Centers for Disease Control and Prevention
Model Performance Evaluation Program

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Report of Results
April 2016
Performance Evaluation Survey

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

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Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for April 2016 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 April 2016.
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The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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***Mycobacterium tuberculosis* Complex Drug Susceptibility Testing MPEP Report for April 2016 Survey**

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 April 2016 are shown in the tables below. Although CDC recommends broth-based methods for routine first-line DST of MTBC isolates, Table 1 provides the results obtained by the reference agar proportion method, except for pyrazinamide, in which MGIT was performed. Table 2 provides molecular results obtained by using DNA sequencing [2].

Table 1. Expected Growth-based Results for April 2016 Survey

Growth-based Results					
	First-Line Drugs				**Second-Line Drugs**
	RMP	INH	EMB	PZA	Resistant to:
2016A	S	R	S	S	STR, ETA
2016B	S	S	S	S	AMK, KAN, CAP
2016C	R	S	S	S	
2016D	S	S	S	S	OFL
2016E	R	S	S	S	STR, KAN

Note—S=susceptible, R=resistant

Table 2. Expected Molecular Results for April 2016 Survey

Mutations Detected in Loci Associated with Resistance					
	rpoB	*katG*	*rrs*	*eis*	*gyrA*
2016A		Ser315Thr			
2016B			A1401G		
2016C	His526Tyr				
2016D					Ser91Pro & Asp94Asn
2016E	Ser531Leu			G-10A	

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 2016 MTBC isolates A, B, C, D, and E in this report.

- The source of data in all tables and figures is the April 2016 MPEP MTBC DST survey.
- The tables indicate the number of reported results (S represents susceptible and R represents resistant) for each drug.
- 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.
- Of the 30 laboratories reporting second-line drug results (with the exception of streptomycin), ten (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

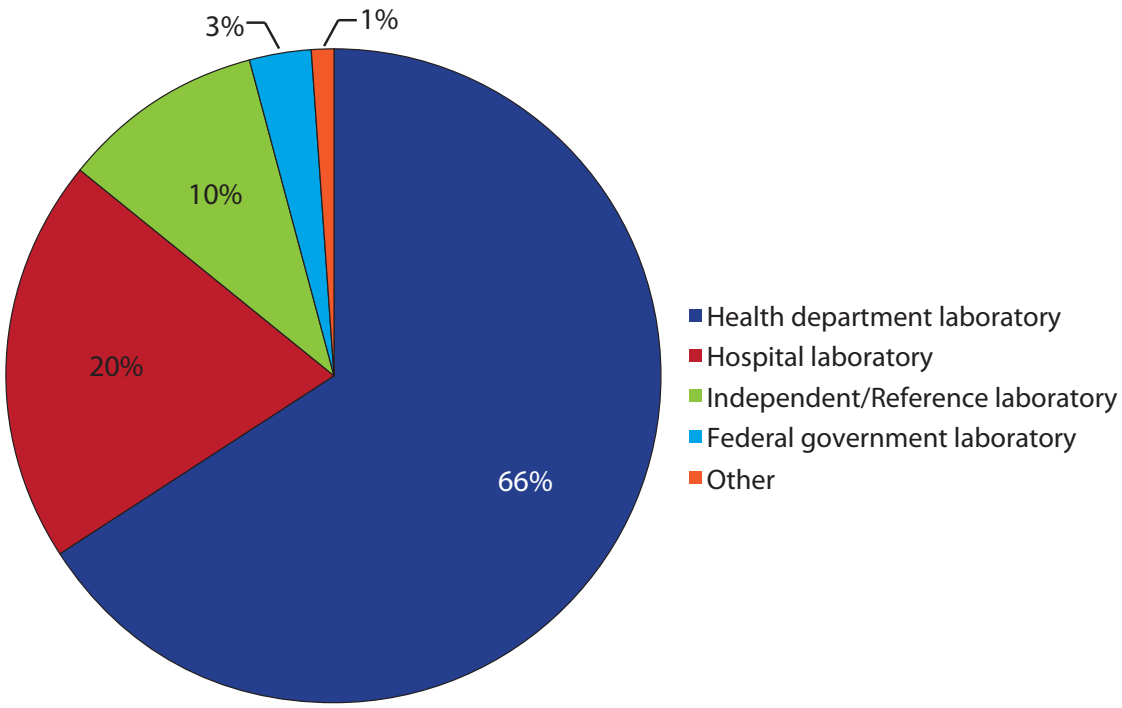
Primary Classification

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)
- 16 (20%): Hospital laboratory
- 8 (10%): Independent/Reference laboratory (non-hospital based)
- 2 (3%): Federal government laboratory
- 1 (1%): Other (quality control manufacturer)

