For accessible version: https://www.cdc.gov/tb/topic/laboratory/mpep/2022/feb-mpepReport.htm.

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Model Performance Evaluation Program
Report of Results
February 2022

Centers for Disease Control and Prevention
National Center for HIV, Viral Hepatitis, STD, and TB Prevention

CS 335864-A

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for February 2022 Survey

Purpose

The purpose of this report is 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 survey sent to participants in February 2022.

Report Content

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Note on Accessibility:
Find descriptions and explanations of figures in Appendix 1: Accessible Explanation of Figures on page 39.

Contents

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™ 960—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
RMP	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

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 tests (DST) for MTBC in the United States. MPEP is a voluntary program, and this report reflects data received from participating laboratory personnel. This aggregate report is prepared in a format that will allow laboratory personnel to compare their 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 isolates.

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, *Nocardiae* spp., and Other Aerobic Actinomycetes" and "M62: 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 INH, RMP, EMB, PZA and 12 second-line anti-tuberculosis drugs [3, 4]. Based on the systematic review data, recommendations were made for adjustments to critical concentrations for RMP, MOX, LVX, AMK, and KAN for some DST methods.

Expected Drug Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in February 2022 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 [5].

Table 1. Expected Growth-based Results for February 2022 Survey

Note—S=susceptible, R=resistant

Isolate	RMP	INH	EMB	PZA	Second-line Drug Resistances:
2022A	S	S	S	S*	
2022B	S*	S	S	S	
2022C	S	R	S	S	OFL, CIP, ETA
2022D	S*	S	S	S	
2022E	S	R	R*	S	STR*, ETA*

*80% consensus for a single categorical result across all methods reported for this drug of either susceptible or resistant was not achieved for these isolates among participating laboratories.

Table 2. Expected Molecular Results (Mutations Detected in Loci Associated with Resistance) for February 2022 Survey

Note—Empty cell=No mutation detected

Isolate	*rpoB*[¥]	*katG*	*inhA*	*embB*	*pncA*	*gyrA*	*ethA*
2022A					Glu37Val		
2022B	His445Leu* (His526Leu)[†]						
2022C			C-15T			Ala90Val	
2022D	Asp435Tyr* (Asp516Tyr)[†]						
2022E		Ser315Thr		Met306Val			Partial deletion

[¥] Mutation is listed using both the *M. tuberculosis* and *E.coli* numbering system [6, 7]

* *M. tuberculosis* numbering system used

[†] *E. coli* numbering system used

Technical Notes

The following information pertains to all of the tables and figures for the 2022 MTBC isolates A, B, C, D, and E included in this report.

- The source of data in all tables and figures is the February 2022 MPEP MTBC DST survey.
- 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.
- Laboratories that use more than one DST method are encouraged to test isolates with each of those methods at either CLSI-recommended or 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 to provide a categorical interpretation of S or R.
- For participant result tables for first- and second-line DST that have drug-method totals equal to 0, results were not received, or the test was not performed.
- VersaTREK™ tables are not included in this report since results were not received for this method for the February MPEP MTBC DST survey.

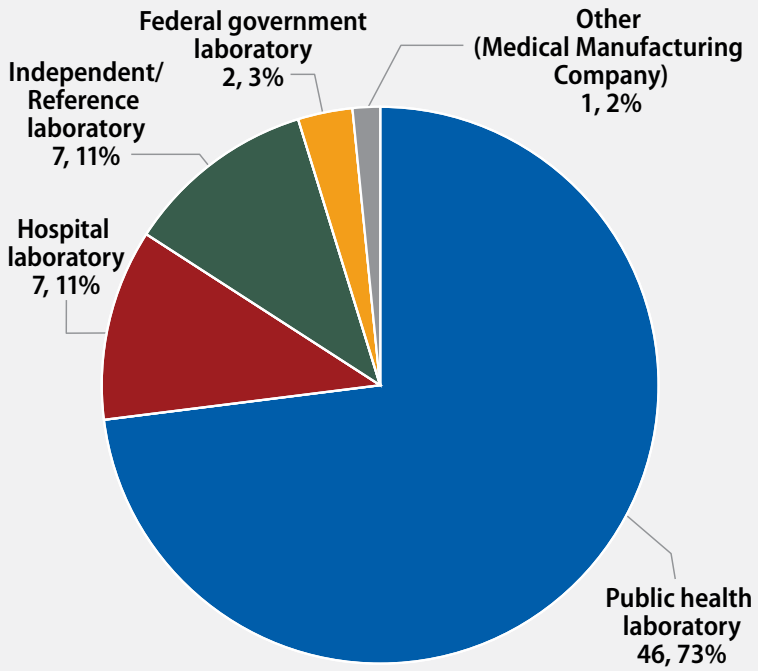
Descriptive Information about Participant Laboratories

Primary Classification

This report contains DST results submitted to CDC by survey participants at 63 laboratories in 33 states.

