CDC Model Performance Evaluation Program (MPEP) for *Mycobacterium tuberculosis* Drug Susceptibility Testing

Attachment 13

Final Aggregate Report

Centers for Disease Control and Prevention
Model Performance Evaluation Program

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Report of Results
February 2017

National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention
Division of Tuberculosis Elimination

CS 283150-A

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for February 2017 Samples 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 2017.

Report Content

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Table of 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. This 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 approved standards, participants should refer to consensus documents published by the Clinical and Laboratory Standards Institute (CLSI), "Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard," M24-A2 [1].

Expected Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in February 2017 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 using DNA sequencing are listed in Table 2 [2].

Table 1. Expected Growth-based Results for February 2017 Survey

Growth-based Results					
	First-Line Drugs				Second-Line Drugs
Isolate	RMP	INH	EMB	PZA	Resistant to:
2017A	S	S	S	S	OFL, CIP
2017B	S	R	S	S	STR
2017C	S	S	S	S	AMK, KAN, CAP
2017D	S	R	S	S	ETA
2017E	S	S	S	S	

Note–S=susceptible, R=resistant

Table 2. Expected Molecular Results for February 2017 Survey

Mutations Detected in Loci Associated with Resistance					
Isolate	*rpoB*	*katG*	*fabG1*	*gyrA*	*rrs*
2017A	Phe514Phe			Asp94Asn	
2017B		Ser315Thr			
2017C					A1401G
2017D	Arg528Arg		Leu203Leu		
2017E					

Abbreviations and Acronyms

AMK	amikacin
AP	agar proportion—performed on Middlebrook 7H10 or 7H11
bp	base pair
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
HMO	Health Maintenance Organization
INH	isoniazid
KAN	kanamycin
LEV	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 *Mycobacterium tuberculosis* MIC plate
STR	streptomycin
TB	tuberculosis
VersaTREK	Thermo Scientific VersaTREK Myco susceptibility
XDR	extensively drug resistant

Technical Notes

The following information pertains to all of the tables and figures for the 2017 MTBC isolates A, B, C, D, and E in this report.

- The source of data in all tables and figures is the February 2017 MPEP MTBC DST survey.
- The number of reported results (S represents susceptible and R represents resistant) for each drug are indicated in each table.
- 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 80 (the number of participating laboratories). This report contains all results reported by participating laboratories.
- Critical concentrations of antituberculosis drugs used for each DST method are listed at the end of this report.
- The Trek Sensititre system allows determination of a minimum inhibitory concentration (MIC) for each drug in the panel. Laboratories using this method must establish breakpoints to provide a categorical interpretation of S or R.
- For 30 laboratories reporting second-line drug results (with the exception of streptomycin), nine (30%) tested all three second-line injectable drugs and at least one fluoroquinolone needed to confidently define XDR TB. The second-line injectable drugs are amikacin, kanamycin, and capreomycin. Fluoroquinolones include ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin.

Descriptive Information about Participant Laboratories

Primary Classification

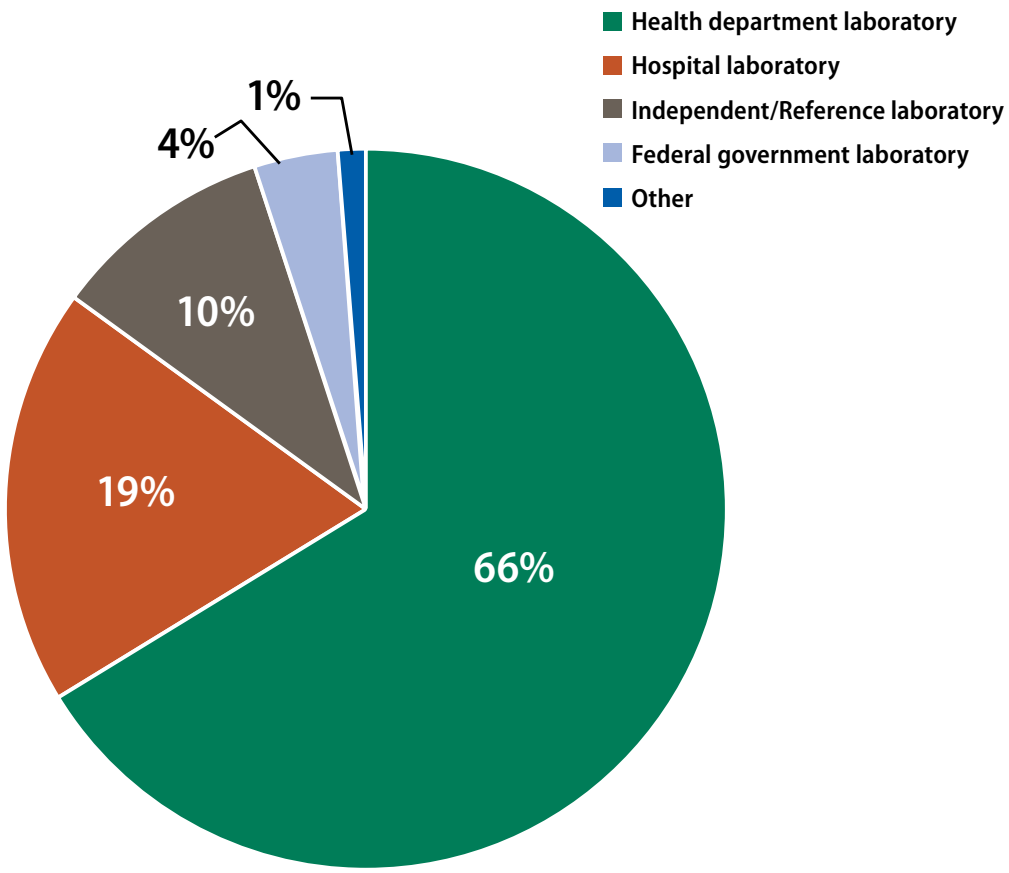
This report contains DST results submitted to CDC by survey participants at 80 laboratories in 36 states.