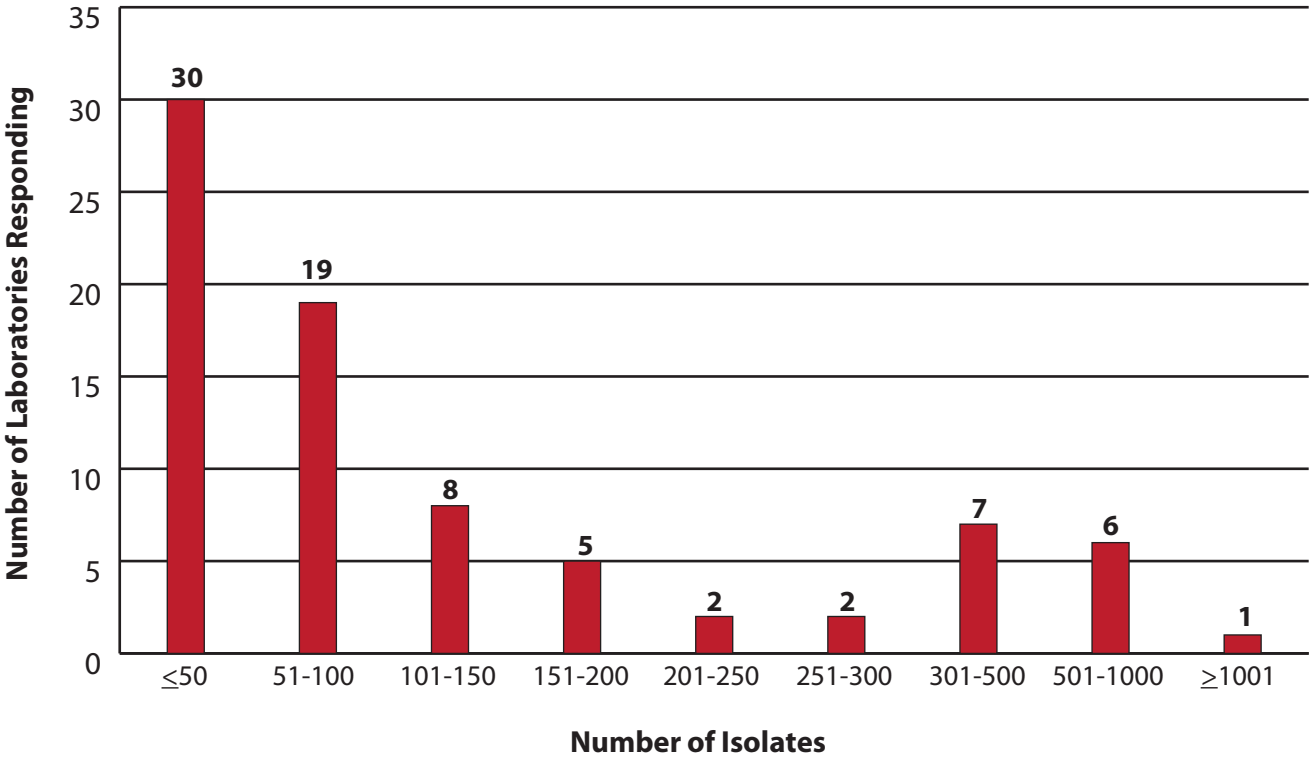
Figure 1. Primary Classification of Participating Laboratories, April 2016



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 80 participants in 2015 (excluding isolates used for quality control) is shown in Figure 2. In 2015, the counts ranged from 0 to 1416 tests. Participants at 30 (38%) laboratories reported testing 50 or fewer DST isolates per year, down from 35 (43%) laboratories in 2014. Laboratories with low MTBC DST volumes are encouraged to consider referral of testing because of concerns about maintaining proficiency [3].