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

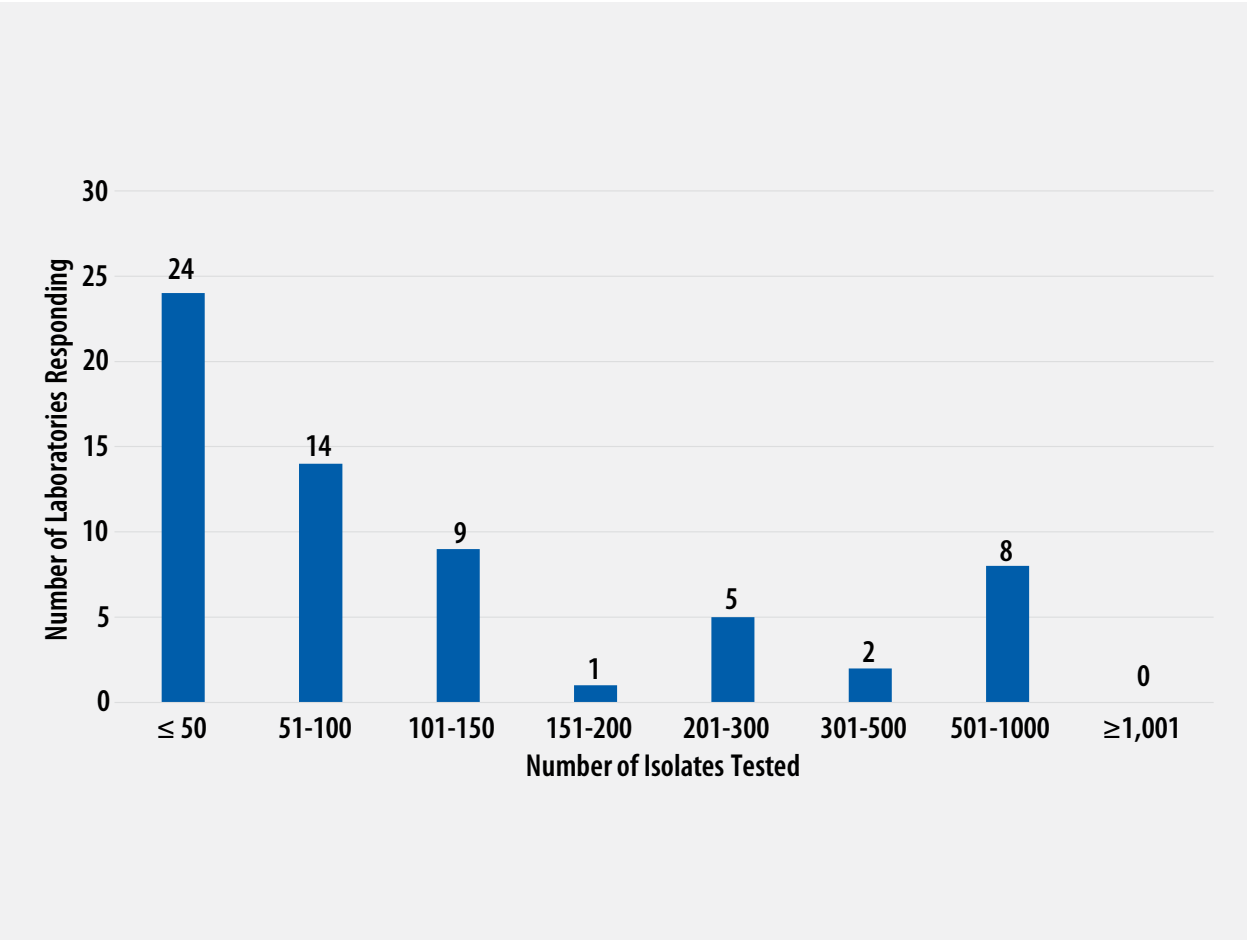
Figure 1. Primary Classification of Participating Laboratories, February 2022



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 63 participants in 2021 (excluding isolates used for quality control) is shown in Figure 2. In 2021, the counts ranged from 0 to 812 tests. Participants at 24 (38%) 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 [8].

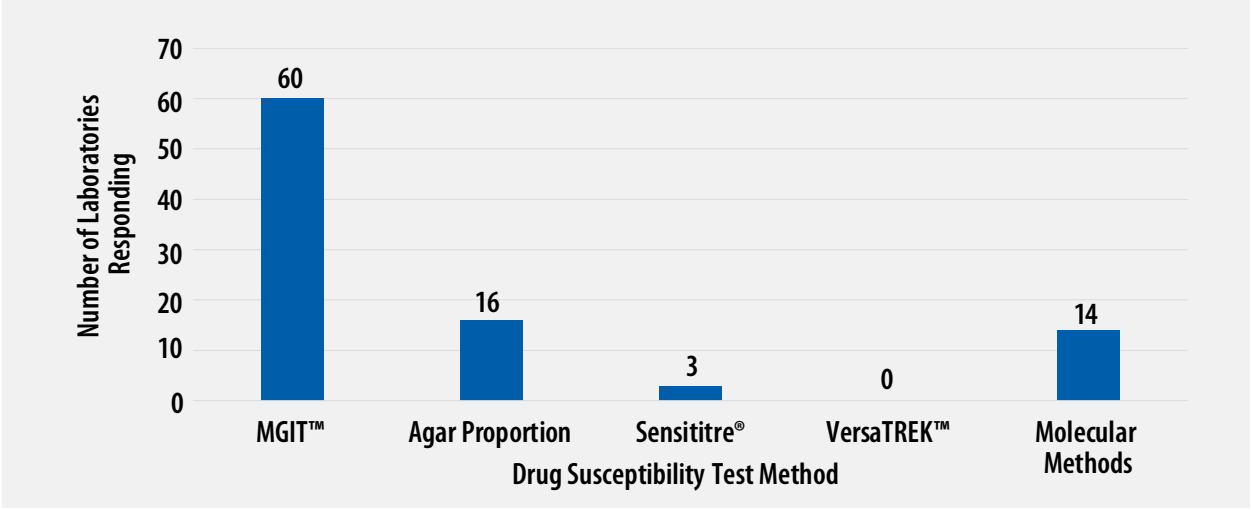
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=63)



MTBC DST Methods Used by Participants

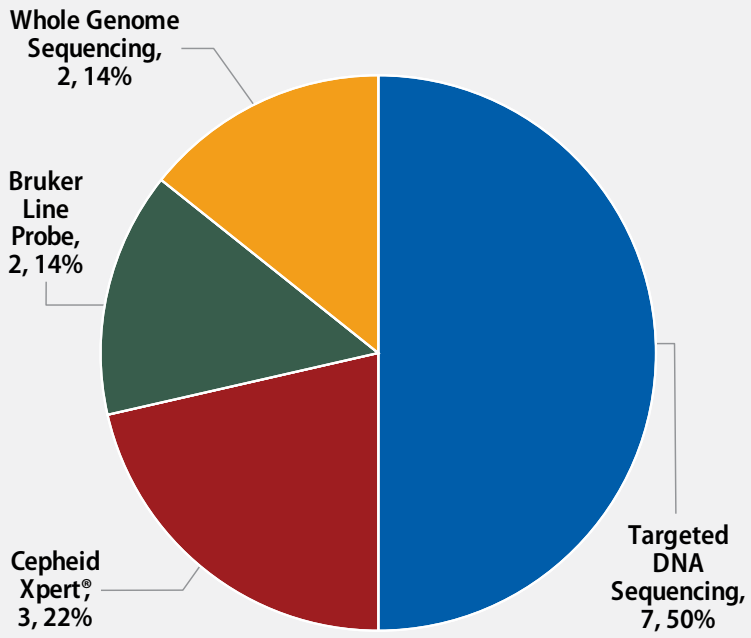
The DST methods that were performed by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Of participating laboratories, 37 (59%) reported results for only one method, 22 (35%) reported two methods, and 4 (6%) noted three susceptibility methods.