The participants were asked to indicate the primary classification of their laboratory (Figure 1). MPEP participants self-classified as:

- **53 (66%):** Health department laboratory (e.g., local, county, state)
- **15 (19%):** Hospital laboratory
- **8 (10%):** Independent/Reference laboratory (non-hospital based)
- **3 (4%):** Federal government laboratory
- **1 (1%):** Other (quality control manufacturer)

Figure 1. Primary Classification of Participating Laboratories, February 2017

Accessible information for all figures is located in Appendix 1, page 30.

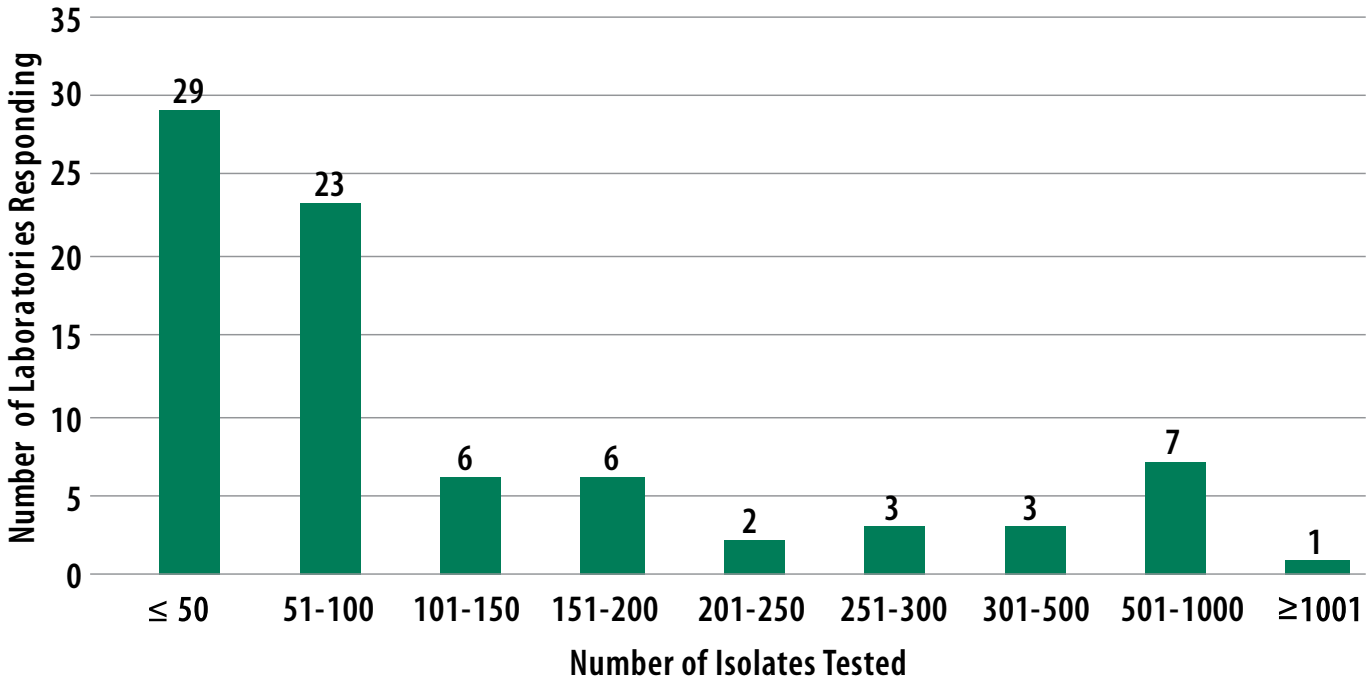


Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 80 participants in 2016 (excluding isolates used for quality control) is shown in Figure 2. In 2016, the counts ranged from 0 to 1,119 tests. Participants at 29 (36%) 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 [3].

Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=80)

Accessible information for all figures is located in Appendix 1, page 30.