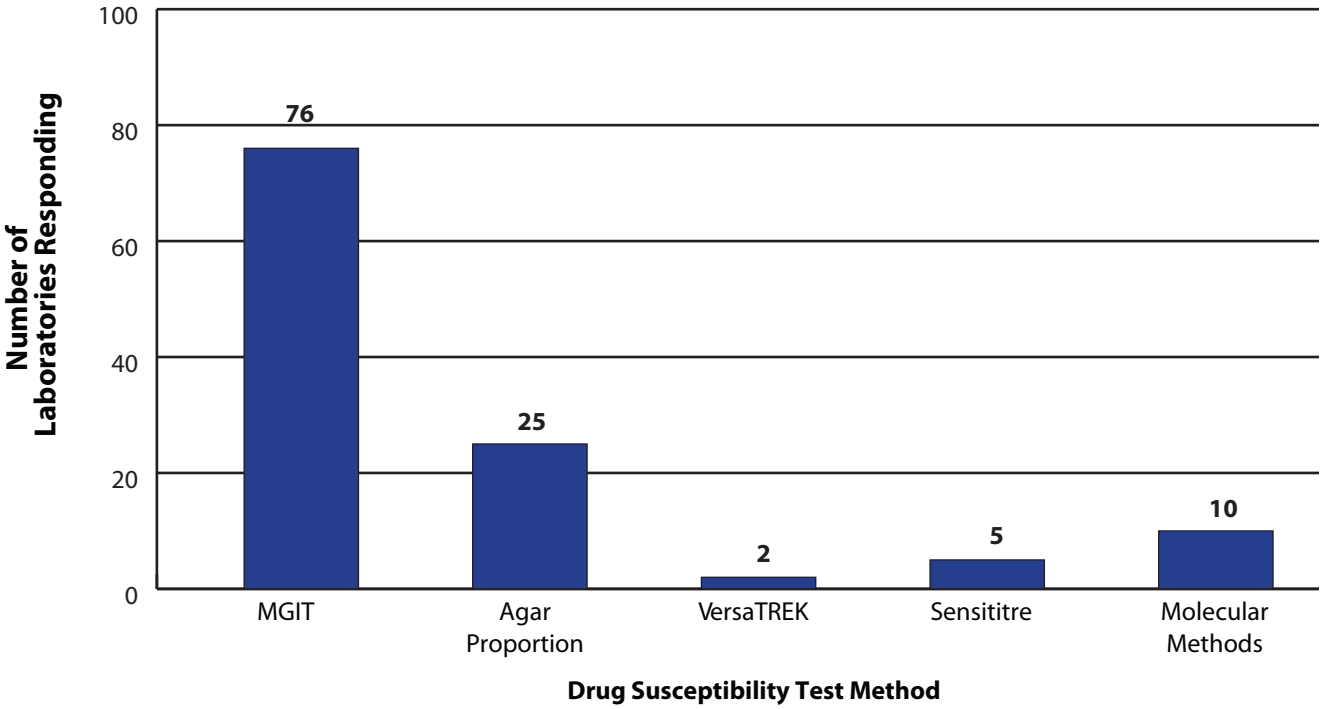
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=80)



MTBC DST Methods Used by Participants

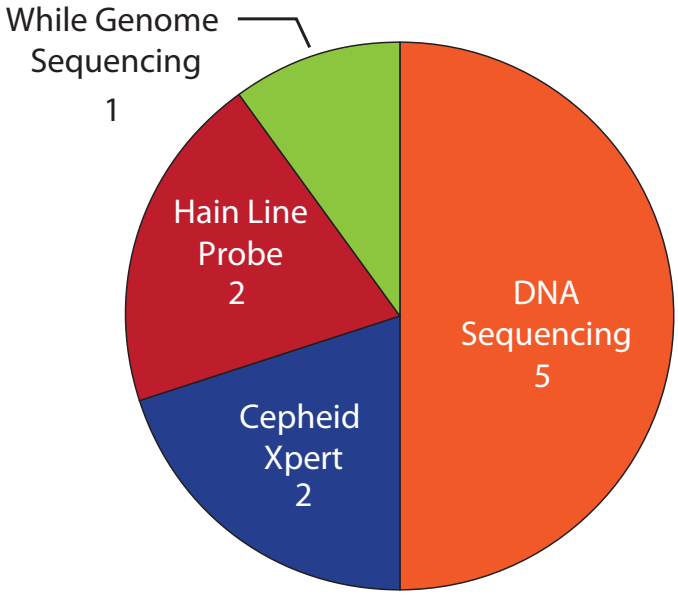
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 47 (59%) laboratories reported results for only one method, 29 laboratories reported two methods, and four laboratories noted three susceptibility methods.

Figure 3. MTBC Drug Susceptibility Test Method Used by Participants (n=118)



Molecular methods reported by ten participants are shown in Figure 4. The method used most frequently by laboratories was DNA sequencing (50%), including pyrosequencing and Sanger sequencing. Two laboratories reported results for the Cepheid Xpert MTB/RIF assay, two reported use of the line probe assays Genotype MTBDRplus and MTBDRsl by Hain Lifescience, and one reported results from whole genome sequencing.

