Centers for Disease Control and Prevention
Model Performance Evaluation Program

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Report of Results
May 2015
Performance Evaluation Survey

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

CS260712

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for May 2015 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 May 2015.
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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 Report for May 2015 Samples 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 May 2015 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 Conventional Results for May 2015 Survey

	Growth-based Results				
	First-Line Drugs				**Second-Line Drugs**
	INH	RMP	EMB	PZA	Resistant to:
2015A	R	S	S	S	
2015B	R	S	S	S	STR
2015C	R	S	S	S	STR
2015D	S	S	S	R	
2015E	R	S	S	S	

Note—S=susceptible, R=resistant

Table 2. Expected Molecular Results for May 2015 Survey

	Mutations Detected				
	First-Line Drugs				
	rpoB	***inhA***	***katG***	***fabG1***	***pncA***
2015A	Arg528Arg			Leu203Leu	
2015B			Ser315Thr		
2015C					
2015D					His57Asp
2015E		C-15T			

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 2015 MTBC isolates A, B, C, D, and E in this report.

- The source of data in all tables and figures is the May 2015 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 83 (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 33 laboratories reporting second-line drug results (with the exception of streptomycin), nine (27%) 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

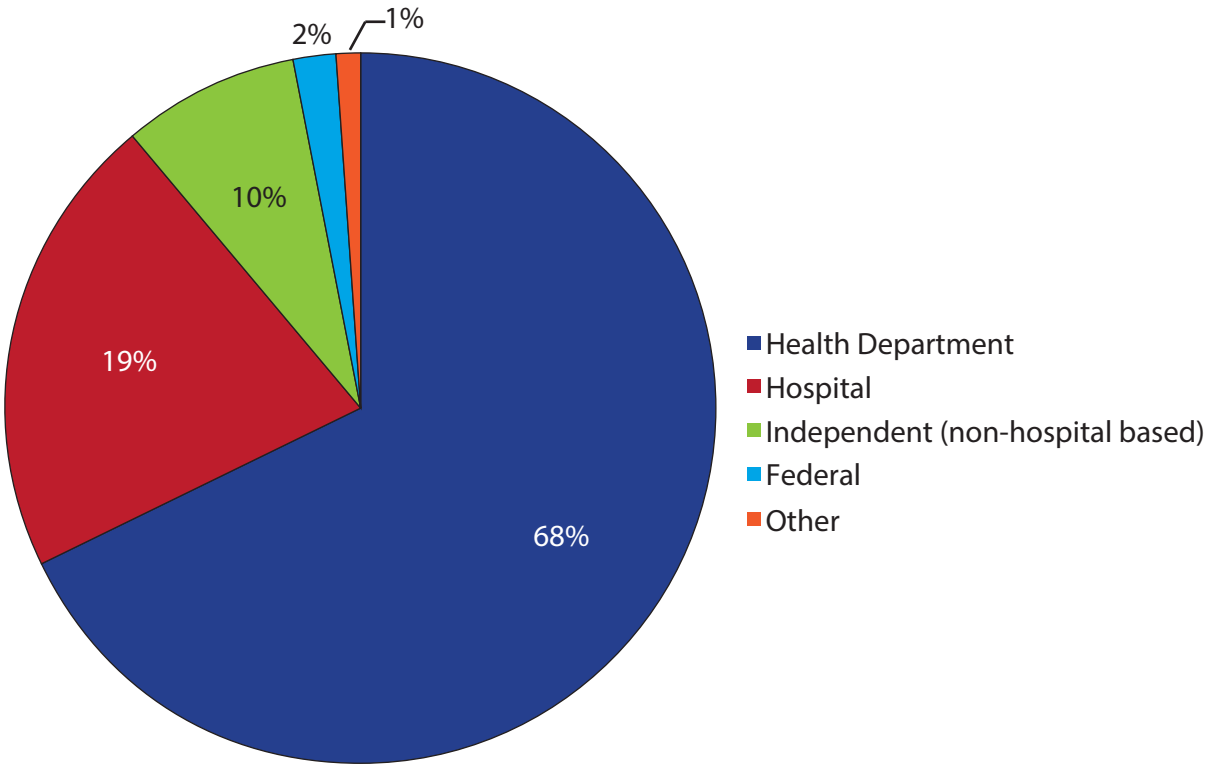
Primary Classification

This report contains DST results submitted to CDC by survey participants at 83 laboratories in 37 states.

