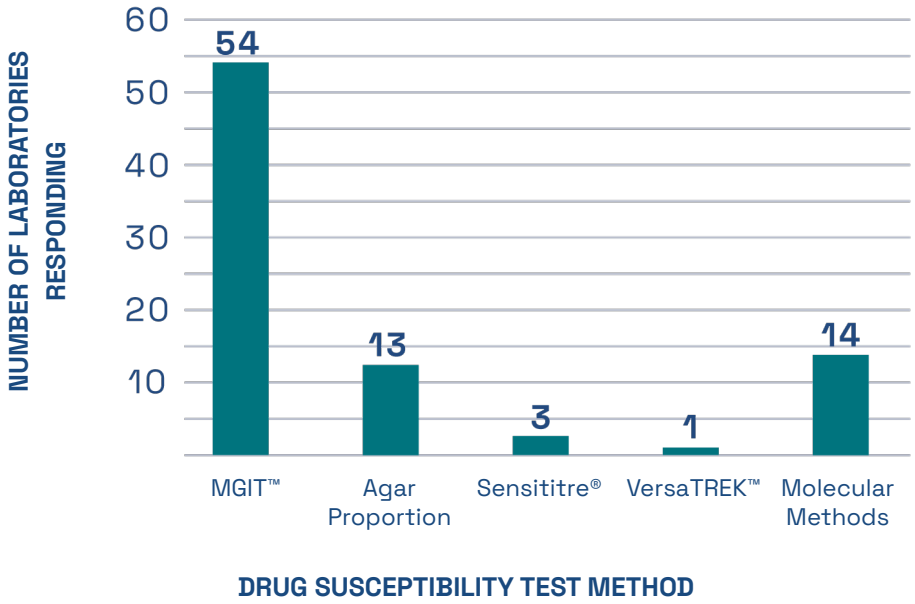
2 **FIGURE 2. DISTRIBUTION OF THE ANNUAL VOLUME OF MTBC ISOLATES TESTED FOR DRUG SUSCEPTIBILITY BY PARTICIPANTS IN PREVIOUS CALENDAR YEAR (N=56)**



MTBC DRUG SUSCEPTIBILITY TEST METHODS PERFORMED BY PARTICIPANTS

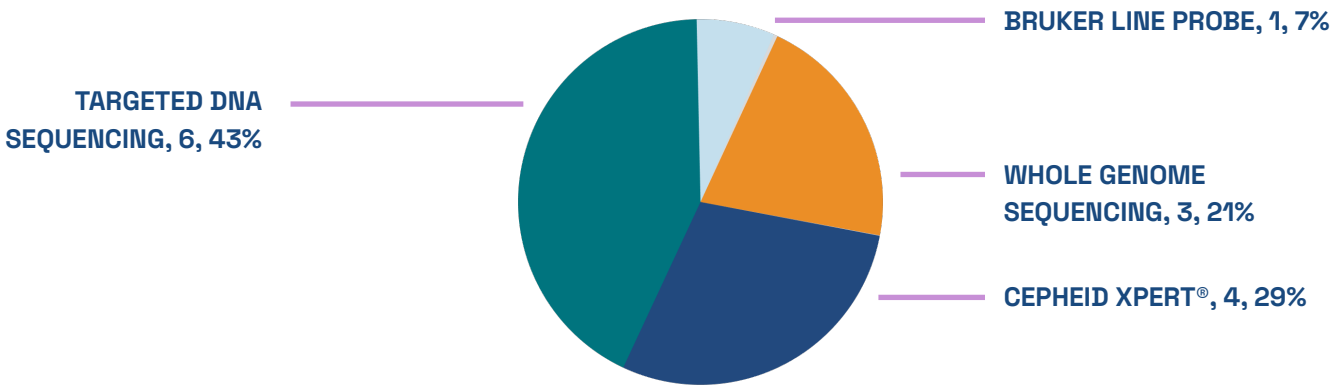
The DST methods that were performed by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Of participating laboratories, 31 (55%) reported results for only one method, 22 (39%) reported two methods, 2 (4%) reported three methods, and 1 (2%) noted four susceptibility methods. Fifty-four (96%) participating laboratories indicated use of MGIT.

3 FIGURE 3. MTBC DRUG SUSCEPTIBILITY TEST METHODS PERFORMED (N=85 RESPONSES)



Molecular methods reported by participants are shown in Figure 4. The method performed most frequently (43%) was targeted DNA sequencing.

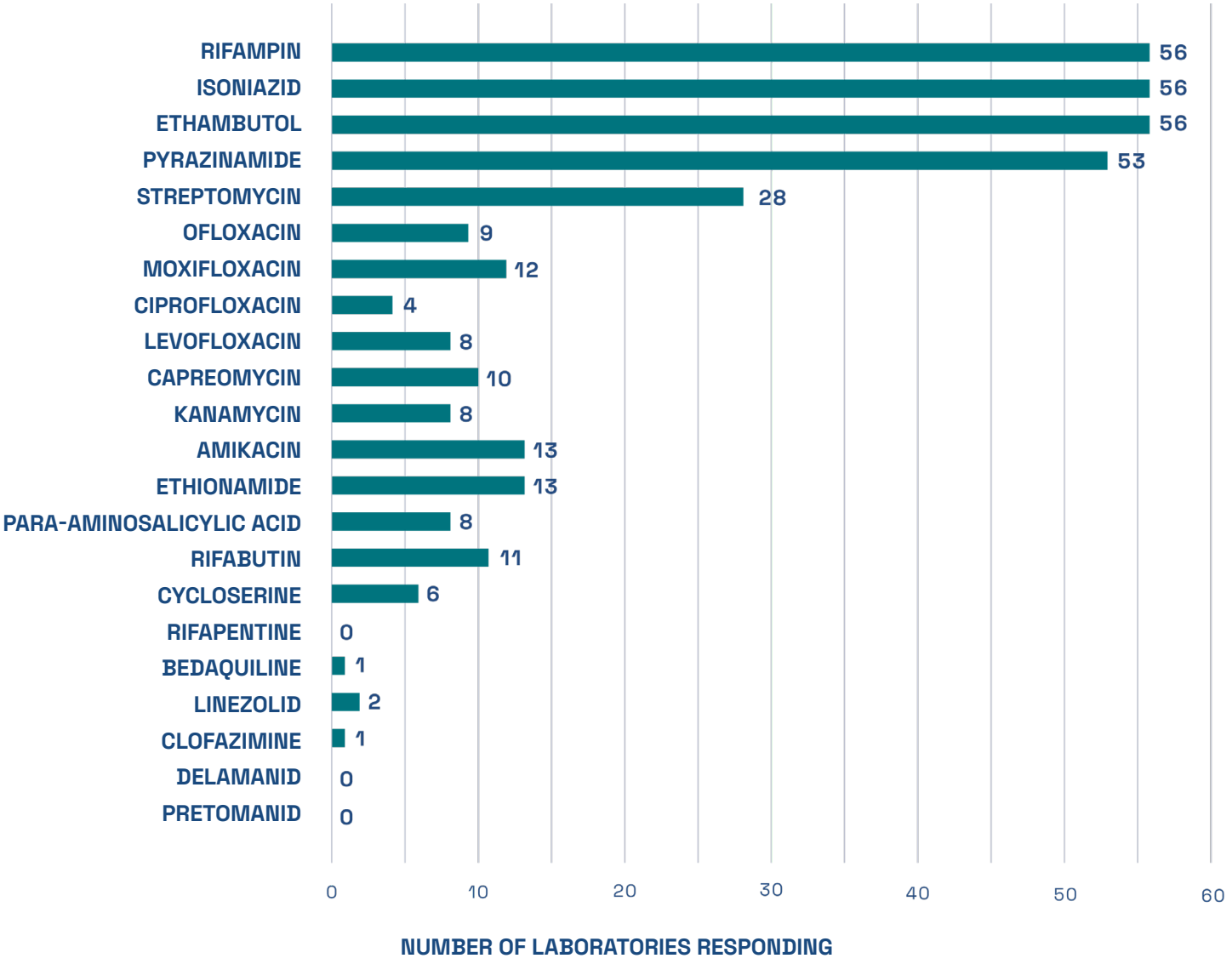
4 MOLECULAR METHOD REPORTED (N=14 RESPONSES)