Figure 3. MTBC Drug Susceptibility Test Method Performed by Participants (n=93)



Molecular methods reported by participants are shown in Figure 4. The method performed most frequently (50%) was targeted DNA sequencing, including pyrosequencing and Sanger sequencing. Three (22%) laboratories reported use of the Cepheid Xpert® MTB/RIF assay, two (14%) reported results for line probe assays, Bruker Genotype MTBDR*plus* and MTBDRsl, and two (14%) reported results from whole genome sequencing.

Figure 4. Molecular Method Reported (n=14)



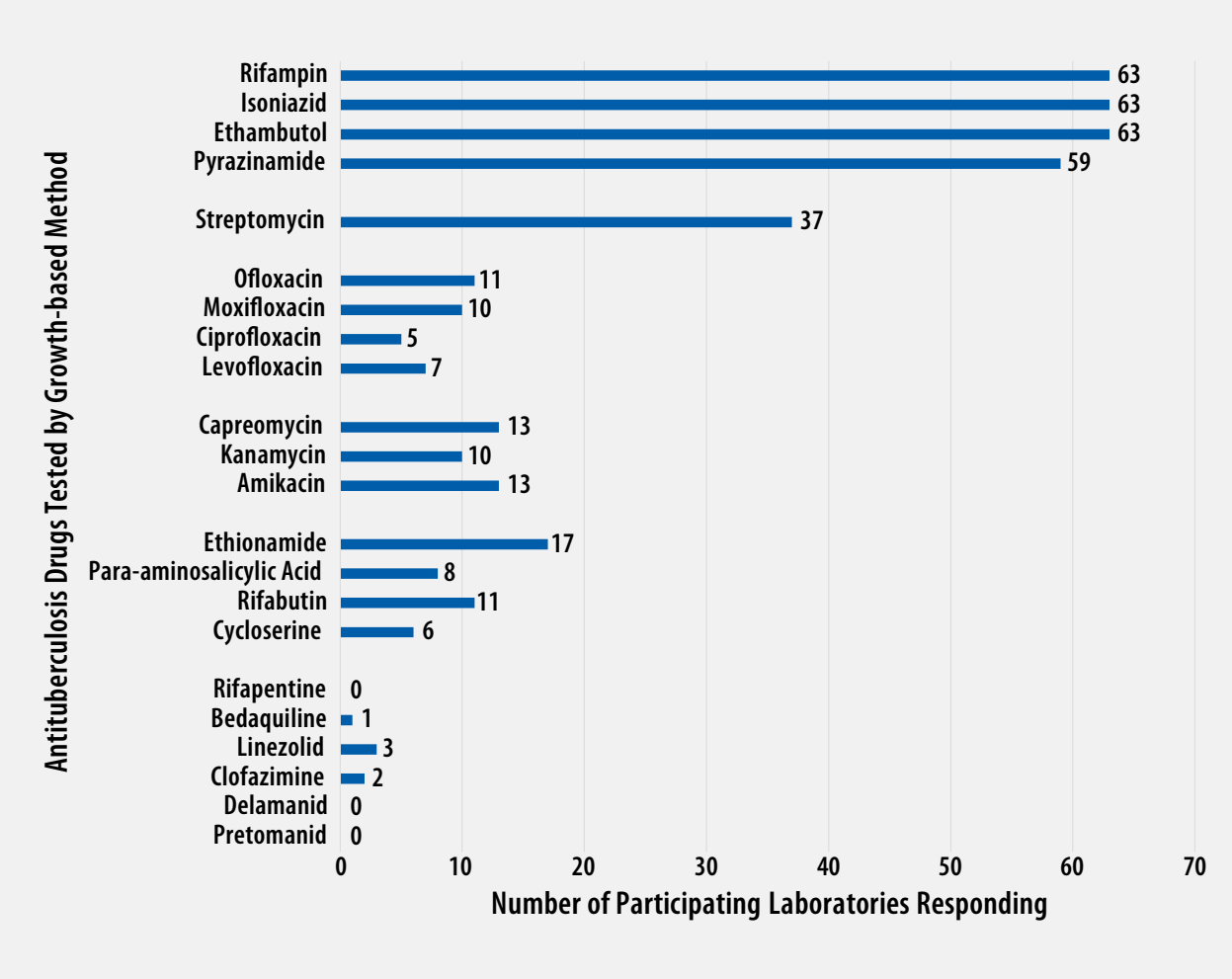
Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the February 2022 survey is presented in Figure 5. CLSI recommends testing a full panel of first-line drugs (rifampin [RMP], 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 may consider the addition of fluoroquinolones to their testing panel as CDC recommends susceptibility testing for fluoroquinolones (e.g., moxifloxacin) with use of the 4-month rifapentine-moxifloxacin treatment regimen; RMP results may be used as a proxy for rifapentine [9].

All participants reported results for three of the first-line drugs (RMP, INH, and EMB) and 59 (94%) also reported results for PZA by growth-based DST methods. One laboratory performs molecular testing for PZA via sequencing of *pncA*, in place of growth-based DST. Twenty laboratories tested at least one fluoroquinolone.

CDC has adopted a new hybrid definition of XDR that includes both the former classification (i.e., MDR with resistance to second-line injectable plus fluoroquinolone) or the revised WHO definition (i.e., MDR plus resistance to fluoroquinolone and either bedaquiline or linezolid) [10, 11]. Twenty laboratories reported second-line drug results other than streptomycin. Five (22%) of these laboratories tested all three second-line injectable drugs (amikacin, kanamycin, and capreomycin) and at least one fluoroquinolone (ofloxacin, ciprofloxacin, levofloxacin, or moxifloxacin) needed to confidently define the former classification of XDR TB. Three laboratories tested at least one fluoroquinolone and either bedaquiline or linezolid to define the WHO's revised XDR TB definition.

Figure 5. Antituberculosis Drugs Tested by Growth-based Method by Participants



Isolate 2022A

Expected Result: Susceptible to all first- and second-line drugs by agar proportion

Isolate 2022A is susceptible to all first- and second-line drugs.

Pyrazinamide

Isolate 2022A was expected to be susceptible to PZA. DNA sequence analysis of *pncA* in Isolate 2022A revealed a A>T point mutation resulting in wild-type glutamate being replaced by valine at codon 37 (Glu37Val). However, isolates with the non-synonymous Glu37Val mutation have been reported to test susceptible to PZA in growth-based assays [12]. Issues with false-resistance to PZA have been reported [13] and remain a concern.

Of those testing PZA for Isolate 2022A, **resistance** was reported by:

- **56% (33/59)** of the results when using MGIT™

Of the 5 molecular results reported for PZA, four (80%) laboratories reported detection of a mutation, with three specifically noting the Glu37Val mutation.