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

- 56 (68%): Health department (city, country, state, regional, or district laboratory)
- 16 (19%): Hospital laboratory
- 8 (10%): Independent (e.g., commercial, commercial manufacturer of reagents, reference laboratory [non-governmental affiliated])
- 2 (2%): Federal government laboratory
- 1 (1%): Other (quality control manufacturer)

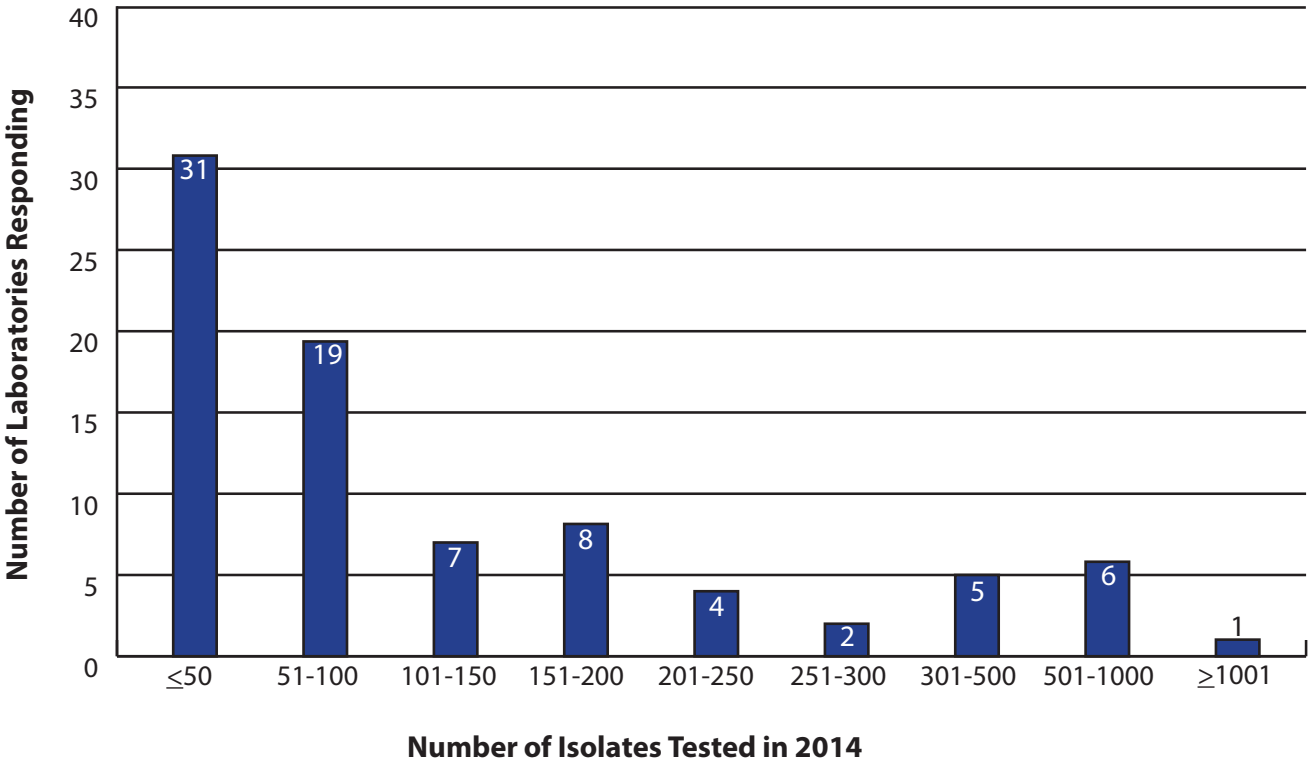
Figure 1. Primary Classification of Participating Laboratories



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 83 participants in 2014 (excluding isolates used for quality control) is shown in Figure 2. In 2014, the counts ranged from 0 to 1100 tests and participants at 31 (37%) 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].