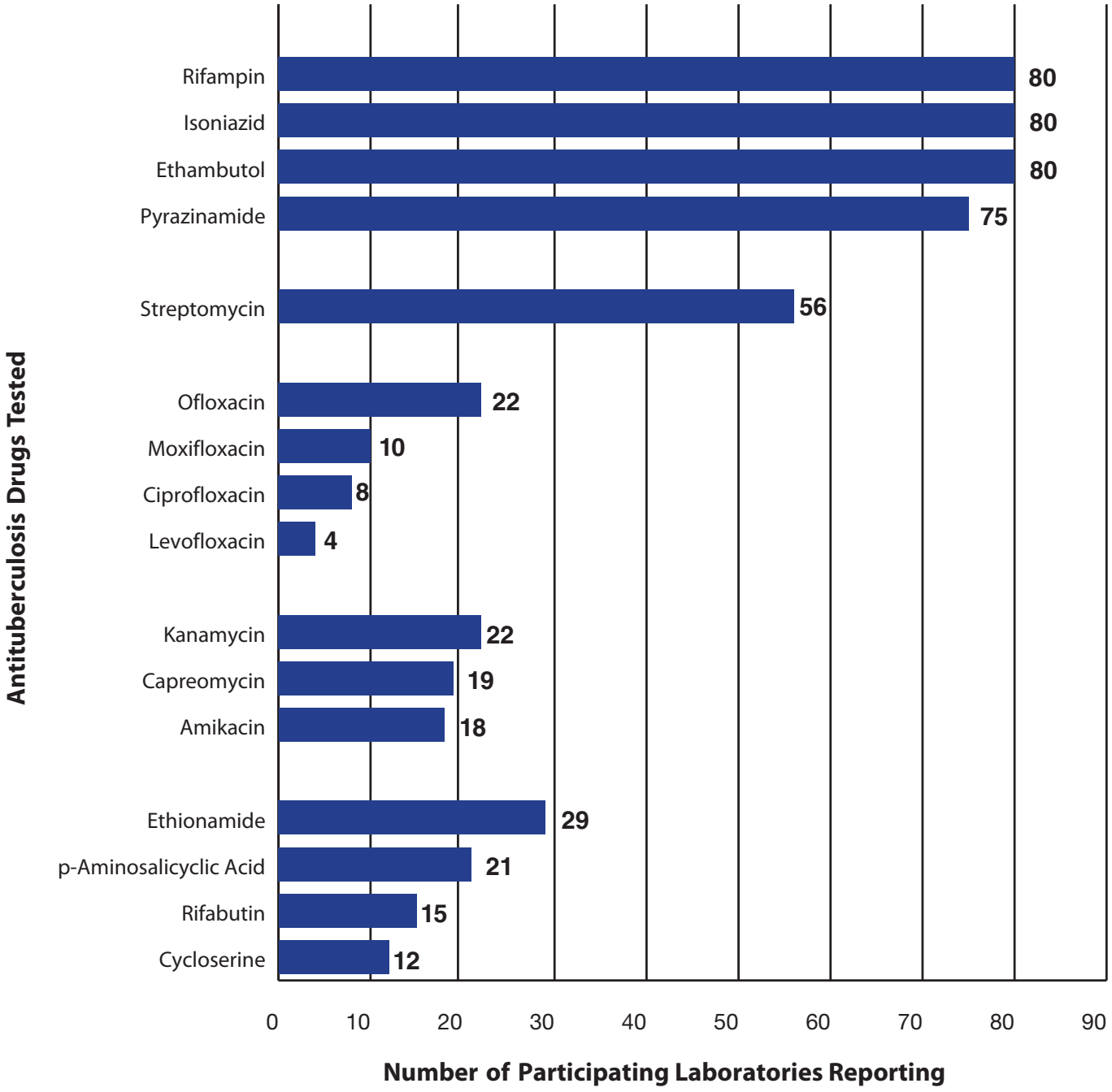
Figure 4. Molecular Method Reported (n=10)



Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the April 2016 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 in the United States. All participants reported results for three of the first-line drugs—RMP, INH, and EMB—and 75 (94%) of the participants also reported results for PZA. The number of laboratories testing second-line drugs has stayed relatively stable since the May 2014 survey despite the overall decrease in participating laboratories.

Figure 5. Antituberculosis Drugs Tested by Participants



Isolate 2016A

Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml, STR 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 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, 4]. 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, 4, 5]. 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 *fabG1* (also known as *mabA*) gene, like *inhA*, is involved in mycolic acid biosynthesis and at least one mutation in this region has been associated with low-level INH resistance [6, 7]. In MTBC, *ahpC* 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 [4].

DNA sequence analysis of *inhA*, *katG*, *fabG1,* and *ahpC* of Isolate 2016A revealed a T>A point mutation at codon 315 in the *kat*G locus resulting in wild-type serine being replaced by threonine (Ser315Thr); *inhA*, *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 2016A, 103 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- 100% (23/23) of the results when using AP
- 100% (73/73) of the results when using MGIT
- 100% (5/5) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

Sixty-seven (100%) results were reported as **resistant** at the higher concentrations of INH.

Of the eight molecular results reported for INH, all (100%) detected a mutation.

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[4, 5]. In MTBC, the genetic basis of the majority of resistance to STR is usually due to mutations in *rrs* or *rpsL*[5, 8]. 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, 9].

Among three methods, 71 results for STR were reported for Isolate 2016A. This isolate was reported as **resistant** to STR by method, as follows:

- 100% (23/23) of the results when using AP
- 100% (44/44) of the results when using MGIT
- 100% (4/4) 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 [10]. 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 [10].

Sequencing of the *inhA* gene revealed wild-type (i.e., no mutations were detected) for Isolate 2016A and sequencing analysis of *ethA* was not performed.

Issues with reproducibility of DST results for ETA have been reported [11] and remain a potential concern.

For Isolate 2016A, 27 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- 62% (13/21) of the results when using AP
- 100% (3/3) of the results when using MGIT
- 67% (2/3) of the results when using Sensititre

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2016A are listed in Tables, 3, 4, and 5.