For internal comparison purposes, this isolate was previously sent as MPEP 2019I where 23% (15/65) of MGIT™ results and 0% (0/1) of VersaTREK™ results were reported as resistant.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2022A are listed in Tables 3–9.

Table 3. Isolate 2022A—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	13	0	13
Isoniazid—Low	13	0	13
Isoniazid—High	12	0	12
Ethambutol	13	0	13

Table 4. Isolate 2022A—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	59	0	59
Isoniazid—Low	59	0	59
Isoniazid—High	22	0	22
Ethambutol	58	1	59
Pyrazinamide	26	33	59

Table 5. Isolate 2022A—Participant Results for First-Line DST by Sensititre®

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

*One additional laboratory reported susceptible for INH by Sensititre® but did not differentiate by INH—Low and INH—High.

Table 6. Isolate 2022A—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	12	0	12
Ofloxacin	6	0	6
Ciprofloxacin	3	0	3
Moxifloxacin	4	0	4
Levofloxacin	3	0	3
Amikacin	8	0	8
Kanamycin	7	0	7
Capreomycin	9	0	9
Ethionamide	11	1	11
Rifabutin	6	0	6
Cycloserine	5	0	5
p-Aminosalicylic acid	5	0	5
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 7. Isolate 2022A—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	25	0	25
Ofloxacin	3	0	3
Ciprofloxacin	1	0	1
Moxifloxacin	5	0	5
Levofloxacin	3	0	3
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1
Rifapentine	0	0	0
Bedaquiline	1	0	1
Linezolid	2	0	2
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 8. Isolate 2022A—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	2	0	2
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
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

* One additional laboratory reported 'No Interpretation' for CYS by Sensititre®.

Table 9. Isolate 2022A—Participant Results for Molecular Testing

Drug	Susceptible	Resistant	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	0	11	11
Isoniazid	0	9	9
Ethambutol	0	5	5
Pyrazinamide	4	1	5
Streptomycin	0	3	3
Ofloxacin	1*	7	8
Ciprofloxacin	1*	7	8
Moxifloxacin	1*	6	7
Levofloxacin	1*	6	7
Amikacin	0	8	8
Kanamycin	0	8	8
Capreomycin	0	8	8
Ethionamide	0	4	4
Cycloserine	0	1	1
p-Aminosalicylic acid	0	1	1
Bedaquiline	0	2	2
Linezolid	0	2	2
Clofazimine	0	2	2
Delamanid	0	1	1
Pretomanid	0	0	0

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

Isolate 2022B

Expected Result: Susceptible to RMP* at 1.0 µg/ml by agar proportion

*80% consensus for a single categorical result across all methods reported for this drug of either susceptible or resistant was not achieved for these isolates among participating laboratories.

Rifampin

Rifampin (RMP) is a bactericidal drug used as part of a standard first-line regimen for the treatment of TB. RMP's mechanism of action is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase [16]. The primary mechanism of resistance is mutations within the 81-bp central region of the *rpoB* gene (i.e., rifampin resistance determining region or RRDR) that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase [17]. Mutations in codons 450, 445, and 435 (E. coli numbering system corresponding to 531, 526, and 516) are among the most frequent mutations in RMP-resistant isolates and serve as predictors of RMP resistance [16, 17]. The activity of RMP on isolates with *rpoB* mutations depends on both the mutation position and the type of amino acid change.

CDC has recommended that RMP resistance detected by the Xpert® MTB/RIF assay be confirmed by DNA sequencing [29]. The Xpert® MTB/RIF assay could generate results that falsely indicate resistance when compared to growth-based methods because of the presence of silent/synonymous mutations [30]. Sequencing of *rpoB* allows for clarification of the result and understanding of possible discordance between rapid molecular and growth-based testing results.

DNA sequence analysis of *rpoB* in Isolate 2022B revealed an A>T point mutation resulting in histidine being replaced by leucine at codon 445 (His445Leu). Isolates with His445Leu mutations are associated with low-level RMP resistance and often test as susceptible in growth-based assays at currently used critical concentrations [4, 14, 15].

For Isolate 2022B, 70 results for RMP were reported and the majority (69%) of reported results differed from the expected result. This isolate was reported **resistant** to RMP by method, as follows:

- **87% (13/15)** of the results when using AP
- **63% (33/52)** of the results when using MGIT™
- **67% (2/3)** of the results when using Sensititre®

Of the 12 molecular results reported for RMP, all (100%) laboratories reported detection of a mutation with seven laboratories specifically noting the His445Leu mutation.

Three of the laboratories performing Sensititre® reported RMP MIC values as 1 µg/ml (n=1), 2 µg/ml (n=-1), and 16 µg/ml (n=1).

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2022B are listed in Tables 10–16.

Four laboratories noted contaminated/no growth for Isolate 2022B and did not report results for at least one antituberculosis drug tested.

Table 10. Isolate 2022B—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	2	13	15
Isoniazid—Low	15	0	15
Isoniazid—High	14	0	14
Ethambutol	15	0	15

Table 11. Isolate 2022B—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	19	33	52*†
Isoniazid—Low	52	1	53†
Isoniazid—High	21	0	21†
Ethambutol	52	0	52†
Pyrazinamide	58	0	58

*One additional laboratory reported intermediate for RMP by MGIT™.
†One additional laboratory reported no interpretation for RMP, INH—Low, INH—High, and EMB by MGIT™.

Table 12. Isolate 2022B—Participant Results for First-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Rifampin	1	2	3
Isoniazid—Low	2	0	2*
Isoniazid—High	0	0	0*
Ethambutol	3	0	3

*One additional laboratory reported susceptible for INH by Sensititre® but did not differentiate by INH—Low and INH—High.

Table 13. Isolate 2022B—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	14	0	14
Ofloxacin	7	0	7
Ciprofloxacin	4	0	4
Moxifloxacin	4	0	4
Levofloxacin	3	0	3
Amikacin	8	0	8
Kanamycin	8	0	8
Capreomycin	9	0	9
Ethionamide	12	1	13
Rifabutin	6	0	6
Cycloserine	5	0	5
p-Aminosalicylic acid	5	0	5
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 14. Isolate 2022B—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	22	0	22
Ofloxacin	3	0	3
Ciprofloxacin	1	0	1
Moxifloxacin	4	0	4
Levofloxacin	3	0	3
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1
Rifapentine	0	0	0
Bedaquiline	1	0	1
Linezolid	2	0	2
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 15. Isolate 2022B—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
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

*One additional laboratory reported 'No Interpretation' for MOX and CYS by Sensititre®.