ANTITUBERCULOSIS DRUGS TESTED BY PARTICIPANTS

The number of participating laboratories that reported testing each antituberculosis drug in the August 2023 panel is presented in Figure 5. CLSI recommends testing a full panel of first-line drugs (rifampin [RIF], isoniazid [INH], ethambutol [EMB], and pyrazinamide [PZA])[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 (FQ) to their testing panel as CDC recommends susceptibility testing for FQ (e.g., moxifloxacin) with use of the alternate 4-month rifapentine-moxifloxacin treatment regimen; RIF may be used as a proxy for rifapentine [11].

5 FIGURE 5. ANTITUBERCULOSIS DRUGS TESTED BY GROWTH-BASED METHOD BY PARTICIPANTS



Isolate 2023F

EXPECTED RESULTS:

Drug	Growth-based*	Molecular*
RIF	S	*rpoB* wild-type
INH	R (low-level†)	*katG* Asp94Asn◊; *inhA* & *fabG1* wild-type
EMB	S	*embB* wild-type
PZA	S	*pncA* wild-type
Fluoroquinolones	R	*gyrA* Asp94Gly; *gyrB* wild-type
STR	R	*rpsL* Lys43Arg; *rrs* wild-type

Note—S=susceptible, R=resistant

* Growth-based expected results performed by agar proportion, except for PZA which was performed by MGIT. Molecular expected results performed by whole genome sequencing.

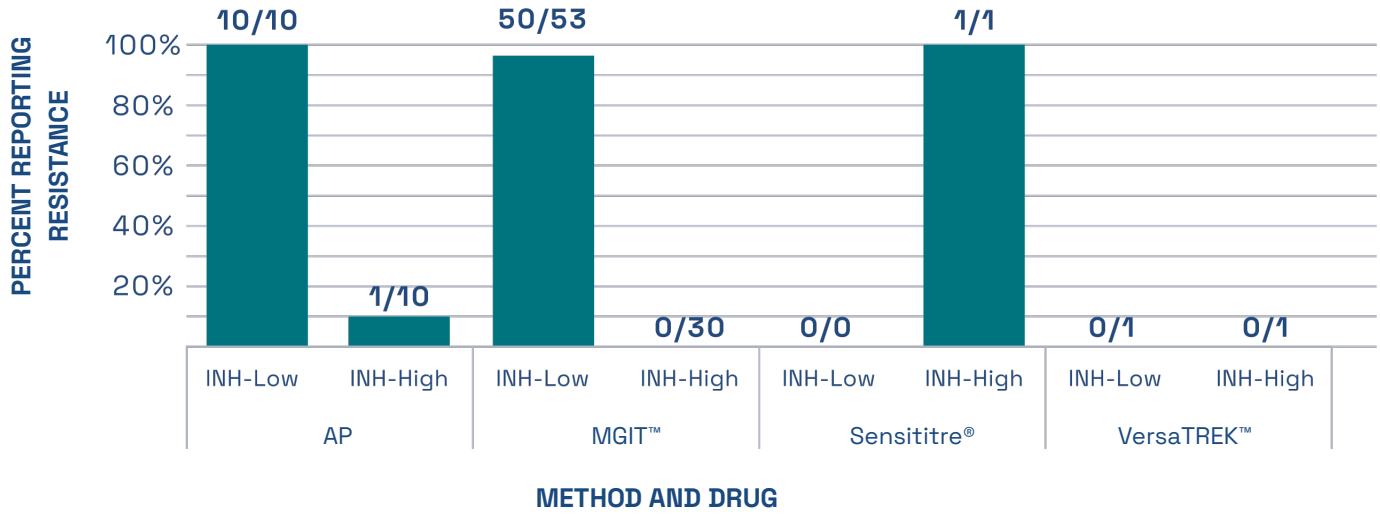
† Resistant at 0.2 µg/ml by agar proportion. See Equivalent Critical Concentration table on page 9 for more information.

◊ Effect of mutation is unknown.

ISONIAZID

DNA sequence analysis of *inhA, katG, fabG1,* and *ahpC* of Isolate 2023F revealed a G>A point mutation in the *katG* locus resulting in wild-type aspartic acid being replaced by asparagine at codon 94 (Asp94Asn); *inhA, fabG1,* and *ahpC* were wild-type (i.e., no mutations were detected). The effect of the *katG* Asp94Asn mutation for this isolate is unknown.