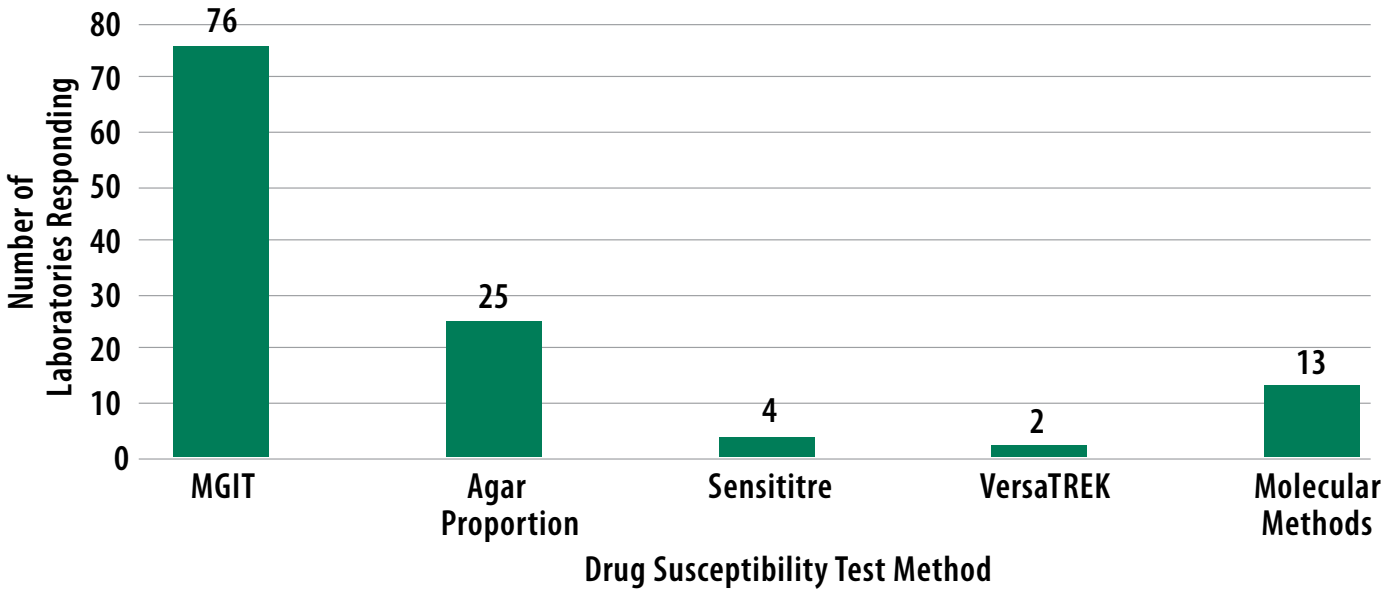


MTBC DST Methods Used by Participants

The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 45 (56%) laboratories reported results for only one method, 30 laboratories reported two methods, and five laboratories noted three susceptibility methods.

Figure 3. MTBC Drug Susceptibility Test Method Used by Participants (n=120)

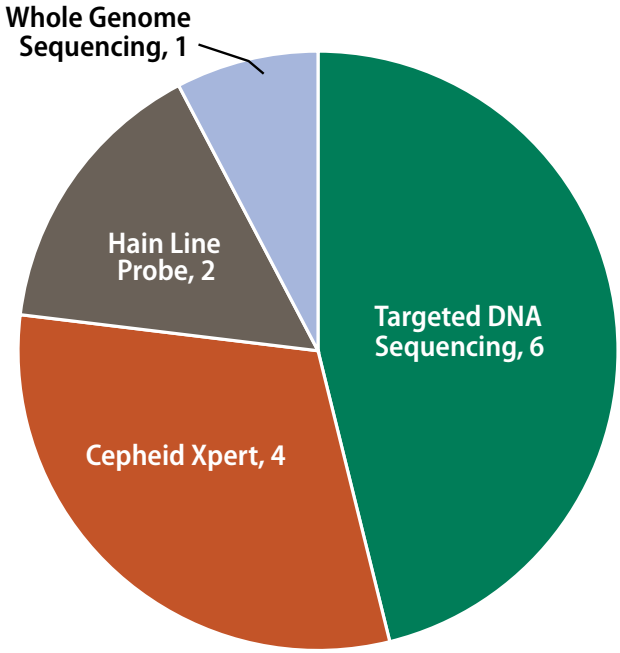
Accessible information for all figures is located in Appendix 1, page 30.



Molecular methods reported by thirteen participants are shown in Figure 4. The method used most frequently by laboratories was targeted DNA sequencing (46%), including pyrosequencing and Sanger sequencing. Four laboratories reported results for the Cepheid Xpert MTB/RIF assay, two reported use of the line probe assays Genotype MTBDR*plus* and MTBDR*sl* by Hain Lifescience, and one reported results from whole genome sequencing.

Figure 4. Molecular Method Reported (n=13)

Accessible information for all figures is located in Appendix 1, page 30.