Table 16. Isolate 2022B—Participant Results for Molecular Testing

Drug	Susceptible	Resistant	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	12	0	12
Isoniazid	1	8	9
Ethambutol	0	5	5
Pyrazinamide	0	5	5
Streptomycin	0	3	3
Ofloxacin	1*	7	8
Ciprofloxacin	1*	7	8
Moxifloxacin	1*	6	7
Levofloxacin	1*	6	7
Amikacin	0	8	8
Kanamycin	0	8	8
Capreomycin	0	8	8
Ethionamide	0	5	5
Cycloserine	0	1	1
p-Aminosalicylic acid	0	1	1
Bedaquiline	0	2	2
Linezolid	0	2	2
Clofazimine	0	2	2
Delamanid	0	1	1
Pretomanid	0	0	0

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

Isolate 2022C

Expected Result: Resistant to INH at 0.2 µg/ml, OFL at 2.0 µg/ml, CIP at 2.0 µg/ml, and ETA at 5.0 µg/ml by agar proportion

Isoniazid

Isoniazid (INH) is the most widely used first-line antituberculosis drug and is a cornerstone of regimens used to treat TB disease and latent TB infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *katG* gene [5, 16]. The target of activated INH is enoyl-acyl-carrier protein reductase (encoded by the *inhA* gene); this binding inhibits cell wall mycolic acid biosynthesis. There are two mechanisms that account for the majority of INH resistance [5, 16, 17]. The most common mechanism, mutations in *katG*, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the *fabG1/inhA* promoter region, which are generally associated with low-level resistance to INH and are less frequent than *katG* mutations. It has been reported that approximately 10–15% of isolates found to be INH-resistant have no mutations detected in either of these loci; however, this percent is decreasing due to the more comprehensive nature of whole genome sequencing [18, 19]. Numerous loci have been investigated to identify additional genes correlated with INH resistance. The *fabG1* (also known as *mabA*) gene, like *inhA*, is involved in mycolic acid biosynthesis and at least one mutation in this region (Leu203Leu) has been associated with low-level INH resistance [20, 21].

DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2022C revealed a C>T point mutation at nucleotide position -15 of the promoter region of the *inhA* gene (C-15T); *katG*, *fabG1*, and *ahpC* were wild-type (i.e., no mutations were detected).

The recommended critical concentration and additional higher concentrations for testing INH using the AP method are 0.2 µg/ml and 1.0 µg/ml, respectively. The equivalent concentrations for MGIT™ and VersaTREK™ are 0.1 µg/ml and 0.4 µg/ml [1].

For Isolate 2022C, 83 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (15/15)** of the results when using AP
- **98% (58/59)** of the results when using MGIT™

Two (4%) results were reported resistant at the higher concentrations of INH. Only 32 (54%) laboratories performing MGIT™ DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a second DST method.

Of the 9 molecular results reported for INH, all (100%) laboratories reported detection of a mutation with seven specifically noting the *inhA* C-15T mutation.

Two of the laboratories performing Sensititre® reported INH MIC values as 0.25 µg/ml (n=2). A third laboratory reported INH MIC value as 0.5 µg/ml (n=1) and noted resistance but since an interpretation was not indicated for INH—Low and INH—High, the result was excluded from Table 19.

Ofloxacin and Ciprofloxacin

Fluoroquinolones (FQs) are one of the most commonly prescribed classes of antibiotic in the United States due to their activity against various types of bacteria. They are an important class of drugs used to treat tuberculosis (TB) [9, 22, 23]. Prolonged treatment with a FQ (>10 days) before a diagnosis of TB is associated with a higher risk of resistance and diagnostic delays [22, 24]. The primary mechanism of action for FQs is the inhibition of DNA synthesis [25] by inhibiting DNA gyrase. The enzyme DNA gyrase generates the activity for cleaving and resealing double-stranded DNA. This action is necessary for DNA replication, transcription, and recombination.

Resistance to FQs has been attributed to point mutations in a 21-bp region, known as the quinolone resistance determining region (QRDR), of the MTBC *gyrA* gene. Mutations in the QRDR, commonly occurring at codons 90, 91, and 94, prevent the drugs from effectively binding DNA gyrase [5, 17, 25]. Mutations in the *gyrB* gene have also been noted with varying rates of resistance,

but high-level resistance is less common without a concurrent *gyrA* mutation [25-27].

Heteroresistance is the result of varying levels of resistance within a population of MTBC due to the presence of sub-populations with differing nucleotides at a locus associated with drug resistance, resulting in both drug-resistant and drug-susceptible organisms [28, 29]. This phenomenon is not limited to FQs but is commonly noted with this drug class.

Studies suggest that the level of resistance, as measured by MIC testing, to ofloxacin (OFL), ciprofloxacin (CIP), levofloxacin (LVX), and moxifloxacin (MOX) is dependent on the mutation and can vary among the FQ [26, 30, 31]. CLSI and WHO currently recommend testing LVX and/or MOX; however, the preferred FQ to be tested is the one used in the treatment regimen [1, 10].

DNA sequencing of *gyrA* in Isolate 2022C detected a C>T point mutation in *gyrA* resulting in wild-type alanine being replaced with valine at codon 90 (Ala90Val). The Ala90Val mutation has been associated with low-level FQ resistance, and the MIC for isolates with this mutation could be close to the critical concentration tested thereby impacting reproducibility [3, 5, 32]. Sequencing of the *gyrB* locus for this isolate revealed no mutations (i.e., wild-type sequence).

Among three growth-based methods, 11 results for OFL were reported for Isolate 2022C. This isolate was reported **resistant** to OFL by method, as follows:

- **100% (7/7)** of the results when using AP
- **100% (3/3)** of the results when using MGIT™
- **100% (1/1)** of the results when using Sensititre®

Participating laboratories also reported results for other FQs (e.g., CIP, LVX, and MOX) for Isolate 2022C; 80% (16/20) of results noted resistance to these additional FQs. The isolate was reported **resistant** to three other FQs by method, as follows:

CIP

- **75% (3/4)** of the results when using AP

LVX

- **100% (3/3)** of the results when using AP
- **100% (3/3)** of the results when using MGIT™

MOX

- **75% (3/4)** of the results when using AP
- **80% (4/5)** of the results when using MGIT™
- **0% (0/1)** of the results when using Sensititre®

A mutation in the *gyrA* gene was detected by six (75%) laboratories that reported molecular testing for OFL and CIP [five (71%) laboratories reported molecular testing for MOX and LVX], with four laboratories noting the Ala90Val mutation.