Table 3. Isolate 2016A—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	23	0	23	73	0	73*	5	0	5	2	0	2
Isoniazid-Low	0	23	23	0	73	73*	0	5	5	0	2	2
Isoniazid-High	0	23	23	0	37	37	0	5	5	0	2	2
Ethambutol	25	0	25	73	0	73*	5	0	5	2	0	2
Pyrazinamide				72	1	73*				1	0	1

Note—S=susceptible, R=resistant

* In addition, one laboratory reported contamination for RMP, INH, EMB, and PZA by MGIT.

Table 4. Isolate 2016A—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	23	23	0	44	44	0	4	4
Ofloxacin	16	0	16	4	0	4	3	0	3
Ciprofloxacin	7	0	7	1	0	1			
Levofloxacin	1	0	1	2	0	2	1	0	1
Moxifloxacin	3	0	3	3	0	3	3	0	3
Amikacin	12	0	12	2	0	2	4	0	4
Kanamycin	17	1	18	1	0	1	3	0	3
Capreomycin	15	0	15	3	0	3	1	0	1
Ethionamide	8	13	21	0	3	3	1	2	3*
Rifabutin	8	0	8	3	0	3	4	0	4
Cycloserine	8	1	9				3	0	3
***p*-Aminosalicylic acid**	16	1	17				4	0	4

Note—S=susceptible, R=resistant

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

Table 5. Isolate 2016A—Participant Results for Molecular Testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	10	10
Isoniazid	8	0	8
Ethambutol	0	4	4
Pyrazinamide	0	2	2
Ofloxacin	0	4	4
Ciprofloxacin	0	4	4
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	0	3	3
Kanamycin	0	4	4
Capreomycin	0	3	3
Ethionamide	1	0	1
Rifabutin	0	2	2

Isolate 2016B

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 genes as follows: *rrs* for AMK; *rrs* and *eis* for KAN; and *rrs* and *tlyA* for CAP [8]. Since these drugs share a molecular target and bind at similar locations, cross-resistance has frequently been detected [2, 12]. The most common mechanism of cross-resistance to all three drugs is due to an A1401G point mutation in the *rrs* gene coding for 16S rRNA [12].

Isolate 2016B was resistant to all of the second-line injectable drugs (AMK, KAN, and CAP) by the AP method and DNA sequence analysis of the *rrs* gene revealed the A1401G mutation.

For Isolate 2016B, 57 results were reported for AMK, KAN, and CAP. The isolate was reported **resistant** to the three second-line injectables by method, as follows:

- 96% (43/45) of the results when using AP
- 100% (6/6) of the results when using MGIT
- 100% (6/6) of the results when using Sensititre

This A1401G mutation in the *rrs* gene was detected by the four laboratories that reported molecular testing for the second-line injectable drugs.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2016B are listed in Tables 6, 7, and 8.

Table 6. Isolate 2016B—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	5	0	5	2	0	2
Isoniazid–Low	21	0	21	74	0	74	5	0	5	2	0	2
Isoniazid–High	21	0	21	22	0	22	5	0	5	2	0	2
Ethambutol	23	0	23	74	0	74	5	0	5	2	0	2
Pyrazinamide				72	2	74				1	0	1

Note—S=susceptible, R=resistant

Table 7. Isolate 2016B—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	44	0	44	4	0	4
Ofloxacin	13	0	13	3	0	3	3	0	3
Ciprofloxacin	6	0	6	1	0	1			
Levofloxacin	1	0	1	2	0	2	1	0	1
Moxifloxacin	2	1	3*	3	0	3	2	0	2#
Amikacin	1	12	13	0	2	2	0	3	3
Kanamycin	0	17	17	0	1	1	0	3	3
Capreomycin	1	14	15	0	3	3			
Ethionamide	21	0	21	2	0	2†	4	0	4
Rifabutin	8	0	8	3	0	3	4	0	4
Cycloserine	9	0	9				2	0	2
***p*-Aminosalicylic acid**	16	0	16				4	0	4

Note—S=susceptible, R=resistant

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

† In addition, one laboratory reported borderline for ETA by MGIT.

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

Table 8. Isolate 2016B—Participant Results for Molecular Testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	10	10
Isoniazid	0	8	8
Ethambutol	0	4	4
Pyrazinamide	0	2	2
Ofloxacin	0	4	4
Ciprofloxacin	0	4	4
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	4	0	4
Kanamycin	4	0	4
Capreomycin	4	0	4
Ethionamide	0	1	1
Rifabutin	0	2	2

Isolate 2016C

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

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 [4]. 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 [5]. 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 [4, 5]. 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 of *rpoB* [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 *rpoB* will allow 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 2016C revealed a C>G point mutation in codon 526 resulting in wild-type histidine being replaced by tyrosine (His526Tyr). Isolates with His526Tyr mutations consistently test resistant to RMP in growth-based assays.

Among four methods, 104 results for RMP were reported for Isolate 2016C. This isolate was reported as **resistant** to RMP by method, as follows:

- 100% (23/23) of the results when using AP
- 100% (74/74) of the results when using MGIT
- 100% (5/5) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

All ten (100%) of the molecular results reported for RMP noted that a mutation was detected.