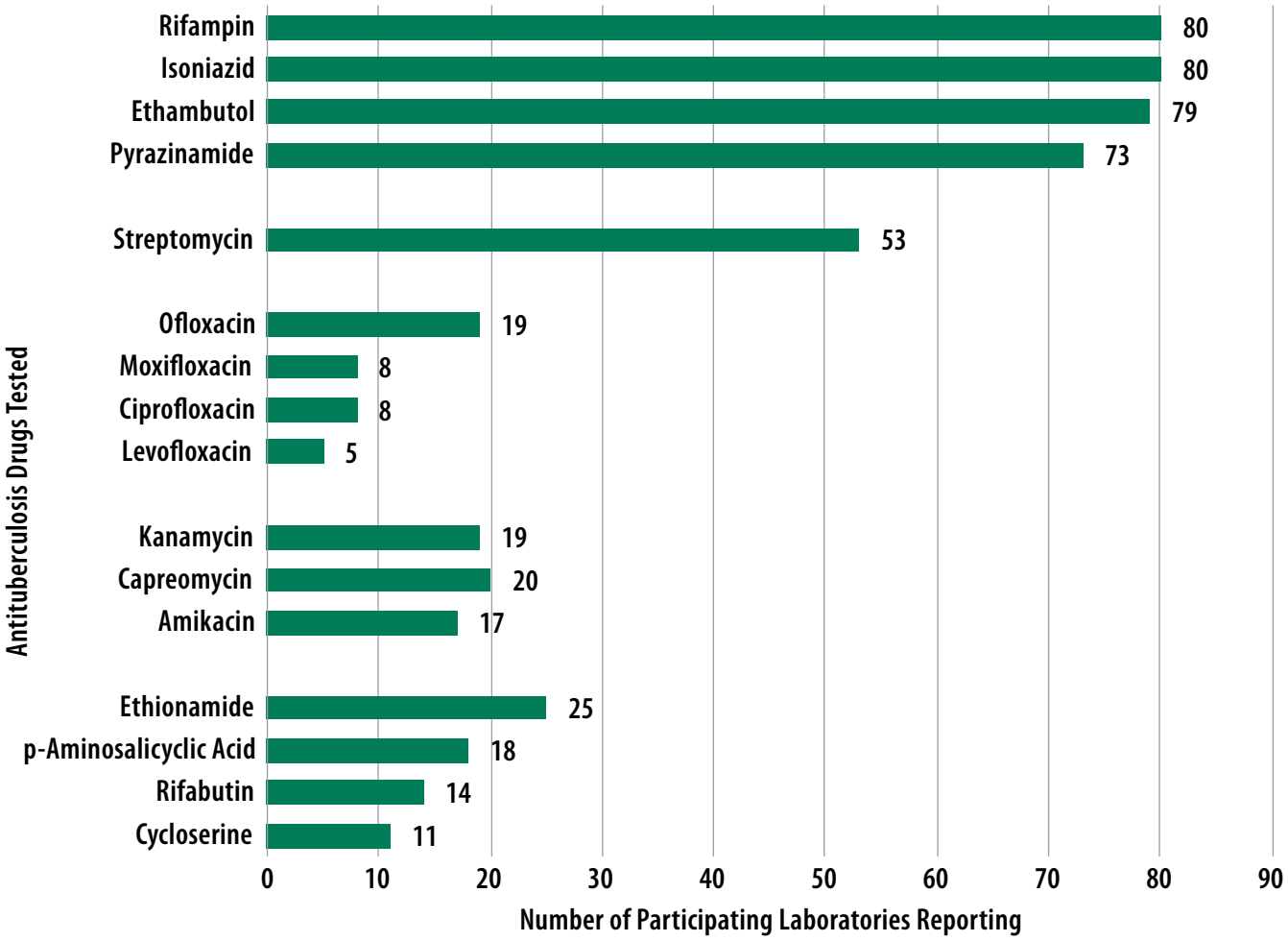


Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the February 2017 survey is shown 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 four-drug antituberculosis therapy currently recommended for most patients. All participants reported results for two of the first-line drugs (RMP and INH), 79 (99%) of the participants reported results for EMB, and 73 (91%) also reported results for PZA. The number of laboratories testing second-line drugs has decreased slightly since the August 2016 survey.

Figure 5. Antituberculosis Drugs Tested by Participants

Accessible information for all figures is located in Appendix 1, page 30.



Isolate 2017A

Expected Result: Resistant to OFL at 2.0 µg/ml by agar proportion

Ofloxacin

Fluoroquinolones (FQ) 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 TB resistant to first-line drugs but also have the potential to become an important part of new TB regimens [4]. In the United States, resistance to FQ is relatively uncommon in strains of MTBC susceptible to first-line drugs, however prolonged treatment with a FQ (>10 days) before a diagnosis of TB is associated with a higher risk for FQ resistance and diagnostic delays [4, 5]. The primary mechanism of action of FQ is the inhibition of DNA synthesis [6] 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 FQ has mainly been attributed to point mutations in a 21-bp region of the MTBC *gyr*A gene, often called the quinolone resistance determining region (QRDR). These mutations, commonly occurring at codons 90, 91, and 94, prevent the drugs from effectively binding DNA gyrase [2, 6, 7]. Mutations in the *gyr*B gene have been noted with varying rates of resistance, but high-level resistance is less common without a concurrent *gyr*A mutation [6].

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 loci associated with drug resistance, resulting in both drug-resistant and drug-susceptible organisms [8, 9]. This phenomenon is not limited to FQ but is commonly noted with this class of drugs.

As newer FQ are assessed for use as antituberculosis drugs, it is important to determine cross-resistance between these and older FQ that are tested in growth-based DST methods. Studies suggest that there may not be full cross-resistance between ofloxacin (OFL), ciprofloxacin (CIP), levofloxacin (LVX), and moxifloxacin (MOX) at the defined critical concentrations and that low- and high-level resistance, as seen with INH, may be applicable to FQ as well, particularly MOX [10, 11].

DNA sequencing of *gyr*A revealed a G>A point mutation in codon 94 resulting in wild-type aspartate being replaced with asparagine (Asp94Asn). Sequencing of *gyr*B was wild-type (i.e., no mutations were detected).

Among three methods, 14 results for OFL were reported for Isolate 2017A. This isolate was reported as **resistant** to OFL by method, as follows:

- **89% (8/9)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Participating laboratories also reported results for other FQ drugs (i.e., CIP, LVF, and MOX) for Isolate 2017A; 83% (15/18) of results noted resistance to these additional FQ. The isolate was reported **resistant** to three other fluoroquinolones by method, as follows:

Ciprofloxacin

- **71% (5/7)** of the results when using AP
- **100% (1/1)** of the results when using MGIT

Moxifloxacin

- **50% (1/2)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Levofloxacin

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

This Asp94Asn mutation in the *gyr*A gene was detected by all (100%) laboratories that reported molecular testing for FQ drugs.

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 [12]. The primary mechanism of resistance is a mutation within the 81-bp central region of the *rpo*B gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase [7]. Mutations in codons 531, 526, and 516 (E. coli numbering system corresponding to 450, 445, and 435 in MTBC) are among the most frequent mutations in RMP-resistant isolates and serve as predictors of RMP resistance [7, 12]. The activity of RMP on isolates with *rpo*B 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 of *rpo*B [13]. 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 [14]. Sequencing of *rpo*B will allow for clarification of the result and understanding of possible discordance between rapid molecular and growth-based testing results.

DNA sequence analysis of *rpo*B in Isolate 2017A revealed a C>T point mutation in codon 514 of the *rpo*B locus. However, this mutation does not result in an amino acid change; phenylalanine remains phenylalanine (Phe514Phe). This synonymous (i.e., silent) mutation in *rpo*B is not considered clinically significant and isolates with this mutation reliably test as RMP-susceptible in growth-based systems. The Xpert MTB/RIF will generate a report of RMP resistance detected for isolates with this mutation.

Among four methods, 97 results for RMP were reported for Isolate 2017A. This isolate was reported as **susceptible** to RMP by method, as follows:

- **100% (17/17)** of the results when using AP
- **100% (74/74)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Eleven (85%) of the molecular results reported for RMP noted that a mutation was detected; six of which noted the silent mutation Phe514Phe. Three laboratories reported Mutation Not Detected, however this may be due to the detection of a silent mutation not associated with resistance.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2017A are listed in Tables, 3, 4, and 5.