6 **FIGURE 6. ISOLATE 2023F: PERCENT OF LABORATORIES REPORTING INH-LOW AND INH-HIGH RESISTANCE, BY GROWTH-BASED METHOD.**



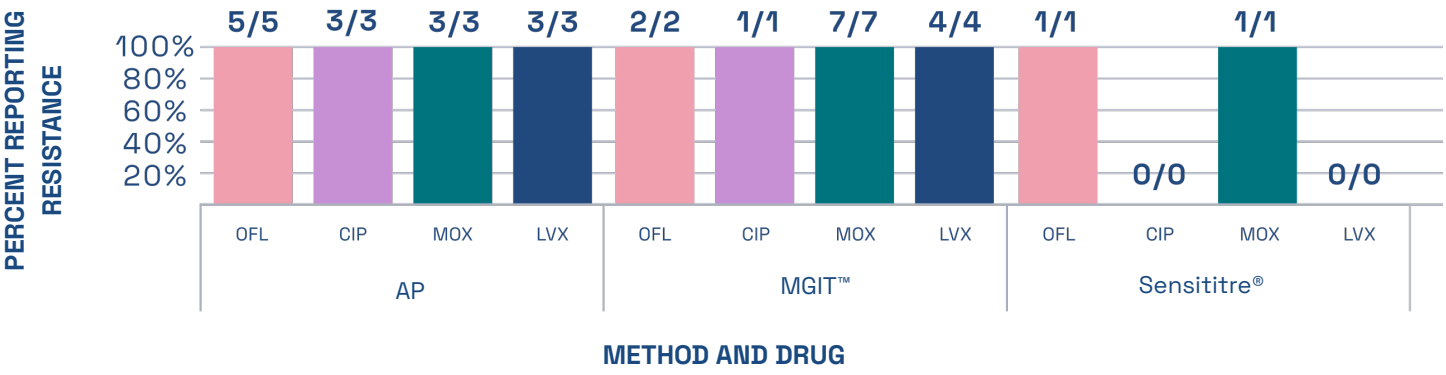
Note—Three laboratories performing Sensititre® reported INH MIC value as 0.25 µg/ml (n=3).

For internal comparison purposes, this isolate was previously sent as MPEP 2021G where 88% (14/16) of AP results, 97% (60/62) of MGIT™ results, 33% (1/3) of Sensititre® results, and 100% (2/2) of VersaTREK™ results were reported as resistant, by method, for the low concentration of isoniazid (INH-Low).

OFLOXACIN AND CIPROFLOXACIN

DNA sequencing of *gyrA* in Isolate 2023F 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 FQ resistance [12, 13].

7 **FIGURE 7. ISOLATE 2023F: PERCENT OF LABORATORIES REPORTING OFL, CIP, MOX, AND LVX RESISTANCE, BY GROWTH-BASED METHOD.**



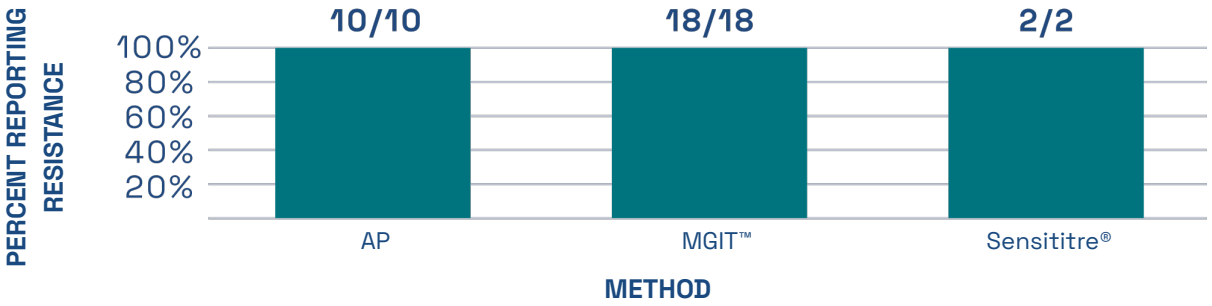
Note—Three of the laboratories performing Sensititre® reported FQ MIC values for OFL as 16 µg/ml (n=2), MOX as 4 µg/ml (n=3), and LVX as 8 µg/ml (n=1).

For internal comparison purposes, this isolate was previously sent as MPEP 2021G where comparable results, by method, were reported as resistant for ofloxacin (OFL), ciprofloxacin (CIP), moxifloxacin (MOX), and levofloxacin (LVX).

STREPTOMYCIN

DNA sequencing analysis revealed a A>G point mutation in *rpsL* resulting in wild-type lysine being replaced by arginine at codon 43 (Lys43Arg). This mutation has been associated with STR resistance [8].

8 **ISOLATE 2023F: PERCENT OF LABORATORIES REPORTING STR RESISTANCE, BY GROWTH-BASED METHOD.**



Note—Three of the laboratories performing Sensititre® reported STR MIC values as 32 µg/ml (n=1) and >32 µg/ml (n=2).

For internal comparison purposes, this isolate was previously sent as MPEP 2021G where comparable results, by method, were reported as resistant for streptomycin (STR).

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2023F are listed in Tables 3–10.

3 TABLE 3. ISOLATE 2023F—PARTICIPANT RESULTS FOR FIRST-LINE DST BY AP

Drug	Susceptible	Resistant	Total
Rifampin	11	0	11
Isoniazid—Low	0	10	10
Isoniazid—High	9	1	10
Ethambutol	10	0	10

4 TABLE 4. ISOLATE 2023F—PARTICIPANT RESULTS FOR FIRST-LINE DST BY MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	53	0	53
Isoniazid—Low	3	50	53
Isoniazid—High	30	0	30
Ethambutol	53	0	53
Pyrazinamide	42	8	50*

* Two additional laboratories reported 'No Interpretation' for PZA by MGIT™.

5 TABLE 5. ISOLATE 2023F—PARTICIPANT RESULTS FOR FIRST-LINE DST BY SENSITITRE®

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

* One additional laboratory reported 'Intermediate for INH-Low and INH-High by Sensititre®.

6 TABLE 6. ISOLATE 2023F—PARTICIPANT RESULTS FOR FIRST-LINE DST BY VERSATREK™

Drug	Susceptible	Resistant	Total
Rifampin	1	0	1
Isoniazid—Low	1	0	1
Isoniazid—High	1	0	1
Ethambutol	1	0	1
Pyrazinamide	1	0	1

7 TABLE 7. ISOLATE 2023F—PARTICIPANT RESULTS FOR SECOND-LINE DST BY AP

Drug	Susceptible	Resistant	Total
Streptomycin	0	10	10
Ofloxacin	0	5	5
Ciprofloxacin	0	3	3
Moxifloxacin	0	3	3
Levofloxacin	0	3	3
Amikacin	7	0	7
Kanamycin	5	0	5
Capreomycin	6	0	6
Ethionamide	7	0	7*
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

* One additional laboratory reported 'No Interpretation' for ETA by AP.