Pyrazinamide

Pyrazinamide (PZA) is an important first-line drug for treatment of TB and is used with INH and RMP. The addition of this drug shortens TB treatment from the previous 9–12 months to 6 months because it kills a population of persistent bacilli in acidic pH environments within the lesions that are not killed by other drugs. PZA-resistant MTBC strains lose pyrazinamidase activity and resistance to PZA is usually caused by nucleotide changes scattered throughout the *pncA* gene. There may be additional mechanisms of resistance to PZA that are still unknown[15], but issues with false resistance to PZA have been reported as well [16] and remain a potential concern.

For Isolate 2016C, DNA sequencing of the *pncA* gene did not reveal a mutation.

Isolate 2016C was expected to be susceptible to PZA; however, of those testing PZA, **resistance** was reported by:

- 32% (24/74) of the results when using MGIT
- 0% (0/1) of the results when using VersaTREK

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2016C are listed in Tables 9, 10, and 11.

Table 9. Isolate 2016C—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	0	23	23	0	74	74	0	5	5	0	2	2
Isoniazid–Low	23	0	23	74	0	74	5	0	5	2	0	2
Isoniazid–High	23	0	23	22	0	22	5	0	5	2	0	2
Ethambutol	25	0	25	74	0	74	5	0	5	2	0	2
Pyrazinamide				50	24	74				1	0	1

Note—S=susceptible, R=resistant

Table 10. Isolate 2016C—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	23	0	23	43	0	43	4	0	4
Ofloxacin	16	0	16	4	0	4	3	0	3
Ciprofloxacin	7	0	7	1	0	1			
Levofloxacin	1	0	1	2	0	2	1	0	1
Moxifloxacin	3	0	3	3	0	3	4	0	4
Amikacin	12	0	12	2	0	2	4	0	4
Kanamycin	18	0	18	1	0	1	2	0	2*
Capreomycin	15	0	15	3	0	3	1	0	1
Ethionamide	22	0	22	3	0	3	4	0	4
Rifabutin	1	7	8	0	3	3	0	4	4
Cycloserine	8	1	9				3	0	3
***p*-Aminosalicylic acid**	17	0	17				4	0	4

Note—S=susceptible, R=resistant

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

Table 11. Isolate 2016C—Participant Results for Molecular Testing

	Molecular Testing		
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	10	0	10
Isoniazid	0	8	8
Ethambutol	0	4	4
Pyrazinamide	0	2	2
Ofloxacin	0	4	4
Ciprofloxacin	0	4	4
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	0	4	4
Kanamycin	0	4	4
Capreomycin	0	4	4
Ethionamide	0	1	1
Rifabutin	2	0	2

Isolate 2016D

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 [17]. 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 [17, 18]. The primary mechanism of action of FQ is the inhibition of DNA synthesis [8] 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, 5, 8]. 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 [8].

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 [19, 20]. 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 [21, 22].

DNA sequencing of *gyr*A in Isolate 2016D revealed a T>C point mutation in codon 91 of *gyr*A resulting in wild-type serine being replaced with proline (Ser91Pro). The Ser91Pro mutation has been associated with FQ resistance [2, 23]. DNA sequencing also revealed a G>A point mutation in codon 94 resulting in wild-type aspartate being replaced with asparagine (Asp94Asn). Sequencing of *gyrB* was wild-type (i.e., no mutations were detected).

Among three methods, 22 results for OFL were reported for Isolate 2016D. This isolate was reported as **resistant** to OFL by method, as follows:

- 93% (14/15) of the results when using AP
- 100% (4/4) of the results when using MGIT
- 100% (3/3) of the results when using Sensititre

Participating laboratories also reported results for other FQ drugs (i.e., CIP, LVF, and MOX) for Isolate 2016D; 95% (18/19) of results noted resistance to these additional FQ.

A mutation in the *gyrA* gene was detected by the four laboratories that reported molecular testing for fluoroquinolones.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2016D are listed in Tables 12, 13, and 14.

Table 12. Isolate 2016D—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	5	0	5	2	0	2
Isoniazid–Low	21	0	21	74	0	74	5	0	5	2	0	2
Isoniazid–High	21	0	21	22	0	22	5	0	5	2	0	2
Ethambutol	23	0	23	74	0	74	5	0	5	2	0	2
Pyrazinamide				74	0	74				1	0	1

Note—S=susceptible, R=resistant

Table 13. Isolate 2016D—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	43	0	43	4	0	4
Ofloxacin	1	14	15	0	4	4	0	3	3
Ciprofloxacin	0	6	6	0	1	1			
Levofloxacin	0	1	1	0	2	2			
Moxifloxacin	0	3	3	1	2	3	0	3	3
Amikacin	12	0	12	2	0	2	4	0	4
Kanamycin	17	0	17	1	0	1	3	0	3
Capreomycin	15	0	15	3	0	3	1	0	1
Ethionamide	21	0	21	3	0	3	4	0	4
Rifabutin	8	0	8	2	0	2*	4	0	4
Cycloserine	9	0	9				3	0	3
***p*-Aminosalicylic acid**	16	0	16				4	0	4