Four laboratories noted no growth for at least one antituberculosis drug tested for Isolate 2017A.

Table 3. Isolate 2017A—Participant Results for First-Line DST

Results by Method for First-Line Drugs											
	AP			MGIT			Sensititre			VersaTREK		
Drug	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	17	0	17	74	0	74	4	0	4	2	0	2
Isoniazid–Low	17	0	17	72	1	73	4	0	4	2	0	2
Isoniazid–High	17	0	17	25	1	26	4	0	4	2	0	2
Ethambutol	18	0	18	72	0	72	4	0	4	2	0	2
Pyrazinamide				59	12	71				1	0	1

Note—S=susceptible, R=resistant

Table 4. Isolate 2017A—Participant Results for Second-Line DST

Results by Method for Second-Line Drugs									
	AP			MGIT			Sensititre		
Drug	S	R	Total	S	R	Total	S	R	Total
Streptomycin	18	0	18	38	0	38	3	0	3
Ofloxacin	1	8	9*	0	3	3	0	2	2
Ciprofloxacin	2	5	7	0	1	1			
Levofloxacin				0	3	3			
Moxifloxacin	1	1	2	0	3	3	0	2	2
Amikacin	9	0	9	3	0	3	3	0	3
Kanamycin	14	0	14	2	0	2	2	0	2
Capreomycin	13	1	14	4	0	4	1	0	1
Ethionamide	16	0	16	5	0	5	3	0	3
Rifabutin	7	0	7	4	0	4	3	0	3
Cycloserine	7	0	7				2	0	2
p-Aminosalicylic acid	12	0	12				3	0	3

Note—S=susceptible, R=resistant

*In addition, one laboratory reported borderline for OFL by AP.

Table 5. Isolate 2017A—Participant Results for Molecular Testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	10	3	13
Isoniazid	0	9	9
Ethambutol	0	5	5
Pyrazinamide	0	4	4
Ofloxacin	5	0	5
Ciprofloxacin	5	0	5
Levofloxacin	4	0	4
Moxifloxacin	4	0	4
Amikacin	0	4	4
Kanamycin	0	5	5
Capreomycin	0	4	4
Ethionamide	0	2	2
Rifabutin	1	1	2

Isolate 2017B

Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml, and STR at 2.0 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 tuberculosis (TB) disease and latent infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *kat*G gene [2, 12]. The target of activated INH is enoyl-acyl-carrier protein reductase (encoded by the *inh*A gene); this binding inhibits cell wall mycolic acid biosynthesis. There are two mechanisms that account for the majority of INH resistance [2, 7, 12]. The most common mechanism, mutations in *kat*G, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the *inh*A gene, which are generally associated with low-level resistance to INH and are less frequent than *kat*G mutations. Approximately 10–15% of isolates found to be INH resistant have no mutations detected in either of these loci. Numerous loci have been investigated to identify additional genes correlated with INH resistance. The *fab*G1 (also known as mabA) gene, like *inh*A, is involved in mycolic acid biosynthesis and at least one mutation in this region has been associated with low-level INH resistance [15, 16]. In MTBC, *ahp*C codes for an alkyl hydroperoxide reductase that is associated with resistance to reactive oxygen and reactive nitrogen intermediates; consequently it was initially believed that mutations in the promoter region could be surrogate markers for INH resistance [12].

DNA sequence analysis of *inh*A, *kat*G, *fab*G1, and *ahp*C of Isolate 2017B revealed a T>A point mutation at codon 315 in the *kat*G locus resulting in wild-type serine being replaced by threonine (Ser315Thr); *inh*A, *fab*G1 and *ahp*C 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 2017B, 101 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (22/22)** of the results when using AP
- **100% (73/73)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Sixty-seven (98%) results were reported as **resistant** at the higher concentrations of INH. Only 40 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.

For the nine molecular results reported for INH, all (100%) reported Mutation Detected.

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[7, 12]. In MTBC, the genetic basis of the majority of resistance to STR is usually due to mutations in *rrs* or *rps*L[6, 7]. CLSI recommended 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, 17].

Among three methods, 63 results for STR were reported for Isolate 2017B. This isolate was reported as **resistant** to STR by method, as follows:

- **100% (22/22)** of the results when using AP
- **100% (38/38)** of the results when using MGIT
- **100% (3/3)** of the results when using Sensititre

Ethionamide

Ethionamide (ETA) is a structural analog of INH. ETA, like INH, targets *inh*A, an enzyme involved in mycolic acid biosynthesis [18]. Resistance to INH and ETA can occur by mutations in the promoter region of the *inh*A gene which are generally associated with low-level resistance to INH. Mutations in *eth*A also confer resistance to ETA, without concomitant resistance to INH [18].

Sequencing analysis of *eth*A was not performed and, as noted above, sequencing of the *inh*A gene revealed wild-type (i.e., no mutations were detected) for the expected result of Isolate 2017B.

Issues with reproducibility of DST results for ETA have been reported [19] and remain a potential concern.

Isolate 2017B was expected to be susceptible to ETA; however, of those testing ETA, **resistance** was reported by method, as follows:

- **100% (19/19)** of the results when using AP
- **100% (4/4)** of the results when using MGIT
- **0% (0/2)** of the results when using Sensititre

For the two molecular results reported for ETA, one (50%) reported Mutation Detected.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2017B are listed in Tables 6, 7, and 8.

One laboratory noted no growth for at least one antituberculosis drug tested for Isolate 2017B.