8 TABLE 8. ISOLATE 2023F—PARTICIPANT RESULTS FOR SECOND-LINE DST BY MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	0	18	18
Ofloxacin	0	2	2
Ciprofloxacin	0	1	1
Moxifloxacin	0	7	7
Levofloxacin	0	4	4
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	1	0	1
Clofazimine	1	0	1

9 TABLE 9. ISOLATE 2023F—PARTICIPANT RESULTS FOR SECOND-LINE DST BY SENSITITRE®

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

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

10 TABLE 10. ISOLATE 2023F—PARTICIPANT RESULTS FOR MOLECULAR TESTING

Drug	Mutation Not Detected	Mutation Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	13	0	13
Isoniazid	5	4*†	9
Ethambutol	6	0	6
Pyrazinamide	5	0	5
Streptomycin	2	3¥	5
Fluoroquinolones (Ofloxacin, Ciprofloxacin, Moxifloxacin, Levofloxacin)	0	8§	8
Amikacin	8	0	8
Kanamycin	8	0	8
Capreomycin	6	0	6
Ethionamide	5	0	5
Cycloserine	2	0	2
p-Aminosalicylic acid	2	0	2
Bedaquiline	3	0	3
Linezolid	3	0	3
Clofazimine	3	0	3
Delamanid	1	0	1
Pretomanid	0	0	0

* Four laboratories specifically noted the detection of *katG* Asp94Asn mutation.

† One laboratory also noted the detection of *katG* Arg463Leu mutation.

¥ Three laboratories noted the detection of *rpsL* Lys43Arg mutation.

§ Seven laboratories noted the detection of *gyrA* Asp94Gly mutation.

Isolate 2023G

EXPECTED RESULTS:

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

Note—S=susceptible, R=resistant

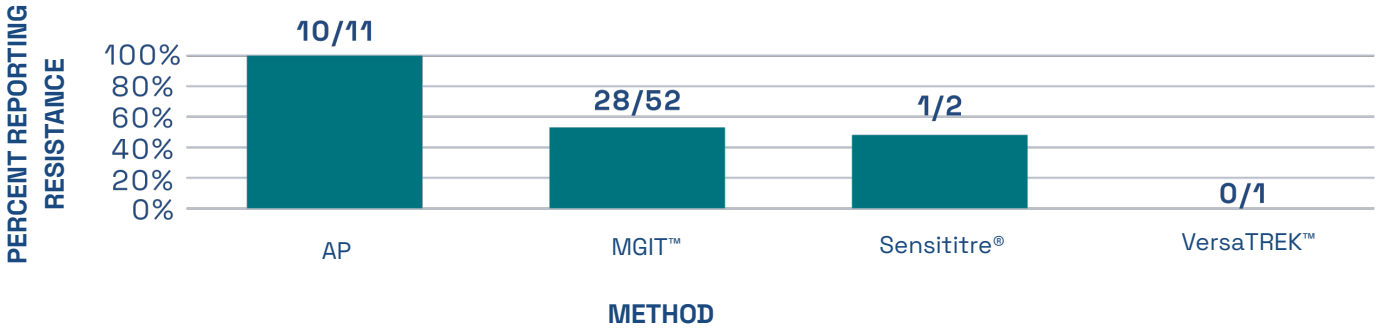
* Growth-based expected results performed by agar proportion, except for PZA which was performed by MGIT. Molecular expected results performed by whole genome sequencing.

† 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.

RIFAMPIN

DNA sequence analysis of *rpoB* in Isolate 2023G 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 RIF resistance and can test as susceptible in growth-based assays [8, 14, 15].

9 **FIGURE 9. ISOLATE 2023G: PERCENT OF LABORATORIES REPORTING RIF RESISTANCE, BY GROWTH-BASED METHOD.**



Note—Two of the laboratories performing Sensititre® reported RIF MIC values as 1.0 µg/ml (n=1) and 16 µg/ml (n=1).

For internal comparison purposes, this isolate was previously sent as MPEP 2022B where 87% (13/15) of AP results, 63% (33/52) of MGIT™ results, and 67% (2/3) of Sensititre® results were reported as RIF resistant, by method.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2023G are listed in Tables 11–18.

11 TABLE 11. ISOLATE 2023G—PARTICIPANT RESULTS FOR FIRST-LINE DST BY AP

Drug	Susceptible	Resistant	Total
Rifampin	1	10	11
Isoniazid—Low	10	0	10
Isoniazid—High	10	0	10
Ethambutol	10	0	10

12 TABLE 12. ISOLATE 2023G—PARTICIPANT RESULTS FOR FIRST-LINE DST BY MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	24	28	52*
Isoniazid—Low	53	0	53
Isoniazid—High	23	0	23
Ethambutol	53	0	53
Pyrazinamide	49	3	52

* One additional laboratory reported 'Intermediate' for RIF by MGIT™.

13 TABLE 13. ISOLATE 2023G—PARTICIPANT RESULTS FOR FIRST-LINE DST BY SENSITITRE®

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

14 TABLE 14. ISOLATE 2023G—PARTICIPANT RESULTS FOR FIRST-LINE DST BY VERSATREK™

Drug	Susceptible	Resistant	Total
Rifampin	1	0	1
Isoniazid—Low	1	0	1
Isoniazid—High	1	0	1
Ethambutol	1	0	1
Pyrazinamide	1	0	1

15 TABLE 15. ISOLATE 2023G—PARTICIPANT RESULTS FOR SECOND-LINE DST BY AP

Drug	Susceptible	Resistant	Total
Streptomycin	9	1	10
Ofloxacin	5	0	5
Ciprofloxacin	3	0	3
Moxifloxacin	3	0	3
Levofloxacin	3	0	3
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

16 TABLE 16. ISOLATE 2023G—PARTICIPANT RESULTS FOR SECOND-LINE DST BY MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	19	0	19
Ofloxacin	2	0	2
Ciprofloxacin	1	0	1
Moxifloxacin	6	0	6*
Levofloxacin	4	0	4
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	4	0	4
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Bedaquiline	1	0	1
Linezolid	1	0	1
Clofazimine	1	0	1

* One additional laboratory reported 'No Interpretation' for MOX by MGIT™.

17 TABLE 17. ISOLATE 2023G—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	0	0	0*
p-Aminosalicylic acid	2	0	2*
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0

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

18 TABLE 18. ISOLATE 2023G—PARTICIPANT RESULTS FOR MOLECULAR TESTING

Drug	Mutation Not Detected	Mutation Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	0	13*	13
Isoniazid	9	0	9
Ethambutol	6	0	6
Pyrazinamide	5	0	5
Streptomycin	4	0	4
Fluoroquinolones (Ofloxacin, Ciprofloxacin, Moxifloxacin, Levofloxacin)	7	1†	8
Amikacin	8	0	8
Kanamycin	8	0	8
Capreomycin	6	0	6
Ethionamide	5	0	5
Cycloserine	2	0	2
p-Aminosalicylic acid	2	0	2
Bedaquiline	3	0	3
Linezolid	3	0	3
Clofazimine	3	0	3
Delamanid	1	0	1
Pretomanid	0	0	0

* Seven laboratories noted the detection of *rpoB* His445Leu mutation. Additionally, one laboratory performing Xpert® MTB/RIF assay noted Probe D did not bind and another laboratory performing Hain line probe assay noted missing wild-type band 7.