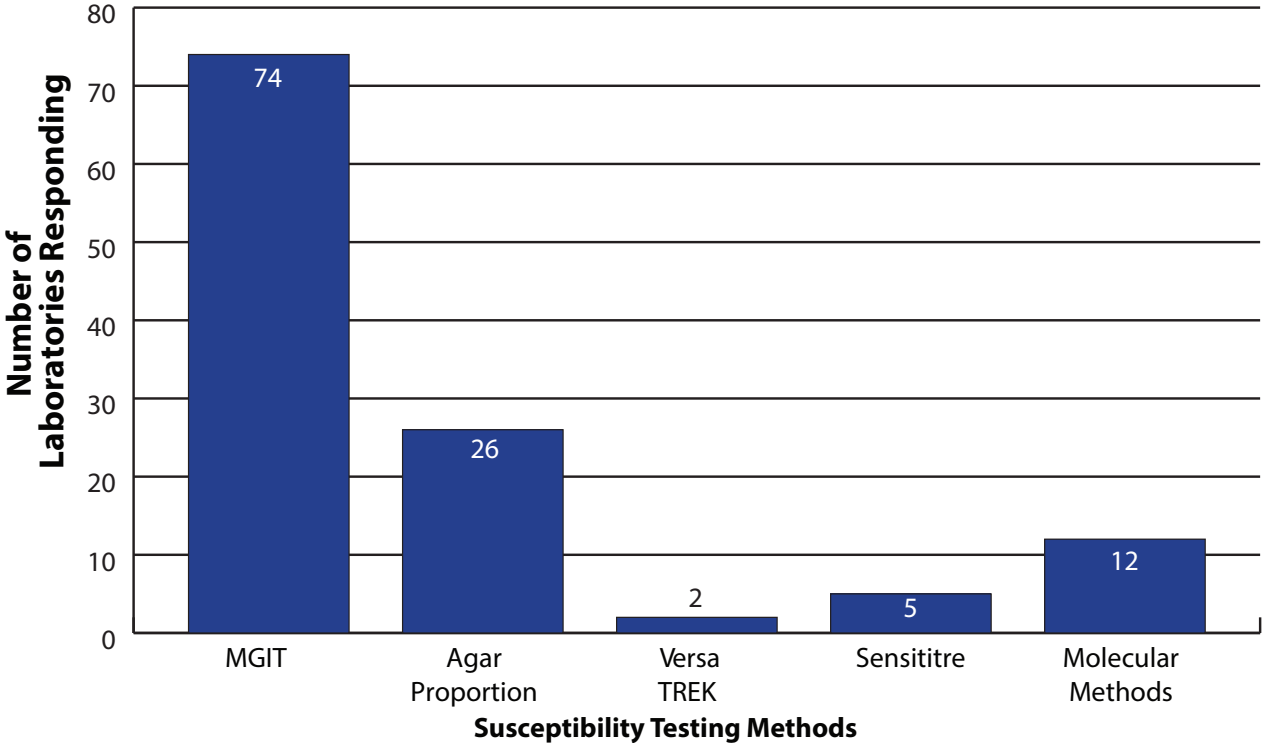
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in 2014 (n=83)



MTBC DST Methods Used by Participants

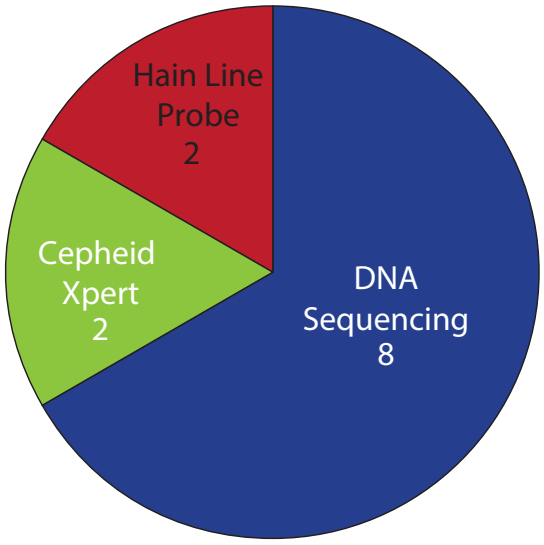
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 49 (59%) laboratories reported results for only one method, 27 laboratories reported two methods, and seven laboratories noted three susceptibility methods.