Two of the laboratories performing Sensititre® reported MIC values for FQs; one of these did not report interpretations. Reported MIC values were as follows: OFL at 8 µg/ml (n=1); MOX at 2 µg/ml (n=1) and 4 µg/ml (n=1); and LVX at 4 µg/ml (n=1).

Ethionamide

Resistance to INH and ethionamide (ETA) can occur by mutations in the *fabG1–inhA* regulatory region, which are generally associated with low-level resistance to INH. Mutations in *ethA* also confer resistance to ETA, without concomitant resistance to INH [33]. Sequencing analysis of *ethA* did not detect a mutation but sequencing of the promoter region of the *inhA* gene revealed a C>T point mutation at nucleotide position -15 (C-15T). This mutation has been associated with ETA resistance [34].

Issues with reproducibility of DST results for ETA have been reported [35] and remain a potential concern.

For Isolate 2022C, 15 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **77% (10/13)** of the results when using AP
- **100% (1/1)** of the results when using MGIT™
- **100% (1/1)** of the results when using Sensititre®

One of the laboratories performing Sensititre® reported ETA MIC value as >40 µg/ml (n=1).

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2022C are listed in Tables 17–23.

One laboratory noted no growth for Isolate 2022C and did not report results for at least one antituberculosis drug tested.

Table 17. Isolate 2022C—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	15	0	15
Isoniazid—Low	0	15	15
Isoniazid—High	13	1	14
Ethambutol	15	0	15

Table 18. Isolate 2022C—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	59	0	59
Isoniazid—Low	1	58	59
Isoniazid—High	32	0	32
Ethambutol	58	1	59
Pyrazinamide	57	1	58

Table 19. Isolate 2022C—Participant Results for First-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Rifampin	3	0	3
Isoniazid—Low	0	0	0*†
Isoniazid—High	0	1	1†
Ethambutol	2	1	3

* One additional laboratory reported intermediate for INH—Low by Sensititre®.

†One additional laboratory reported resistant for INH by Sensititre® but did not differentiate by INH—Low and INH—High.

Table 20. Isolate 2022C—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	14	0	14
Ofloxacin	0	7	7
Ciprofloxacin	1	3	4
Moxifloxacin	1	3	4
Levofloxacin	0	3	3
Amikacin	8	0	8
Kanamycin	8	0	8
Capreomycin	9	0	9
Ethionamide	3	10	13
Rifabutin	6	0	6
Cycloserine	5	0	5
p-Aminosalicylic acid	5	0	5
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 21. Isolate 2022C—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	25	0	25
Ofloxacin	0	3	3
Ciprofloxacin	0	0	0*
Moxifloxacin	1	4	5
Levofloxacin	0	3	3
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	0	1	1
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1
Rifapentine	0	0	0
Bedaquiline	1	0	1
Linezolid	1	0	1
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

* One additional laboratory reported intermediate for CIP by MGIT™.

Table 22. Isolate 2022C—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	1	0	1*
Levofloxacin	0	0	0*
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	1	0	1
Ethionamide	0	1	1
Rifabutin	2	0	2
Cycloserine	0	0	0*
p-Aminosalicylic acid	2	0	2
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

*One additional laboratory reported 'No Interpretation' for LVX, MOX, and CYC by Sensititre®.

Table 23. Isolate 2022C—Participant Results for Molecular Testing

Drug	Susceptible	Resistant	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	0	11	11
Isoniazid	9	0	9
Ethambutol	0	5	5
Pyrazinamide	3	3	6
Streptomycin	0	3	3
Ofloxacin	6	2	8
Ciprofloxacin	6	2	8
Moxifloxacin	5	2	7
Levofloxacin	5	2	7
Amikacin	0	8	8
Kanamycin	0	8	8
Capreomycin	0	8	8
Ethionamide	5	0	5
Cycloserine	0	1	1
p-Aminosalicylic acid	0	1	1
Bedaquiline	0	2	2
Linezolid	0	2	2
Clofazimine	0	2	2
Delamanid	0	1	1
Pretomanid	0	0	0

Isolate 2022D

Expected Result: Susceptible to RMP* at 1.0 µg/ml by agar proportion

*80% consensus for a single categorical result across all methods reported for this drug of either susceptible or resistant was not achieved for these isolates among participating laboratories.

Rifampin

DNA sequence analysis of *rpoB* in Isolate 2022D revealed a G>T point mutation resulting in wild-type serine being replaced by leucine in MTB codon 435 (Asp435Tyr). Isolates with Asp435Tyr (Asp516Tyr in E. coli numbering system) mutations are associated with low-level RMP resistance and can test as susceptible in growth-based assays [14, 15].

For Isolate 2022D, 74 results for RMP were reported. This isolate was reported **susceptible** to RMP by method, as follows:

- **100% (12/12)** of the results when using AP
- **100% (59/59)** of the results when using MGIT™
- **67% (2/3)** of the results when using Sensititre®

Of the 11 molecular results reported for RMP, all (100%) laboratories reported detection of a mutation in *rpoB*. Seven laboratories specifically noted the Asp435Tyr mutation.

Three of the laboratories performing Sensititre® reported RMP MIC values as 0.25 µg/ml (n=1), 1 µg/ml (n=1), and 16 µg/ml (n=1).

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2022D are listed in Tables 24–30.

Table 24. Isolate 2022D—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	12	0	12*
Isoniazid—Low	13	0	13
Isoniazid—High	12	0	12
Ethambutol	13	0	13

* One additional laboratory reported No Interpretation for RMP by AP.

Table 25. Isolate 2022D—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	59	0	59
Isoniazid—Low	59	0	59
Isoniazid—High	22	0	22
Ethambutol	59	0	59
Pyrazinamide	58	1	59

Table 26. Isolate 2022D—Participant Results for First-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Rifampin	2	1	3
Isoniazid—Low	2	0	2*
Isoniazid—High	0	0	0*
Ethambutol	3	0	3

*One additional laboratory reported susceptible for INH by Sensititre® but did not differentiate by INH—Low and INH—High.

Table 27. Isolate 2022D—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	12	0	12
Ofloxacin	6	0	6
Ciprofloxacin	3	0	3
Moxifloxacin	4	0	4
Levofloxacin	3	0	3
Amikacin	8	0	8
Kanamycin	7	0	7
Capreomycin	9	0	9
Ethionamide	12	0	12
Rifabutin	6	0	6
Cycloserine	5	0	5
p-Aminosalicylic acid	5	0	5
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 28. Isolate 2022D—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	25	0	25
Ofloxacin	3	0	3
Ciprofloxacin	1	0	1
Moxifloxacin	4	0	4
Levofloxacin	3	0	3
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1
Rifapentine	0	0	0
Bedaquiline	1	0	1
Linezolid	2	0	2
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 29. Isolate 2022D—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	0	0	0*
p-Aminosalicylic acid	2	0	2
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	1	0	1
Clofazimine	0	0	0
Delamanid	0	0	0
Pretomanid	0	0	0

* One additional laboratory reported 'No Interpretation' for MOX, LVX, and CYS by Sensititre®.