† This laboratory noted the detection of a *gyrA* mutation not associated with FQ resistance.

Isolate 2023H

EXPECTED RESULTS:

Drug	Growth-based*	Molecular*
RIF	S	*rpoB* Arg447Arg†
INH	R (low-level◊)¥	*fabG1* Leu203Leu; *katG* & *inhA* wild-type
EMB	S	*embB* wild-type
PZA	S	*pncA* wild-type
Fluoroquinolones	S	*gyrA* & *gyrB* wild-type

Note—S=susceptible, R=resistant

* Growth-based expected results performed by agar proportion, except for PZA which was performed by MGIT. Molecular expected results performed by whole genome sequencing.

† Mutation not associated with resistance [8].

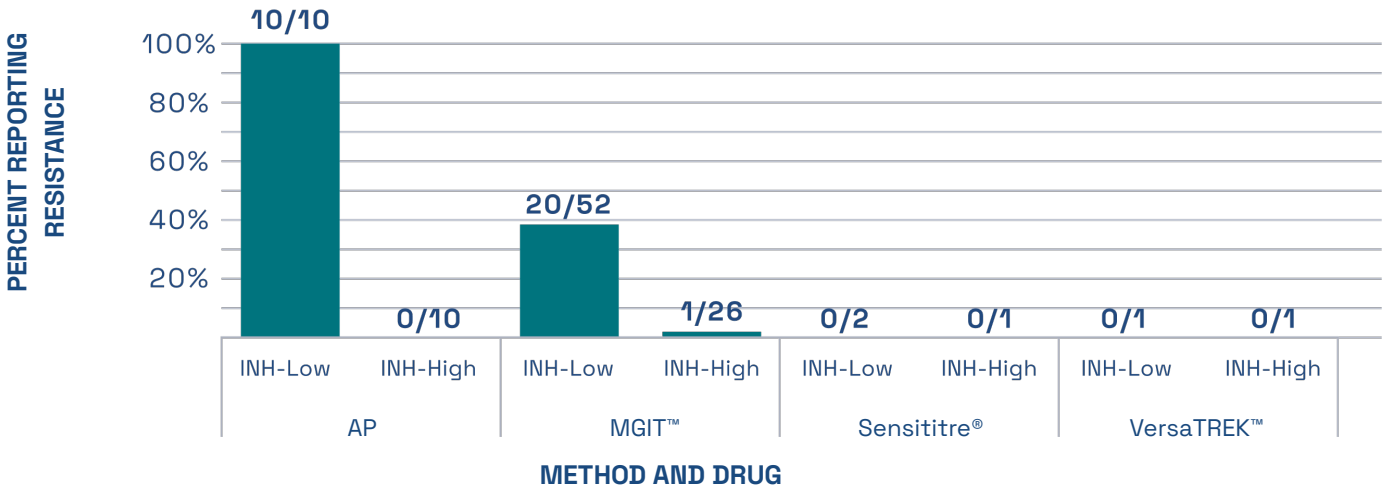
◊ Resistant at 0.2 µg/ml by agar proportion. See Equivalent Critical Concentration table on page 9 for more information.

¥ 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.

ISONIAZID

DNA sequence analysis of *fabG1* in Isolate 2023H revealed a G>A point mutation in codon 203 in wild-type leucine being replaced by leucine (Leu203Leu). Within *fabG1*, the silent/synonymous mutation (i.e., nucleotide change but no corresponding change in amino acid) Leu203Leu has been found to confer INH resistance [16]. Although synonymous mutations were previously believed to not play a role in drug resistance, the Leu203Leu mutation demonstrates that synonymous mutations could be associated with resistance depending on the specific gene and the location of the mutation.

10 **FIGURE 10. ISOLATE 2023H: PERCENT OF LABORATORIES REPORTING INH-LOW AND INH-HIGH RESISTANCE, BY GROWTH-BASED METHOD.**



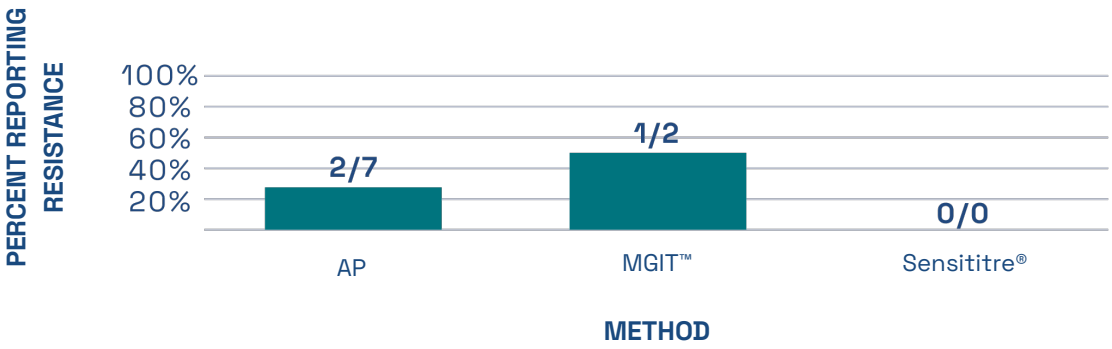
Note—Three of the laboratories performing Sensititre® reported INH MIC values as 0.06 µg/ml (n=2) and 0.12 µg/ml (n=2).

For internal comparison purposes, this isolate was previously sent as MPEP 2020I. For 2020I, 82% (14/17) of AP results, 55% (32/58) of MGIT™ results, 0% (0/3) of Sensititre® results, and 100% (2/2) of VersaTREK™ results were reported as INH resistant, by method.

ETHIONAMIDE

Resistance to ETA is commonly due to mutations in the *ethA* gene or mutations in *fabG1* or *inhA* resulting in cross-resistance with INH. DNA sequencing analysis revealed the *fabG1* Leu203Leu mutation; *ethA* was wild-type (i.e., no mutations were detected).

11 **FIGURE 11. ISOLATE 2023H: PERCENT OF LABORATORIES REPORTING ETA RESISTANCE, BY GROWTH-BASED METHOD.**



Note—Two of the laboratories performing Sensititre® reported an ETA MIC value as 2.5 µg/ml (n=2).

For internal comparison purposes, this isolate was previously sent as MPEP 2020I where 71% (10/14) of AP results, 67% (2/3) of MGIT™ results, and 0% (0/1) of Sensititre® results were reported as ETA resistant, by method.