Figure 3. MTBC Susceptibility Test Method Used by Participants (n=119)



Molecular methods reported by participants are shown in Figure 4. The method used most frequently by laboratories was DNA sequencing (67%), including pyrosequencing and Sanger sequencing. Two laboratories reported results for the Cepheid Xpert MTB/RIF assay and two reported use of the line probe assays Genotype MTBDR*plus* and MTBDR*sl* by Hain Lifescience.

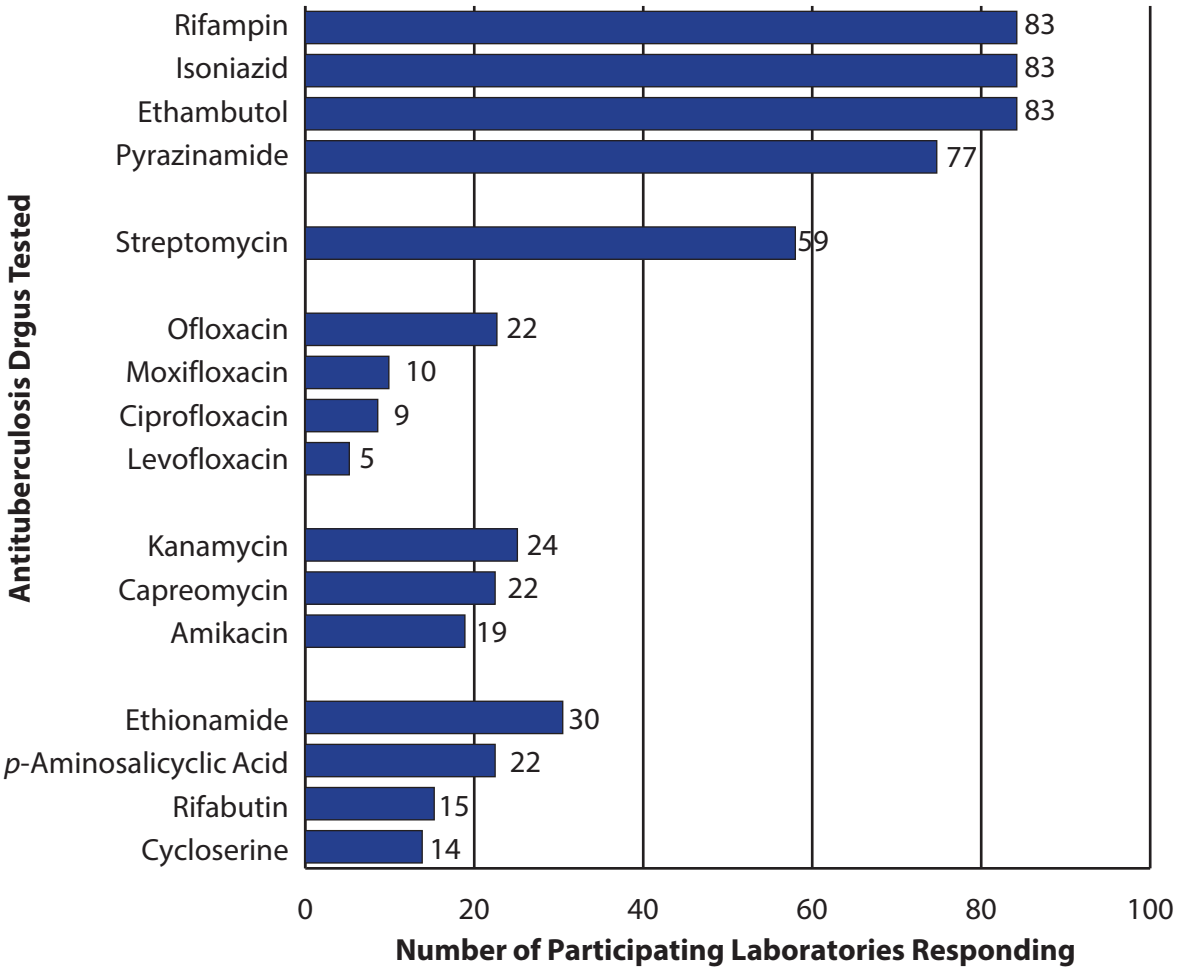
Figure 4. Molecular Method Reported (n=12)



Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in May 2015 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 77 (93%) of the participants also reported results for PZA. There has been a slight increase in the number of laboratories testing second-line drugs since the May 2014 survey. The number of laboratories performing Sensititre, which includes second-line drugs, has also increased; however, the overall increase in second-line testing cannot only be attributable to use of this test.

Figure 5. Antituberculosis Drugs Tested by Participants



Isolate 2015A

Expected Result: Resistant to INH at 0.2 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 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 (*inh*A) which is required for mycolic acid biosynthesis. There are two described 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. There are approximately 10–15% of isolates found to be INH resistant with no mutations detected in either of these loci. In these isolates, 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]. Within *fabG1*, the silent/synonymous mutation (i.e., nucleotide change but no corresponding change in amino acid) Leu203Leu has been found to confer INH resistance through the formation of an alternative promoter thereby increasing the transcriptional levels of *inhA* [7]. 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 *inhA*, *katG*, and *fabG1* for Isolate 2015A revealed a G>A point mutation at codon 203 resulting in the silent/synonymous mutation Leu203Leu; *inhA* and *katG* 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.

For Isolate 2015A, 100 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- 83% (19/23) of the results when using AP
- 50% (35/70) of the results when using MGIT
- 20% (1/5) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

Sixty-three (97%) results were reported as **susceptible** at the higher concentrations of INH.

Of the nine laboratories reporting molecular results for INH, none (0%) reported detection of a mutation.

Rifampin

Rifampin (RMP) is a bactericidal drug used for the treatment of TB caused by organisms known or presumed to be susceptible to this drug. RMP's mechanism of action is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase [4]. More than 96% of RMP-resistant isolates contain a mutation in the 81-bp central region of the *rpo*B gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase. The activity of RMP on RMP-resistant isolates 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 should be confirmed by DNA sequencing of *rpoB* [8]. 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 [9]. Sequencing of *rpoB* will allow for clarifying the result and understanding possible discordance between the rapid molecular and the growth-based testing results.

DNA sequence analysis of *rpoB* in Isolate 2015A revealed a C>T point mutation in codon 528 of the *rpoB* locus. However, this mutation does not result in an amino acid change; arginine remains arginine (Arg528Arg). Unlike the *fabG1* silent mutation in 2015A that was associated with INH resistance, the Arg528Arg silent mutation in *rpoB* 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 *rpoB* will allow for clarifying the result and understanding discordance between the Xpert result and results from growth-based testing.

Of the 107 RMP results reported for Isolate 2015A, **susceptible** was reported by:

- 100% (25/25) 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

Six (60%) of the ten laboratories reporting molecular testing results for RMP detected a mutation, all of which noted that it was a silent mutation by sequencing.

Ethionamide

Ethionamide (ETA) is a structural analog of INH. Both drugs target *inh*A, an enzyme involved in mycolic acid biosynthesis [10]. Resistance to INH and ETA can occur by mutations in *fabG1–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 [10]. The silent/synonymous mutation Leu203Leu was detected in the *fabG1* gene for Isolate 2015A.

Of the 28 results reported for ETA for Isolate 2015A, **resistance** was reported by:

- 60% (12/20) of the results when using AP
- 40% (2/5) 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 2015A are listed in Tables 3, 4, and 5.