Table 6. Isolate 2017B—Participant Results for First-Line DST

Results by Method for First-Line Drugs												
	AP			MGIT			Sensititre			VersaTREK		
Drug	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Rifampin	22	0	22	74	0	74	4	0	4	2	0	2
Isoniazid–Low	0	22	22	0	73	73	0	4	4	0	2	2
Isoniazid–High	0	22	22	1	39	40	0	4	4	0	2	2
Ethambutol	23	1	24	73	0	73	4	0	4	2	0	2
Pyrazinamide				69	3	72				1	0	1

Note—S=susceptible, R=resistant

Table 7. Isolate 2017B—Participant Results for Second-Line DST

Results by Method for Second-Line Drugs									
	AP			MGIT			Sensititre		
Drug	S	R	Total	S	R	Total	S	R	Total
Streptomycin	0	22	22	0	38	38	0	3	3
Ofloxacin	14	0	14	4	0	4	2	0	2
Ciprofloxacin	7	0	7	1	0	1			
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	3	0	3	2	0	2
Amikacin	11	0	11	3	0	3	3	0	3
Kanamycin	16	0	16	2	0	2	2	0	2
Capreomycin	14	1	15	4	0	4			
Ethionamide	0	19	19	0	4	4	2	0	2*
Rifabutin	7	0	7	3	0	3	2	0	2*
Cycloserine	8	1	9				1	0	1*
***p*-Aminosalicylic acid**	15	0	15				2	0	2*

Note—S=susceptible, R=resistant

*In addition, one laboratory reported borderline for CAP, ETA, RBT, CYC, and PAS by Sensititre.

Table 8. Isolate 2017B—Participant Results for Molecular Testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	13	13
Isoniazid	9	0	9
Ethambutol	0	5	5
Pyrazinamide	0	4	4
Ofloxacin	0	5	5
Ciprofloxacin	0	5	5
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	0	4	4
Kanamycin	0	5	5
Capreomycin	0	4	4
Ethionamide	1	1	2
Rifabutin	2	0	2

Isolate 2017C

Expected Result: Resistant to AMK at 4.0 µg/ml, CAP at 10.0 µg/ml, and KAN at 5.0 µg/ml by agar proportion

Second-line Injectables

The second-line injectable drugs include a cyclic-peptide antibiotic, capreomycin (CAP), and two aminoglycoside antibiotics, kanamycin (KAN) and amikacin (AMK). All three drugs inhibit protein synthesis and the primary mechanisms of resistance occur due to mutations in the following genes: *rrs* for AMK; *rrs* and *eis* for KAN; and *rrs* and *tly*A for CAP [6]. Since these drugs share a molecular target and bind at similar locations, cross-resistance has frequently been observed for mutations in the *rrs* that codes for 16S rRNA [2, 20]. The most common *rrs* mutation for cross-resistance to all three drugs is the A1401G point mutation [20].

Isolate 2017C was resistant to all of the second-line injectable drugs (AMK, KAN, and CAP) by the AP method and DNA sequence analysis of *rrs* revealed the A1401G mutation.

For Isolate 2017C, 54 results were reported for AMK, KAN, and CAP. The isolate was reported **resistant** to the three second-line injectables by method, as follows:

Amikacin

- **100% (11/11)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Capreomycin

- **100% (15/15)** of the results when using AP
- **100% (4/4)** of the results when using MGIT

Kanamycin

- **100% (15/15)** of the results when using AP
- **100% (2/2)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

This A1401G mutation in the *rrs* gene was detected by all (100%) laboratories that reported molecular testing for AMK, KAN, and CAP.

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2017C are listed in Tables 9, 10, and 11.

One laboratory noted no growth for at least one antituberculosis drug tested for Isolate 2017C.

Table 9. Isolate 2017C—Participant Results for First-Line DST

Results by Method for First-Line Drugs											
	AP			MGIT			Sensititre			VersaTREK		
Drug	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	20	0	20	74	0	74	4	0	4	2	0	2
Isoniazid–Low	20	0	20	72	1	73	4	0	4	2	0	2
Isoniazid–High	20	0	20	26	1	27	4	0	4	2	0	2
Ethambutol	19	2	21	73	0	73	4	0	4	2	0	2
Pyrazinamide				69	2	71*				1	0	1

Note—S=susceptible, R=resistant

*In addition, one laboratory reported borderline for PZA by MGIT.

Table 10. Isolate 2017C—Participant Results for Second-Line DST

Results by Method for Second-Line Drugs									
	AP			MGIT			Sensititre		
Drug	S	R	Total	S	R	Total	S	R	Total
Streptomycin	20	0	20	39	0	39	3	0	3
Ofloxacin	13	0	13	3	0	3	1	0	1*
Ciprofloxacin	6	0	6	1	0	1			
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	3	0	3	1	0	1*
Amikacin	0	11	11	0	3	3	0	2	2
Kanamycin	0	15	15	0	2	2	0	2	2
Capreomycin	0	15	15	0	4	4			
Ethionamide	18	0	18	4	0	4	2	0	2*
Rifabutin	7	0	7	3	0	3	2	0	2*
Cycloserine	9	0	9						
***p*-Aminosalicylic acid**	14	0	14				2	0	2*

Note—S=susceptible, R=resistant

*In addition, one laboratory reported borderline for OFL, MOX, ETA, RBT, CYC, and PAS by Sensititre.

Table 11. Isolate 2017C—Participant Results for Molecular Testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	0	13	13
Isoniazid	0	9	9
Ethambutol	0	5	5
Pyrazinamide	0	4	4
Ofloxacin	0	5	5
Ciprofloxacin	0	5	5
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	4	0	4
Kanamycin	5	0	5
Capreomycin	4	0	4
Ethionamide	0	2	2
Rifabutin	0	2	2

Isolate 2017D

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

Isoniazid

As previously noted, resistance to INH most commonly occurs due to mutations in the katG gene or the promoter region of the *inh*A gene, however, mutations in *fab*G1 and *ahp*C can also cause resistance. Within *fab*G1, the silent/synonymous mutation (i.e., nucleotide change but no corresponding change in amino acid) Leu203Leu has been found to confer INH resistance through the formation of an alternative promoter, thereby increasing the transcriptional levels of *inh*A [16]. Although silent mutations were previously believed to not play a role in drug resistance, the Leu203Leu mutation demonstrates that silent mutations could be associated with resistance depending on the specific gene and the location of the mutation.