Note—S=susceptible, R=resistant

* In addition, one laboratory reported borderline for RBT by MGIT

Table 14. Isolate 2016D—Participant Results for Molecular Testing

	Molecular Testing		
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	0	10	10
Isoniazid	0	8	8
Ethambutol	0	4	4
Pyrazinamide	1*	1	2
Ofloxacin	4	0	4
Ciprofloxacin	4	0	4
Levofloxacin	4	0	4
Moxifloxacin	4	0	4
Amikacin	0	4	4
Kanamycin	0	4	4
Capreomycin	0	4	4
Ethionamide	0	1	1
Rifabutin	0	2	2

* One laboratory noted the mutation detected was a silent mutation for PZA.

Isolate 2016E

Expected Result: Resistant to RMP at 1.0 µg/ml, STR at 2.0 µg/ml, and KAN at 5.0 µg/ml by agar proportion

Rifampin

DNA sequence analysis of *rpoB* in Isolate 2016E revealed a C>T point mutation in codon 531 resulting in wild-type serine being replaced by leucine (Ser531Leu). Isolates with Ser531Leu mutations consistently test resistant to RMP in growth-based assays.

Among four methods, 104 results for RMP were reported for Isolate 2016E. This isolate was reported as **resistant** to RMP by method, as follows:

- 100% (23/23) of the results when using AP
- 100% (74/74) of the results when using MGIT
- 100% (5/5) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

All ten (100%) of the molecular results reported for RMP noted that a mutation was detected.

Streptomycin

Among three methods, 70 results for STR were reported for Isolate 2016E. This isolate was reported as **resistant** to STR by method, as follows:

- 91% (20/22) of the results when using AP
- 98% (43/44) of the results when using MGIT
- 100% (4/4) of the results when using Sensititre

Second-line injectables/Kanamycin

As noted for Isolate 2016B, the most common mechanism of cross-resistance to all three second-line injectable drugs is due to an A1401G point mutation in the *rrs* gene [12]. However, low-level KAN resistance, without AMK or CAP resistance, is associated with mutations in the promoter region of the *eis* gene which results in the overexpression of the encoded aminoglycoside acetyltransferase [2, 24].

DNA sequence analysis of the *rrs* and *eis* genes of Isolate 2016E revealed a G-10A mutation in the promoter region of *eis*; *rrs* was wild-type (i.e., no mutations were detected).

Among three methods, 22 results for KAN were reported for Isolate 2016E. This isolate was reported as **resistant** to KAN by method, as follows:

- 83% (15/18) of the results when using AP
- 100% (1/1) of the results when using MGIT
- 100% (3/3) of the results when using Sensititre

This G-10A mutation in the *eis* gene was detected by one of the four laboratories that reported molecular testing for KAN.

Ethionamide

As noted in 2016A, there is concern regarding reproducibility of DST results for ETA. Isolate 2016E was expected to be susceptible to ETA; however, of those testing ETA, **resistance** was reported by:

- 67% (14/21) of the results when using AP
- 100% (3/3) of the results when using MGIT
- 67% (2/3) of the results when using Sensititre

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2016E are listed in Tables 15, 16, and 17.

Table 15. Isolate 2016E—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	0	23	23	0	74	74	0	5	5	0	2	2
Isoniazid–Low	23	0	23	74	0	74	5	0	5	2	0	2
Isoniazid–High	23	0	23	23	0	23	5	0	5	2	0	2
Ethambutol	22	2	24*	74	0	74	5	0	5	2	0	2
Pyrazinamide				74	0	74				1	0	1

Note—S=susceptible, R=resistant

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

Table 16. Isolate 2016E—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	2	20	22*	1	43	44	0	4	4
Ofloxacin	15	1	16	4	0	4	3	0	3
Ciprofloxacin	7	0	7	1	0	1			
Levofloxacin	1	0	1	2	0	2	1	0	1
Moxifloxacin	2	1	3	3	0	3	2	0	2#
Amikacin	12	0	12	2	0	2	4	0	4
Kanamycin	3	15	18	0	1	1	0	3	3
Capreomycin	15	0	15	3	0	3	1	0	1
Ethionamide	7	14	21†	0	3	3	1	2	3#
Rifabutin	2	6	8	0	3	3	0	4	4
Cycloserine	9	0	9				3	0	3
***p*-Aminosalicylic acid**	17	0	17				4	0	4

Note—S=susceptible, R=resistant

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

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

\# In addition, one laboratory reported borderline for MOX and ETA by Sensititre.

Table 17. Isolate 2016E—Participant Results for Molecular Testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	10	0	10
Isoniazid	0	8	8
Ethambutol	0	4	4
Pyrazinamide	0	2	2
Ofloxacin	0	4	4
Ciprofloxacin	0	4	4
Levofloxacin	0	4	4
Moxifloxacin	0	4	4
Amikacin	0	4	4
Kanamycin	1	3	4
Capreomycin	0	4	4
Ethionamide	1	0	1
Rifabutin	2	0	2

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 Drug		
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.