Table 30. Isolate 2022D—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	11	0	11
Isoniazid	0	9	9
Ethambutol	1	4	5
Pyrazinamide	0	5	5
Streptomycin	0	3	3
Ofloxacin	1*	7	8
Ciprofloxacin	1*	7	8
Moxifloxacin	1*	6	7
Levofloxacin	1*	6	7
Amikacin	0	8	8
Kanamycin	0	8	8
Capreomycin	0	8	8
Ethionamide	0	5	5
Cycloserine	0	1	1
p-Aminosalicylic acid	0	1	1
Bedaquiline	0	2	2
Linezolid	0	2	2
Clofazimine	0	2	2
Delamanid	0	1	1
Pretomanid	0	0	0

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

Isolate 2022E

Expected Result: Resistant to INH at 0.2 µg/ml, EMB* at 5.0 µg/ml, ETA at 5.0 µg/ml, and STR at 2.0 µg/ml by agar proportion

*80% consensus for a single categorical result across all methods reported for this drug of either susceptible or resistant was not achieved for these isolates among participating laboratories.

Isoniazid

As previously noted, resistance to INH most commonly occurs due to mutations in the *katG* gene or the promoter region of the *inhA* gene; however, mutations in *fabG1* can also cause resistance with the role of mutations in *ahpC* remaining less clear. DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2022E revealed a G>C point mutation in the *katG* locus resulting in wild-type serine being replaced by threonine at codon 315 (Ser315Thr); *inhA*, *fabG1*, and *ahpC* were wild-type (i.e., no mutations were detected).

For Isolate 2022E, 75 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (15/15)** of the results when using AP
- **100% (59/59)** of the results when using MGIT™
- **100% (1/1)** of the results when using Sensititre®

Forty-seven or 100% of results at the higher concentrations of INH were reported as resistant. Only 32 (54%) laboratories performing MGIT™ DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a second DST method.

Of the 9 molecular results reported for INH, all (100%) laboratories reported detection of a mutation with 8 laboratories specifically noting the *katG* Ser315Thr mutation.

Two of the laboratories performing Sensititre® reported INH MIC values as 4 µg/ml (n=2). A third laboratory reported INH MIC value as 4 µg/ml (n=1) and noted 'Resistant' but since an interpretation was not indicated for INH—Low and INH—High, the result was excluded from Table 33.

For internal comparison purposes, this isolate was previously sent as MPEP 2020H where 100% (82/82) of results were reported as resistant.

Ethambutol

Ethambutol (EMB) is an important first-line drug for the treatment of TB and is used in combination with INH, RMP and PZA to prevent emergence of drug resistance. EMB is a bacteriostatic agent that is active against growing bacilli and has no effect on non-replicating bacilli [16, 17]. EMB targets the arabinosyl transferases (*embCAB* operon), thereby inhibiting the biosynthesis of the cell wall components arabinogalactan and lipoarabinomannan [36].

Issues with false-susceptibility with some growth-based methods for EMB, particularly in broth-based media, have been reported and remain a potential concern. Probable causes include the bacteriostatic nature of the drug, reduced drug activity in culture, and an isolate MIC for EMB falling close to the critical concentration tested [37-39].

Sequence analysis of EMB-resistant clinical isolates has shown that EMB resistance is associated primarily with missense (non-synonymous) mutations within the EMB resistance determining region of the gene *embB* at codons 306, 406, and 497 [5, 36].

DNA sequence analysis of *embB* of Isolate 2022E revealed a A>G point mutation in the *embB* gene resulting in wild-type methionine being replaced by valine at codon 306 (Met306Val). Certain *embB* mutations at the 306 codon, such as Met306Val and Met306Leu, are associated with EMB resistance [5].

For Isolate 2022E, 75 EMB results were reported. This isolate was reported **resistant** to EMB by method, as follows:

- **93% (14/15)** of the results when using AP
- **19% (11/59)** of the results when using MGIT™
- **100% (1/1)** of the results when using Sensititre®

Of the 5 molecular results reported for EMB, all laboratories reported detection of a mutation and specifically noted the Met306Val mutation.

Three of the laboratories performing Sensititre® reported EMB MIC values as 2.5 µg/ml (n=1) and 8 µg/ml (n=2).

For internal comparison purposes, this isolate was previously sent as MPEP 2020H where 88% (15/17) of AP results, 16% (10/61) of MGIT™ results, 100% (3/3) of Sensititre® results, and 50% (1/2) of VersaTREK™ results were reported as resistant.

Ethionamide

As previously noted, 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 a partial deletion of *ethA*; *inhA* and *fabG1* were wild-type (i.e., no mutations were detected).

For Isolate 2022E, 17 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **46% (6/13)** of the results when using AP
- **67% (2/3)** of the results when using MGIT™
- **100% (1/1)** of the results when using Sensititre®

Of the 5 molecular results reported for ETA, 2 (40%) laboratories reported detection of a mutation specifically noting an *ethA* deletion.

One of the laboratories performing Sensititre® reported an ETA MIC value as 10 µg/ml (n=1).

For internal comparison purposes, this isolate was previously sent as MPEP 2020H where 64% (9/14) of AP results, 100% (3/3) of MGIT™ results, and 0% (0/1) of Sensititre® results were reported as resistant.

Streptomycin

Streptomycin (STR) belongs to the aminoglycoside class of drugs and its primary mechanism of action is to inhibit protein synthesis by preventing the initiation of translation by binding to the 16s rRNA [16, 17]. In MTBC, the genetic basis of the majority of resistance to STR is usually due to mutations in *rrs* or *rpsL* [17, 25]. CLSI recommends testing STR as a second-line drug based on American Thoracic Society's categorization of STR as a second-line drug for treatment due to increased resistance in many parts of the world [1, 40].

DNA sequencing analysis did not reveal a mutation in *rrs* or *rpsL*; other mechanisms of resistance may exist.

Among three methods, 41 results for STR were reported for Isolate 2022E. This isolate was reported **resistant** to STR by method, as follows:

- **64% (9/14)** of the results when using AP
- **36% (9/25)** of the results when using MGIT™
- **50% (1/2)** of the results when using Sensititre®

Two of the laboratories performing Sensititre® reported STR MIC values as 1 µg/ml (n=1) and 8 µg/ml (n=2).