DNA sequence analysis of *inh*A, *kat*G, *fab*G1, and *ahp*C for Isolate 2017D revealed a G>A point mutation at codon 203 resulting in the synonymous/silent mutation Leu203Leu; *inh*A, *kat*G, and *ahp*C were wild-type (i.e., no mutations were detected).

For Isolate 2017D, 98 INH results were reported. This isolate was reported **resistant** to low-level INH by method, as follows:

- **90% (18/20)** of the results when using AP
- **17% (12/72)** of the results when using MGIT
- **25% (1/4)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

For the nine molecular results reported for INH, two (22%) laboratories reported Mutation Detected noting it was a silent mutation.

Ethionamide

Resistance to INH and ETA can occur by mutations in the *fab*G1–*inh*A regulatory region, which are generally associated with low-level resistance to INH. Mutations in *eth*A also confer resistance to ETA, without concomitant resistance to INH [18].

Sequencing analysis of *eth*A was not performed and as previously noted, sequencing of the *inh*A gene revealed wild-type (i.e., no mutations were detected). The synonymous/silent mutation Leu203Leu was detected in the *fab*G1 locus for Isolate 2017D.

For Isolate 2017D, 25 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **95% (18/19)** of the results when using AP
- **100% (4/4)** of the results when using MGIT
- **0% (0/2)** of the results when using Sensititre

Rifampin

DNA sequence analysis of *rpo*B in Isolate 2017D revealed a C>T point mutation in codon 528 of the *rpo*B locus. However, this mutation does not result in an amino acid change; arginine remains arginine (Arg528Arg). Unlike the *fab*G1 silent mutation in this isolate that was associated with INH resistance, the Arg528Arg synonymous (i.e., silent) mutation in *rpo*B is not considered clinically significant and isolates with this mutation reliably test as RMP-susceptible in growth-based systems.

The Xpert MTB/RIF could generate a report of RMP resistance detected for isolates with this mutation. Sequencing of *rpo*B will allow for clarifying the result and understanding discordance between the Xpert result and results from growth-based testing.

Among four methods, 101 results for RMP were reported for Isolate 2017D. This isolate was reported as **susceptible** to RMP by method, as follows:

- **100% (21/21)** of the results when using AP
- **100% (74/74)** of the results when using MGIT
- **100% (4/4)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Of the thirteen molecular results reported for RMP, four (31%) reported Mutation Detected; however, five laboratories noted that a silent mutation was detected as a comment.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2017D are listed in Tables 12, 13, and 14.

One laboratory noted no growth for at least one antituberculosis drug tested for Isolate 2017D.

Table 12. Isolate 2017D—Participant Results for First-Line DST

Results by Method for First-Line Drugs											
	AP			MGIT			Sensititre			VersaTREK		
Drug	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	21	0	21	74	0	74	4	0	4	2	0	2
Isoniazid–Low	2	18	20	60	12	72	3	1	4	0	2	2
Isoniazid–High	21	0	21	28	1	29	4	0	4	2	0	2
Ethambutol	21	0	21*	73	0	73	4	0	4	2	0	2
Pyrazinamide				66	6	72				1	0	1

Note—S=susceptible, R=resistant

* In addition, one laboratory reported borderline for EMB by AP.

Table 13. Isolate 2017D—Participant Results for Second-Line DST

Results by Method for Second-Line Drugs									
	AP			MGIT			Sensititre		
Drug	S	R	Total	S	R	Total	S	R	Total
Streptomycin	21	0	21	39	0	39	3	0	3
Ofloxacin	14	0	14	3	0	3	1	1	2
Ciprofloxacin	7	0	7	1	0	1			
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	3	0	3	1	1	2
Amikacin	11	1	12	3	0	3	3	0	3
Kanamycin	16	0	16	2	0	2	1	0	1*
Capreomycin	15	1	16	4	0	4	1	0	1
Ethionamide	1	18	19	0	4	4	2	0	2*
Rifabutin	8	0	8	3	0	3	3	0	3
Cycloserine	9	0	9				1	0	1
***p*-Aminosalicylic acid**	15	0	15				2	0	2

Note—S=susceptible, R=resistant

* In addition, one laboratory reported borderline for KAN and ETA by Sensititre.

Table 14. Isolate 2017D—Participant Results for Molecular Testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	4	9	13
Isoniazid	2	7	9
Ethambutol	0	5	5
Pyrazinamide	0	4	4
Ofloxacin	0	5	5
Ciprofloxacin	0	5	5
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	0	4	4
Kanamycin	0	5	5
Capreomycin	0	5	5
Ethionamide	2	0	2
Rifabutin	1	1	2

Isolate 2017E

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

Isolate 2017E is susceptible to all first- and second-line drugs.

Most (99%) results were reported susceptible for this isolate across all methods.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2017E are listed in Tables 15, 16, and 17.

Two laboratories noted no growth for at least one antituberculosis drug tested for Isolate 2017E.