RIFAMPIN

DNA sequence analysis of *rpoB* in Isolate 2023I revealed a C>T point mutation in codon 447 of *rpoB* resulting in wild-type arginine being replaced by arginine (Arg447Arg). The Arg447Arg synonymous (i.e., silent) mutation in *rpoB* is not considered clinically significant and isolates with this mutation reliably test as RIF-susceptible in growth-based systems [17]. However, Xpert® MTB/RIF assay could indicate RIF resistance for this isolate and sequencing of *rpoB* should be performed [18].

For internal comparison purposes, this isolate was previously sent as MPEP 2020I where 0% (0/17) of AP results, 0% (0/60) of MGIT™ results, 0% (0/3) of Sensititre® results, and 0% (0/2) of VersaTREK™ results were reported as RIF resistant, by method.

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2023H are listed in Tables 19–26.

19 TABLE 19. ISOLATE 2023H—PARTICIPANT RESULTS FOR FIRST-LINE DST BY AP

Drug	Susceptible	Resistant	Total
Rifampin	11	0	11
Isoniazid—Low	0	10	10
Isoniazid—High	10	0	10
Ethambutol	10	0	10

20 TABLE 20. ISOLATE 2023H—PARTICIPANT RESULTS FOR FIRST-LINE DST BY MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	53	0	53
Isoniazid—Low	32	20	53*
Isoniazid—High	25	1	26
Ethambutol	52	1	53
Pyrazinamide	50	1	51

* One additional laboratory reported 'Intermediate' for INH-Low by MGIT™.

21 TABLE 21. ISOLATE 2023H—PARTICIPANT RESULTS FOR FIRST-LINE DST BY SENSITITRE®

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

22 TABLE 22. ISOLATE 2023H—PARTICIPANT RESULTS FOR FIRST-LINE DST BY VERSATREK™

Drug	Susceptible	Resistant	Total
Rifampin	1	0	1
Isoniazid—Low	1	0	1
Isoniazid—High	1	0	1
Ethambutol	1	0	1
Pyrazinamide	1	0	1

23 TABLE 23. ISOLATE 2023H—PARTICIPANT RESULTS FOR SECOND-LINE DST BY AP

Drug	Susceptible	Resistant	Total
Streptomycin	10	0	10
Ofloxacin	5	0	5
Ciprofloxacin	3	0	3
Moxifloxacin	3	0	3
Levofloxacin	3	0	3
Amikacin	7	0	7
Kanamycin	5	0	5
Capreomycin	6	0	6
Ethionamide	5	2	7*
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

* One additional laboratory reported 'No Interpretation' for ETA by AP.

24 TABLE 24. ISOLATE 2023H—PARTICIPANT RESULTS FOR SECOND-LINE DST BY MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	18	0	18
Ofloxacin	2	0	2
Ciprofloxacin	1	0	1
Moxifloxacin	6	0	6
Levofloxacin	4	0	4
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	2	0	2
Ethionamide	1	1	2
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0
Bedaquiline	1	0	1
Linezolid	1	0	1
Clofazimine	1	0	1

25 TABLE 25. ISOLATE 2023H—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	0	0	0*
p-Aminosalicylic acid	2	0	2*
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0

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

26 TABLE 26. ISOLATE 2023H—PARTICIPANT RESULTS FOR MOLECULAR TESTING

Drug	Mutation Not Detected	Mutation Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	8	5*	13
Isoniazid	3	6†	9
Ethambutol	6	0	6
Pyrazinamide	5	0	5
Streptomycin	5	0	5
Fluoroquinolones (Ofloxacin, Ciprofloxacin, Moxifloxacin, Levofloxacin)	7	1¥	8
Amikacin	8	0	8
Kanamycin	8	0	8
Capreomycin	6	0	6
Ethionamide	1	4§	5
Cycloserine	2	0	2
p-Aminosalicylic acid	2	0	2
Bedaquiline	3	0	3
Linezolid	3	0	3
Clofazimine	3	0	3
Delamanid	1	0	1
Pretomanid	0	0	0

* Five laboratories noted the detection of *rpoB* Arg447Arg mutation.

† Six laboratories noted the detection of *fabG1* Leu203Leu mutation.

¥ This laboratory noted the detection of a *gyrA* mutation not associated with FQ resistance.

§ Four laboratories noted the detection of *fabG1* Leu203Leu mutation also associated with ETA resistance.

Isolate 2023I

EXPECTED RESULTS:

Drug	Growth-based*	Molecular*
RIF	R†	*rpoB* Asp435Tyr
INH	S	*katG*, *inhA*, & *fabG1* wild-type
EMB	S	*embB* wild-type
PZA	S	*pncA* wild-type
Fluoroquinolones	S	*gyrA* & *gyrB* wild-type

Note—S=susceptible, R=resistant

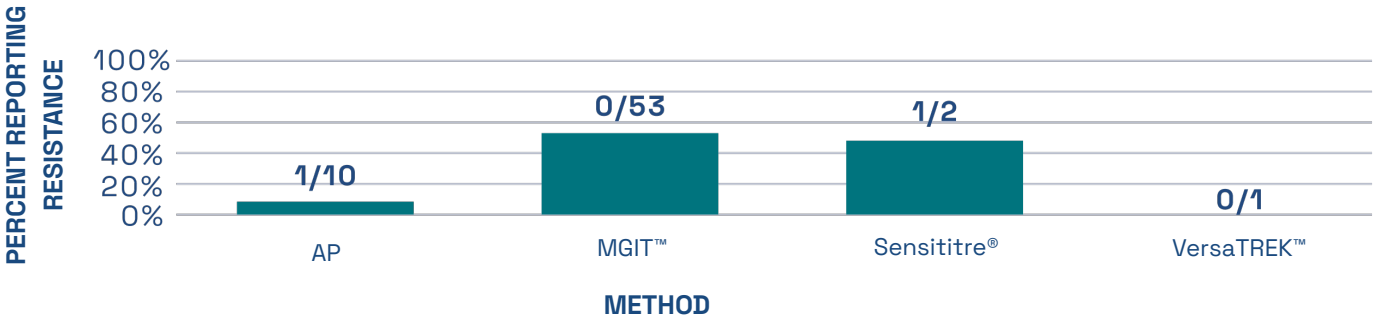
* Growth-based expected results performed by agar proportion, except for PZA which was performed by MGIT. Molecular expected results performed by whole genome sequencing.

† 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.

RIFAMPIN

DNA sequence analysis of *rpoB* in Isolate 2023I revealed a G>T point mutation in codon 435 of *rpoB* resulting in wild-type aspartic acid being replaced by tyrosine (Asp435Tyr). Isolates with Asp435Tyr mutations are associated with low-level RIF resistance and can test as susceptible in growth-based assays [8, 14, 15]. Although this mutation is associated with low-level RIF resistance, participating laboratories' growth-based DST did not detect resistance; this is likely due to the RIF critical concentration being too high and consideration should be given to evaluating a lower critical concentration [5, 19, 20].