Table 3. Isolate 2015A—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	25	0	25	73	0	73	5	0	5	2	0	2
Isoniazid-Low	4	19	23*	35	35	70*#	4	1	5	0	2	2
Isoniazid-High	25	0	25	32	2	34	5	0	5	2	0	2
Ethambutol	25	0	25	73	0	73	5	0	5	2	0	2
Pyrazinamide				76	1	77				1	0	1

Note—S=susceptible, R=resistant

* In addition, two laboratories reported borderline for INH, one by AP and one by MGIT.

\# In addition, one laboratory reported contamination for INH by MGIT.

Table 4. Isolate 2015A—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	24	0	24	45	0	45	4	0	4
Ofloxacin	14	0	14	3	0	3	3	0	3
Ciprofloxacin	6	0	6	2	0	2			
Levofloxacin	1	0	1	2	0	2	1	0	1
Moxifloxacin	3	0	3	4	0	4	3	0	3
Amikacin	12	0	12	4	0	4	4	0	4
Kanamycin	19	0	19	1	0	1	3	0	3
Capreomycin	16	1	17	5	0	5	1	0	1
Ethionamide	8	12	20	3	2	5	1	2	3*
Rifabutin	8	0	8	3	0	3	4	0	4
Cycloserine	8	1	9				3	0	3
p-Aminosalicylic acid	16	0	16	1	0	1	4	0	4

Note—S=susceptible, R=resistant

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

Table 5. Isolate 2015A—Participant Results for Molecular Testing

	Molecular Testing		
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	6*	4	10
Isoniazid	0	9	9
Ethambutol	0	1	1
Pyrazinamide	0	3	3
Ofloxacin	0	2	2
Ciprofloxacin	0	2	2
Levofloxacin	0	1	1
Moxifloxacin	0	1	1
Amikacin	0	2	2
Kanamycin	0	2	2
Capreomycin	0	2	2
Ethionamide	0	1	1
Rifabutin	1	0	1

* Six laboratories noted the mutation detected was a silent mutation

Isolate 2015B

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

As previously noted, resistance to INH most commonly occurs due to mutations in the *kat*G gene or the promoter region of the *inh*A gene, however, mutations in *fabG1* can also cause resistance. DNA sequence analysis of *inh*A, *kat*G, and *fabG1* of Isolate 2015B revealed a T>A point mutation at codon 315 in the *kat*G locus resulting in serine being replaced by threonine (Ser315Thr); *inh*A and *fabG1* were wild-type (i.e., no mutations were detected).

For Isolate 2015B, 103 INH results were reported at the critical concentration. This isolate was reported **resistant** to INH by method, as follows:

- 96% (24/25) of the results when using AP
- 100% (71/71) of the results when using MGIT
- 100% (5/5) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

Seventy-three (97%) results were reported as **resistant** at the higher concentrations of INH.

All nine (100%) laboratories performing molecular testing for INH reported that a mutation was detected.

Streptomycin

Streptomycin (STR) belongs to the aminoglycoside class of drugs and its primary mechanism of action is to inhibit the initiation of translation by binding to the 16S rRNA [4, 5]. In *M. tuberculosis*, the genetic basis of resistance to STR is usually due to mutations in *rrs* or *rps*L [5].

Among three methods, 74 results for STR were reported for this isolate. This isolate was reported as **resistant** to STR by method, as follows:

- 96% (24/25) of the results when using AP
- 96% (43/45) of the results when using MGIT
- 100% (4/4) of the results when using Sensititre

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2015B are listed in Tables 6, 7, and 8.