References

1. CLSI, *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard - Second Edition in CLSI Document M24 A-2. 2011, Clinical and Laboratory Standards Institute: Wayne, PA.*

2. *Campbell, P.J., et al., Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis. Antimicrob Agents Chemother, 2011.* 55(5): p. 2032-41.

3. APHL, *TB Drug Susceptibility Testing Expert Panel Meeting Summary Report. 2007, Association of Public Health Laboratories: Washington, D.C.*

4. *Almeida Da Silva, P.E. and J.C. Palomino, Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs. J Antimicrob Chemother, 2011.* 66(7): p. 1417-30.

5. Zhang, Y. and W.W. Yew, *Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis, 2009.* 13(11): p. 1320-30.

6. Ramaswamy, S.V., et al., *Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother, 2003.* 47(4): p. 1241-50.

7. Ando, H., et al., *A silent mutation in mabA confers isoniazid resistance on Mycobacterium tuberculosis. Mol Microbiol, 2014.* 91(3): p. 538-47.

8. Zhang, Y. and W.W. Yew, *Mechanisms of drug resistance in Mycobacterium tuberculosis: update 2015. Int J Tuberc Lung Dis, 2015.* 19(11): p. 1276-89.

9. Centers for Disease Control and Prevention, *Treatment of Tuberculosis, American Thoracic Society, CDC, and Infectious Diseases Society of America. 2003, MMWR. p. 4,11,19-20.*

10. *Morlock, G.P., et al., ethA, inhA, and katG loci of ethionamide-resistant clinical Mycobacterium tuberculosis isolates. Antimicrob Agents Chemother, 2003.* 47(12): p. 3799-805.

11. Varma-Basil, M. and R. Prasad, *Dilemmas with ethionamide susceptibility testing of Mycobacterium tuberculosis: A microbiologist & physician's nightmare. Indian J Med Res, 2015.* 142(5): p. 512-4.

12. Maus, C.E., B.B. Plikaytis, and T.M. Shinnick, *Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis. Antimicrob Agents Chemother, 2005.* 49(8): p. 3192-7.

13. *Availability of an assay for detecting Mycobacterium tuberculosis, including rifampin-resistant strains, and considerations for its use - United States, 2013. MMWR Morb Mortal Wkly Rep, 2013.* 62(41): p. 821-7.

14. Van Deun, A., et al., *Rifampin drug resistance tests for tuberculosis: challenging the gold standard. J Clin Microbiol, 2013.* 51(8): p. 2633-40.

15. Ramirez-Busby, S.M. and F. Valafar, *Systematic Review of Mutations in Pyrazinamidase Associated with Pyrazinamide Resistance in Mycobacterium tuberculosis Clinical Isolates. Antimicrob Agents Chemother, 2015.* 59(9): p. 5267-77.

16. Chedore, P., et al., *Potential for erroneous results indicating resistance when using the Bactec MGIT 960 system for testing susceptibility of Mycobacterium tuberculosis to pyrazinamide. J Clin Microbiol, 2010.* 48(1): p. 300-1.

17. Devasia, R.A., et al., *Fluoroquinolone resistance in Mycobacterium tuberculosis: the effect of duration and timing of fluoroquinolone exposure. Am J Respir Crit Care Med, 2009.* 180(4): p. 365-70.

18. Chen, T.C., et al., *Fluoroquinolones are associated with delayed treatment and resistance in tuberculosis: a systematic review and meta-analysis. Int J Infect Dis, 2011.* 15(3): p. e211-6.

19. Eilertson, B., et al., *High proportion of heteroresistance in gyrA and gyrB in fluoroquinolone-resistant Mycobacterium tuberculosis clinical isolates. Antimicrob Agents Chemother, 2014.* 58(6): p. 3270-5.

20. Rinder, H., K.T. Mieskes, and T. Loscher, *Heteroresistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis, 2001.* 5(4): p. 339-45.

21. Willby, M., et al., *Correlation between GyrA substitutions and ofloxacin, levofloxacin, and moxifloxacin cross-resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother, 2015.* 59(9): p. 5427-34.

22. Kam, K.M., et al., *Stepwise decrease in moxifloxacin susceptibility amongst clinical isolates of multidrug-resistant Mycobacterium tuberculosis: correlation with ofloxacin susceptibility. Microb Drug Resist, 2006.* 12(1): p. 7-11.

23. Maruri, F., et al., *A systematic review of gyrase mutations associated with fluoroquinolone-resistant Mycobacterium tuberculosis and a proposed gyrase numbering system. Journal of Antimicrobial Chemotherapy, 2012.* 67(4): p. 819-831.

24. Zaunbrecher, M.A., et al., *Overexpression of the chromosomally encoded aminoglycoside acetyltransferase eis confers kanamycin resistance in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A, 2009.* 106(47): p. 20004-9.