12 **FIGURE 12. ISOLATE 2023I: PERCENT OF LABORATORIES REPORTING RIF RESISTANCE, BY GROWTH-BASED METHOD.**



Note—Two of the laboratories performing Sensititre® reported RIF MIC values as 0.5 µg/ml (n=1) and 2 µg/ml (n=1).

For internal comparison purposes, this isolate was previously sent as MPEP 2022D where 0% (0/12) of AP results, 0% (0/59) of MGIT™ results, and 33% (1/3) of Sensititre® results were reported as RIF resistant, by method.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2023I are listed in Tables 27–34.

27 TABLE 27. ISOLATE 2023I—PARTICIPANT RESULTS FOR FIRST-LINE DST BY AP

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

28 TABLE 28. ISOLATE 2023I—PARTICIPANT RESULTS FOR FIRST-LINE DST BY MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	53	0	53
Isoniazid—Low	53	0	53
Isoniazid—High	23	0	23
Ethambutol	53	0	53
Pyrazinamide	42	8	50*

* One additional laboratory reported 'No Interpretation' for PZA by MGIT™.

29 TABLE 29. ISOLATE 2023I—PARTICIPANT RESULTS FOR FIRST-LINE DST BY SENSITITRE®

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

30 TABLE 30. ISOLATE 2023I—PARTICIPANT RESULTS FOR FIRST-LINE DST BY VERSATREK™

Drug	Susceptible	Resistant	Total
Rifampin	1	0	1
Isoniazid—Low	1	0	1
Isoniazid—High	1	0	1
Ethambutol	1	0	1
Pyrazinamide	1	0	1

31 TABLE 31. ISOLATE 2023I—PARTICIPANT RESULTS FOR SECOND-LINE DST BY AP

Drug	Susceptible	Resistant	Total
Streptomycin	9	0	9
Ofloxacin	5	0	5
Ciprofloxacin	3	0	3
Moxifloxacin	3	0	3
Levofloxacin	3	0	3
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

32 TABLE 32. ISOLATE 2023I—PARTICIPANT RESULTS FOR SECOND-LINE DST BY MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	18	0	18
Ofloxacin	2	0	2
Ciprofloxacin	1	0	1
Moxifloxacin	6	0	6
Levofloxacin	4	0	4
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	1	0	1
Clofazimine	1	0	1

33 TABLE 33. ISOLATE 2023I—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	0	0	0*
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

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

34 TABLE 34. ISOLATE 2023I—PARTICIPANT RESULTS FOR MOLECULAR TESTING

Drug	Mutation Not Detected	Mutation Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	2	11*	13
Isoniazid	8	1†	9
Ethambutol	5	1¥	6
Pyrazinamide	5	0	5
Streptomycin	5	0	5
Fluoroquinolones (Ofloxacin, Ciprofloxacin, Moxifloxacin, Levofloxacin)	7	1§	8
Amikacin	8	0	8
Kanamycin	8	0	8
Capreomycin	6	0	6
Ethionamide	5	0	5
Cycloserine	2	0	2
p-Aminosalicylic acid	2	0	2
Bedaquiline	3	0	3
Linezolid	2	1‡	3
Clofazimine	3	0	3
Delamanid	1	0	1
Pretomanid	0	0	0

* Eight laboratories noted the detection of *rpoB* Asp435Tyr mutation. Additionally, one laboratory performing Hain line probe assay noted missing wild-type bands 3 and 4.

† This laboratory noted the detection of *katG* Arg463Leu mutation.

¥ Although only one laboratory reported a mutation detected, two laboratories noted an *embB* Glu378Ala mutation in the comments.

§ This laboratory noted the detection of a *gyrA* mutation not associated with FQ resistance.

‡ This laboratory noted the detection of *rrl* 982G>A mutation.

Isolate 2023J

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
Fluoroquinolones	S	*gyrA* & *gyrB* wild-type

Note—S=susceptible, R=resistant

* Growth-based expected results performed by agar proportion, except for PZA which was performed by MGIT. Molecular expected results performed by whole genome sequencing.

PAN-SUSCEPTIBLE

Isolate 2023J was expected to be susceptible to all first- and second-line drugs.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2023J are listed in Tables 35–42.

One laboratory noted contaminated/no growth for Isolate 2023J and did not report results for at least one antituberculosis drug tested.

35 TABLE 35. ISOLATE 2023J—PARTICIPANT RESULTS FOR FIRST-LINE DST BY AP

Drug	Susceptible	Resistant	Total
Rifampin	11	0	11
Isoniazid—Low	10	0	10
Isoniazid—High	10	0	10
Ethambutol	10	0	10

36 TABLE 36. ISOLATE 2023J—PARTICIPANT RESULTS FOR FIRST-LINE DST BY MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	53	0	53
Isoniazid—Low	53	0	53
Isoniazid—High	23	0	23
Ethambutol	53	0	53
Pyrazinamide	49	2	51

37 TABLE 37. ISOLATE 2023J—PARTICIPANT RESULTS FOR FIRST-LINE DST BY SENSITITRE®

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

38 TABLE 38. ISOLATE 2023J—PARTICIPANT RESULTS FOR FIRST-LINE DST BY VERSATREK™

Drug	Susceptible	Resistant	Total
Rifampin	1	0	1
Isoniazid—Low	1	0	1
Isoniazid—High	1	0	1
Ethambutol	1	0	1
Pyrazinamide	0	0	0

39 TABLE 39. ISOLATE 2023J—PARTICIPANT RESULTS FOR SECOND-LINE DST BY AP

Drug	Susceptible	Resistant	Total
Streptomycin	10	0	10
Ofloxacin	5	0	5
Ciprofloxacin	3	0	3
Moxifloxacin	3	0	3
Levofloxacin	3	0	3
Amikacin	7	0	7
Kanamycin	5	0	5
Capreomycin	4	2	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

40 TABLE 40. ISOLATE 2023J—PARTICIPANT RESULTS FOR SECOND-LINE DST BY MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	18	0	18
Ofloxacin	2	0	2
Ciprofloxacin	1	0	1
Moxifloxacin	6	0	6
Levofloxacin	4	0	4
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	1	0	1
Clofazimine	1	0	1

41 TABLE 41. ISOLATE 2023J—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	0	0	0*
p-Aminosalicylic acid	2	0	2*
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0

* One additional laboratory reported 'No Interpretation' for OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre®.