Table 6. Isolate 2015B—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	26	0	26	72	0	72*	5	0	5	2	0	2
Isoniazid–Low	1	24	25	0	71	71*	0	5	5	0	2	2
Isoniazid–High	2	24	26	0	42	42	0	5	5	0	2	2
Ethambutol	25	1	26	72	0	72*	5	0	5	2	0	2
Pyrazinamide				73	3	76*				1	0	1

Note—S=susceptible, R=resistant

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

Table 7. Isolate 2015B—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	1	24	25	2	43	45*	0	4	4
Ofloxacin	15	0	15	4	0	4	3	0	3
Ciprofloxacin	7	0	7	2	0	2			
Levofloxacin	1	0	1	2	0	2	1	0	1
Moxifloxacin	3	0	3	4	0	4	3	0	3
Amikacin	12	0	12	5	0	5	4	0	4
Kanamycin	20	0	20	1	0	1	3	0	3
Capreomycin	17	0	17	5	0	5	1	0	1
Ethionamide	9	13	22	1	5	6	3	1	4
Rifabutin	8	0	8	2	0	2	4	0	4
Cycloserine	8	1	9				3	0	3
p-Aminosalicylic acid	17	0	17	2	0	2	4	0	4

Note—S=susceptible, R=resistant

* In addition, one laboratory reported contamination for STR by MGIT.

Table 8. Isolate 2015B—Participant Results for Molecular Testing

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

Isolate 2015C

Expected Result: Resistant to INH at 0.2 µg/ml and STR at 2.0 µg/ml by agar proportion

Isoniazid

For Isolate 2015C, DNA sequence analysis of *inh*A, *kat*G, and *fabG1* revealed no mutations; this is known to occur in approximately 10–15% of isolates found to be INH resistant.

For Isolate 2015C, 103 INH results were reported at the critical concentration. This isolate was reported **resistant** to INH by method, as follows:

- 92% (23/25) of the results when using AP
- 93% (67/72) of the results when using MGIT
- 75% (3/4) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

Seventy (95%) results were reported as **susceptible** at the higher concentrations of INH.

Of the nine laboratories reporting molecular results for INH, none (0%) reported detection of a mutation.

Streptomycin

Among three methods, 72 results for STR were reported for this isolate. This isolate was reported as **resistant** to STR by method, as follows:

- 74% (17/23) of the results when using AP
- 89% (41/46) 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 participant for Isolate 2015C are listed in Tables 9, 10, and 11.

Table 9. Isolate 2015C—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	26	0	26	73	0	73	5	0	5	2	0	2
Isoniazid–Low	2	23	25	5	67	72	1	3	4*	0	2	2
Isoniazid–High	24	2	26	39	2	41	5	0	5	2	0	2
Ethambutol	25	1	26	72	1	73	5	0	5	2	0	2
Pyrazinamide				75	1	76				1	0	1

Note—S=susceptible, R=resistant

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

Table 10. Isolate 2015C—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	6	17	23*	5	41	46	1	2	3#
Ofloxacin	15	0	15	3	0	3	2	0	2#
Ciprofloxacin	7	0	7	2	0	2			
Levofloxacin	1	0	1	2	0	2	1	0	1
Moxifloxacin	3	0	3	4	0	4	3	0	3
Amikacin	12	0	12	4	0	4	4	0	4
Kanamycin	19	0	19	1	0	1	3	0	3
Capreomycin	17	0	17	5	0	5	1	0	1
Ethionamide	16	6	22	1	4	5	3	1	4
Rifabutin	8	0	8	2	0	2	4	0	4
Cycloserine	7	2	9				3	0	3
p-Aminosalicylic acid	17	0	17	1	0	1	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 STR and OFL by Sensititre.

Table 11. Isolate 2015C—Participant Results for Molecular Testing

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

Isolate 2015D

Expected Result: *Mycobacterium bovis*; Resistant to PZA at 100.0 µg/ml by MGIT

Pyrazinamide

Pyrazinamide (PZA) is an important first-line drug for treatment of TB and is used with INH and RIF. 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 [11]. PZA is a prodrug that requires conversion to its active form, pyrazinoic acid, by the pyrazinamidase encoded by the *pnc*A gene of *M. tuberculosis*. PZA-resistant *M. tuberculosis* strains lose pyrazinamidase activity and resistance to PZA is usually caused by nucleotide changes scattered throughout the *pncA* gene. However, there may be additional mechanisms of resistance to PZA that are still unknown[12].