For internal comparison purposes, this isolate was previously sent as MPEP 2020H where 76% (11/14) of AP results, 48% (16/33) of MGIT™ results, and 100% (1/1) of Sensititre® results were reported as resistant.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2022E are listed in Tables 31–37.

Table 31. Isolate 2022E—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	15	0	15
Isoniazid—Low	0	15	15
Isoniazid—High	0	14	14
Ethambutol	1	14	15

Table 32. Isolate 2022E—Participant Results for First-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Rifampin	59	0	59
Isoniazid—Low	0	59	59
Isoniazid—High	0	32	32
Ethambutol	48	11	59
Pyrazinamide	58	1	59

Table 33. Isolate 2022E—Participant Results for First-Line DST by Sensititre®

Drug	Susceptible	Resistant	Total
Rifampin	3	0	3
Isoniazid—Low	0	1	1*
Isoniazid—High	0	1	1*
Ethambutol	0	3	3

*One additional laboratory reported resistant for INH by Sensititre® but did not differentiate by INH—Low and INH—High.

Table 34. Isolate 2022E—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	5	9	14
Ofloxacin	7	0	7
Ciprofloxacin	4	0	4
Moxifloxacin	4	0	4
Levofloxacin	3	0	3
Amikacin	8	0	8
Kanamycin	8	0	8
Capreomycin	9	0	9
Ethionamide	7	6	13
Rifabutin	6	0	6
Cycloserine	5	0	5
p-Aminosalicylic acid	5	0	5
Rifapentine	0	0	0
Bedaquiline	0	0	0
Linezolid	0	0	0
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 35. Isolate 2022E—Participant Results for Second-Line DST by MGIT™

Drug	Susceptible	Resistant	Total
Streptomycin	16	9	25
Ofloxacin	3	0	3
Ciprofloxacin	1	0	1
Moxifloxacin	5	0	5
Levofloxacin	3	0	3
Amikacin	3	0	3
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	1	2	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	1	0	1
Rifapentine	0	0	0
Bedaquiline	1	0	1
Linezolid	2	0	2
Clofazimine	1	0	1
Delamanid	0	0	0
Pretomanid	0	0	0

Table 36. Isolate 2022E—Participant Results for Second-Line DST by Sensititre®

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

Table 37. Isolate 2022E—Participant Results for Molecular Testing

Drug	Susceptible	Resistant	Total
Rifamycins (Rifampin, Rifabutin, Rifapentine)	0	11	11
Isoniazid	9	0	9
Ethambutol	5	0	5
Pyrazinamide	2	3	5
Streptomycin	0	3	3
Ofloxacin	1*	7	8
Ciprofloxacin	1*	7	8
Moxifloxacin	1*	6	7
Levofloxacin	1*	6	7
Amikacin	0	8	8
Kanamycin	0	8	8
Capreomycin	0	8	8
Ethionamide	2	3	5
Cycloserine	0	1	1
p-Aminosalicylic acid	0	1	1
Bedaquiline	0	2	2
Linezolid	0	2	2
Clofazimine	0	2	2
Delamanid	0	1	1
Pretomanid	0	0	0

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

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.

†CLSI critical concentrations for RMP differ from revised WHO recommendation of 0.5 µg/ml published in 2021 [1, 41].

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, 3].

- 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.[3].

‡ Critical concentrations as indicated in WHO 2018 Technical Report on critical concentrations [3].

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.

†CLSI critical concentrations for RMP differ from revised WHO recommendation of 0.5 µg/ml published in 2021 [41].

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
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 [2, 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

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Appendix 1: Accessible Explanations of Figures

Figure 1. The primary classification of the 63 laboratories participating in the February 2022 MPEP survey is shown in this pie chart. The largest slice represents 46 laboratories, or 73% of 63 that have self-classified as a health department laboratory. The next major slice signifies 7 laboratories, or 11% of 63 that self-classified as hospital laboratories. The remaining three slices of the pie chart represent 7, or 11% of 63 that self-classified as independent laboratories; 2, or 3% of 63 that self-classified as federal government laboratories; and 1 laboratory, or 2% of 63 that self-classified as other: medical manufacturer. [return to Figure 1]

Figure 2. The annual volume of MTBC isolates tested for drug susceptibility by participating laboratories (N=63) in 2021 is displayed in this vertical bar graph. The vertical y–axis is the number of laboratories responding and ranges from 0 to 30 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, 24 laboratories tested less than or equal to 50 isolates per year; 14 laboratories tested between 51 to 100 isolates per year; 9 laboratories tested between 101 to 150 isolates per year; 1 laboratory tested between 151 to 200 isolates per year; 5 laboratories tested between 201 to 300 isolates per year; 2 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. [return to Figure 2]

Figure 3. The drug susceptibility testing methods performed by MPEP participants (N=93) is displayed in this vertical bar graph. The vertical y-axis is the number of laboratories reporting with ranges from 0 to 70, 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: 60 performed MGIT™, 16 performed agar proportion, 3 performed Sensititre®, 0 performed VersaTREK™, and 14 performed molecular methods. [return to Figure 3]

Figure 4. The molecular methods performed by MPEP participants (N=14) are displayed in this pie chart. The largest slice represents the 7 laboratories that perform targeted DNA sequencing. The next three slices represent 3 laboratories that performed the Cepheid Xpert® MTB/RIF assay, 2 laboratories that performed Bruker line probe assays, and 2 laboratories that performed whole genome sequencing. [return to Figure 4]

Figure 5. The antituberculosis drugs tested 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 70, 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. 63 laboratories tested rifampin; 63 laboratories tested isoniazid; 63 laboratories tested ethambutol; 59 laboratories tested pyrazinamide; 37 laboratories tested streptomycin; 11 laboratories tested ofloxacin; 10 laboratories tested moxifloxacin; 5 laboratories tested ciprofloxacin; 7 laboratories tested levofloxacin; 13 laboratories tested capreomycin; 10 laboratories tested kanamycin; 13 laboratories tested amikacin; 17 laboratories tested ethionamide; 8 laboratories tested PAS; 11 laboratories tested rifabutin; 6 laboratories tested cycloserine; 0 laboratories tested rifapentine; 1 laboratory tested bedaquiline; 3 laboratories tested linezolid; 2 laboratories tested clofazimine; 0 laboratories tested delamanid; and 0 laboratories tested pretomanid. [return to Figure 5]

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