42 TABLE 42. ISOLATE 2023J—PARTICIPANT RESULTS FOR MOLECULAR TESTING

Drug	Mutation Not Detected	Mutation Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	10	3*	13
Isoniazid	9	0	9
Ethambutol	6	0	6
Pyrazinamide	6	0	6
Streptomycin	5	0	5
Fluoroquinolones (Ofloxacin, Ciprofloxacin, Moxifloxacin, Levofloxacin)	7	1†	8
Amikacin	8	0	8
Kanamycin	8	0	8
Capreomycin	6	0	6
Ethionamide	5	0	5
Cycloserine	2	0	2
p-Aminosalicylic acid	2	0	2
Bedaquiline	3	0	3
Linezolid	3	0	3
Clofazimine	3	0	3
Delamanid	1	0	1
Pretomanid	0	0	0

* Three laboratories noted the detection of *rpoB* Pro454Ser mutation, located outside the rifampin resistance determining region.

† This laboratory noted the detection of a *gyrA* mutation not associated with FQ resistance.

References

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Appendix 1: Accessible Explanations of Figures

Figure 1. The primary classification of the 56 laboratories participating in the August 2023 MPEP panel is shown in this pie chart. The largest slice represents 44 laboratories, or 79% of 56 that have self-classified as a health department laboratory. The next major slice signifies 6 laboratories, or 11% of 56 that self-classified as hospital laboratories. The remaining two slices of the pie chart represent 3, or 5% of 56 that self-classified as independent laboratories; and 3, or 5% of 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 2022 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 5. Along the horizontal x-axis are eight vertical bars representing the number of isolates tested per year. From left to right, 18 laboratories tested less than or equal to 50 isolates per year; 13 laboratories tested between 51 to 100 isolates per year; 5 laboratories tested between 101 to 150 isolates per year; 4 laboratories tested between 151 to 200 isolates per year; 5 laboratories tested between 201 to 300 isolates per year; 3 laboratories tested between 301 to 500 isolates per year; 8 laboratories tested between 501 to 1000 isolates per year; and 0 laboratories tested greater than or equal to 1,001 isolates per year.

Figure 3. The drug susceptibility testing methods performed by MPEP participants (N=85) is 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: 54 performed MGIT™, 13 performed agar proportion, 3 performed Sensititre®, 1 performed VersaTREK™, and 14 performed molecular methods.

Figure 4. The molecular methods performed by MPEP participants (N=14) are displayed in this pie chart. The largest slice represents the 6 laboratories that performed targeted DNA sequencing. The next three slices represent 4 laboratories that performed the Cepheid Xpert® MTB/RIF assay, 3 laboratories that performed whole genome sequencing, and 1 laboratory that performed Bruker line probe assays.

Figure 5. The antituberculosis drugs tested by growth-based method by MPEP participants is 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. 56 laboratories tested rifampin; 56 laboratories tested isoniazid; 56 laboratories tested ethambutol; 53 laboratories tested pyrazinamide; 28 laboratories tested streptomycin; 9 laboratories tested ofloxacin; 12 laboratories tested moxifloxacin; 4 laboratories tested ciprofloxacin; 8 laboratories tested levofloxacin; 10 laboratories tested capreomycin; 8 laboratories tested kanamycin; 13 laboratories tested amikacin; 13 laboratories tested ethionamide; 8 laboratories tested PAS; 11 laboratories tested rifabutin; 6 laboratories tested cycloserine; 0 laboratories tested rifapentine; 1 laboratory tested bedaquiline; 2 laboratories tested linezolid; 1 laboratory tested clofazimine; 0 laboratories tested delamanid; and 0 laboratories tested pretomanid.

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Figure 6. The percent of laboratories reporting resistance to isoniazid (low and high concentrations), by growth-based method, for isolate 2023F 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 10 of 10 (100%) reporting resistance and INH-High is 1 of 10 (10%) reporting resistance; laboratories performing MGIT for INH-Low is 50 of 53 (94%) reporting resistance and INH-High is 0 of 30 (0%) reporting resistance; laboratories performing Sensititre for INH-Low is 0 of 0 (0%) reporting resistance and INH-High is 1 of 1 (100%) reporting resistance; and laboratories performing VersaTREK for INH-Low is 0 of 1 (0%) reporting resistance and INH-High is 0 of 1 (0%) reporting resistance.

Figure 7. The percent of laboratories reporting resistance to ofloxacin, ciprofloxacin, moxifloxacin, and levofloxacin, by growth-based method, for isolate 2023F 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 3 of 3 (100%) reporting resistance, MOX is 3 of 3 (100%) reporting resistance, LVX is 3 of 3 (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 7 of 7 (100%) reporting resistance, LVX is 4 of 4 (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 8. The percent of laboratories reporting resistance to streptomycin, by growth-based method, for isolate 2023F 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 10 of 10 (100%) reporting resistance; laboratories performing MGIT for streptomycin is 18 of 18 (100%) reporting resistance; and laboratories performing Sensititre for streptomycin is 2 of 2 (100%) reporting resistance.

Figure 9. The percent of laboratories reporting resistance to rifampin, by growth-based method, for 2023G 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 10 of 11 (91%) reporting resistance; laboratories performing MGIT for rifampin is 28 of 52 (54%) reporting resistance; laboratories performing Sensititre for rifampin is 1 of 2 (50%) reporting resistance; and laboratories performing VersaTREK for rifampin is 0 of 1 (0%) reporting resistance.

Figure 10. The percent of laboratories reporting resistance to isoniazid (low and high concentrations), by growth-based method, for isolate 2023H 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 10 of 10 (100%) reporting resistance and INH-High is 0 of 10 (0%) reporting resistance; laboratories performing MGIT for INH-Low is 20 of 52 (38%) reporting resistance and INH-High is 1 of 26 (4%) reporting resistance; laboratories performing Sensititre for INH-Low is 0 of 2

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(0%) reporting resistance and INH-High is 0 of 1 (0%) reporting resistance; and laboratories performing VersaTREK for INH-Low is 0 of 1 (0%) reporting resistance and INH-High is 0 of 1 (0%) reporting resistance.

Figure 11. The percent of laboratories reporting resistance to ethionamide, by growth-based method, for isolate 2023H 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 2 of 7 (29%) reporting resistance; laboratories performing MGIT for ethionamide is 1 of 2 (50%) reporting resistance; and laboratories performing Sensititre for ethionamide is 0 of 0 (0%) reporting resistance.

Figure 12. The percent of laboratories reporting resistance to rifampin, by growth-based method, for 2023I 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 1 of 10 (10%) reporting resistance; laboratories performing MGIT for rifampin is 0 of 53 (0%) reporting resistance; laboratories performing Sensititre for rifampin is 1 of 2 (50%) reporting resistance; and laboratories performing VersaTREK for rifampin is 0 of 1 (0%) reporting resistance.