Table 15. Isolate 2017E—Participant Results for First-Line DST

Results by Method for First-Line Drugs												
	AP			MGIT			Sensititre			VersaTREK		
Drug	S	R	Total	S	R	Total	S	R	Total	S	R	Total
Rifampin	20	0	20	72	0	72	4	0	4	2	0	2
Isoniazid–Low	20	0	20	71	0	71	4	0	4	2	0	2
Isoniazid–High	20	0	20	26	0	26	4	0	4	2	0	2
Ethambutol	20	1	21	71	0	71	4	0	4	2	0	2
Pyrazinamide				70	1	71				1	0	1

Note—S=susceptible, R=resistant

Table 16. Isolate 2017E—Participant Results for Second-Line DST

Results by Method for Second-Line Drugs									
	AP			MGIT			Sensititre		
Drug	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Streptomycin	20	0	20	37	0	37	3	0	3
Ofloxacin	13	0	13	3	0	3	2	0	2
Ciprofloxacin	6	0	6	1	0	1			
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	3	0	3	1	0	1†
Amikacin	10	1	11	3	0	3	3	0	3
Kanamycin	15	0	15	2	0	2	2	0	2
Capreomycin	14	1	15	4	0	4	1	0	1
Ethionamide	16	1	17*	4	0	4	3	0	3
Rifabutin	7	0	7	3	0	3	3	0	3
Cycloserine	8	1	9				2	0	2
***p*-Aminosalicylic acid**	14	0	14				2	0	2

Note—S=susceptible, R=resistant

*In addition, one laboratory reported borderline for ETA by AP.

†In addition, one laboratory reported borderline for MOX by Sensititre.

Table 17. Isolate 2017E—Participant Results for Molecular Testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	3*	11	14
Isoniazid	0	9	9
Ethambutol	0	5	5
Pyrazinamide	0	4	4
Ofloxacin	0	5	5
Ciprofloxacin	0	5	5
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	0	4	4
Kanamycin	0	5	5
Capreomycin	0	4	4
Ethionamide	0	2	2
Rifabutin	0	2	2

*Three laboratories noted detection of Pro535Ser mutation.

Equivalent Critical Concentrations (Concentrations listed as µg/ml)

Agar Proportion

	7H10 agar	7H11 agar
First-Line Drugs		
Isoniazid	0.2 and 1.0*	0.2 and 1.0*
Rifampin	1.0	1.0
Ethambutol	5.0 and 10.0*	7.5
Pyrazinamide	Not recommended	Not recommended
Second-Line Drugs		
Streptomycin	2.0 and 10.0	2.0 and 10.0
Amikacin	4.0	-†
Capreomycin	10.0	10.0
Kanamycin	5.0	6.0
Levofloxacin	1.0	-†
Moxifloxacin	0.5	0.5
Ofloxacin	2.0	2.0
Ethionamide	5.0	10.0
Rifabutin	0.5	0.5
***p*-Aminosalicylic acid**	2.0	8.0

NOTE—Critical concentrations as indicated in CLSI M24-A2 document [1]
* The higher concentration of INH and EMB should be tested as second-line drugs after resistance at the critical concentration is detected.
† Breakpoints for establishing susceptibility have not be determined.

Broth Based Media

	MGIT	VersaTREK
First-Line Drugs		
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
Second-Line Drugs		
Streptomycin	1.0 (and 4.0*)	

NOTE—Critical concentrations as indicated in applicable manufacturer package inserts
*The higher concentration of INH, EMB, and STR should be tested after resistance at the critical concentration is detected.

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Appendix 1: Accessible Explanations of Figures

Figure 1. Primary Classification of Participating Laboratories, February 2017. The primary classification of the 80 laboratories participating in the February 2017 MPEP survey is shown in this pie chart. The largest slice, at 66%, represents 53 laboratories that have self-classified as a health department laboratory. The next major slice signifies 15 hospital laboratories. The remaining three slices of the pie chart represent 8 independent laboratories, 3 federal government laboratories, and 1 laboratory that identified as a quality control manufacturer.

Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year. The annual volume of MTBC isolates tested for drug susceptibility by participating laboratories (N=80) in 2015 is displayed in this vertical bar graph. The vertical y –axis is the number of laboratories responding and ranges from 0 to 35 using increments of 5. Along the horizontal x-axis are nine vertical bars representing the number of isolates tested per year. From left to right, 29 laboratories tested less than or equal to 50 isolates per year; 23 laboratories tested between 51 to 100 isolates per year; 6 laboratories tested between 101 to 150 isolates per year; 6 laboratories tested between 151 to 200 isolates per year; 2 laboratories tested between 201 to 250 isolates per year; 3 laboratories tested between 251 to 300 isolates per year; 3 laboratories tested between 301 to 500 isolates per year; 7 laboratories tested between 501 to 1000 isolates per year, and 1 laboratory tested between greater than or equal to 1001 isolates per year.

Figure 3. MTBC Drug Susceptibility Test Method Used by Participants. The drug susceptibility testing methods used by MPEP participants (N=120) is displayed in this vertical bar graph. The vertical y-axis is the number of laboratories reporting and ranges from 0 to 80, 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: 76 used MGIT, 25 used agar proportion, 4 used Sensititre, 2 used VersaTREK, and 13 used a molecular method.

Figure 4. Molecular Method Reported. The molecular methods used by MPEP participants (N=13) are displayed in this pie chart. The largest slice represents the 6 laboratories that perform targeted DNA sequencing. The next three slices represent 4 laboratories that use the Cepheid Xpert TB/RIF assay, 2 laboratories that reported results for the Hain line probe assays, and the 1 laboratory that reported results by whole genome sequencing.

Figure 5. Antituberculosis Drugs Tested by Participants. 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 and ranges from 0 to 90, by increments of 10. There are 16 horizontal bars with each bar representing the number of laboratories reporting a result for a particular drug for susceptibility testing. 80 laboratories tested rifampin; 80 laboratories tested isoniazid; 79 laboratories tested ethambutol; laboratories tested pyrazinamide; 53 laboratories tested streptomycin; 19 laboratories tested ofloxacin; 8 laboratories tested moxifloxacin; 8 laboratories tested ciprofloxacin; 5 laboratories tested levofloxacin; 19 laboratories tested kanamycin; 20 laboratories tested capreomycin; 17 laboratories tested amikacin; 25 laboratories tested ethionamide; 18 laboratories tested PAS; 14 laboratories tested rifabutin; and 11 laboratories tested cycloserine.

Notes:

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