Unlike *M. tuberculosis*, *M. bovis* has an inherent resistance to PZA caused by a characteristic single point mutation of C>G at nucleotide position 169 of the *pncA* gene resulting in aspartic acid replacing histidine at codon 57 (His57Asp) in the *M. bovis* pyrazinamidase. This substitution causes defective pyrazinamidase activity and confers natural PZA resistance in *M. bovis* strains, including BCG substrains [13, 14]. DNA sequence analysis of *pncA* in Isolate 2015D confirmed the His57Asp mutation.

The recommended concentrations for testing PZA are 100 µg/ml for MGIT and 300 µg/ml for VersaTREK.

For Isolate 2015D, 78 PZA results were reported. This isolate was reported **resistant** to PZA by method, as follows:

- 99% (74/75) of the results when using MGIT
- 100% (1/1) of the results when using VersaTREK

A mutation was detected by all four of the laboratories that reported molecular testing for PZA, with two laboratories noting the His57Asp mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2015D are listed in Tables 12, 13, and 14.

Table 12. Isolate 2015D—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	24	0	24*	73	0	73	5	0	5	2	0	2
Isoniazid–Low	23	1	24*	70	0	70#	5	0	5	2	0	2
Isoniazid–High	23	0	23*	30	0	30	5	0	5	2	0	2
Ethambutol	24	0	24*	73	0	73	5	0	5	2	0	2
Pyrazinamide				1	74	75†				0	1	1

Note—S=susceptible, R=resistant

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

\# In addition, one laboratory reported contamination for INH by MGIT.

† In addition, one laboratory reported contamination for PZA by MGIT.

Table 13. Isolate 2015D—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	24	0	24*	45	1	46	4	0	4
Ofloxacin	14	0	14*	3	0	3	3	0	3
Ciprofloxacin	6	0	6*	2	0	2			
Levofloxacin	1	0	1*	2	0	2	1	0	1
Moxifloxacin	2	0	2*	4	0	4	2	0	2#
Amikacin	12	0	12*	4	0	4	4	0	4
Kanamycin	18	0	18*	1	0	1	3	0	3
Capreomycin	16	0	16*	5	0	5	1	0	1
Ethionamide	17	3	20*	5	0	5	4	0	4
Rifabutin	8	0	8*	2	0	2	4	0	4
Cycloserine	9	0	9*				4	0	4
p-Aminosalicylic acid	16	0	16*	1	0	1	3	0	3

Note—S=susceptible, R=resistant

* In addition, one laboratory reported contamination for STR, OFL, CIP, LEV, MOX, AMK. KAN, CAP, ETA, RBT, CYS, and PAS by AP.

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

Table 14. Isolate 2015D—Participant Results for Molecular Testing

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

* One laboratory noted the mutations detected were silent mutations for EMB and KAN.

\# Two laboratories noted the mutation detected was a silent mutation for PZA.

Isolate 2015E

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

Isoniazid

DNA sequence analysis of *inh*A, *kat*G, and *fabG1* for Isolate 2015E revealed a C>T point mutation at nucleotide position -15 of the promotor region of the *inhA* gene (C-15T); *katG* and *fabG1* were wild-type (i.e., no mutations were detected).

For Isolate 2015E, 103 INH results were reported. This isolate was reported **resistant** to INH at the critical concentration by method, as follows:

- 67% (16/24) of the results when using AP
- 94% (68/72) of the results when using MGIT
- 60% (3/5) of the results when using Sensititre
- 100% (2/2) of the results when using VersaTREK

Seventy-three (97%) results were reported as **susceptible** at the higher concentrations of INH.

All nine (100%) laboratories performing molecular testing for INH reported detection of a mutation.

Ethionamide

As previously noted, 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. A point mutation (C-15T) was detected in the promoter region for Isolate 2015E.

Of the 32 results reported for ETA for Isolate 2015E, **resistance** was reported by:

- 77% (17/22) of the results when using AP
- 83% (5/6) of the results when using MGIT
- 75% (3/4) of the results when using Sensititre

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2015E are listed in Tables 15, 16, and 17.

Table 15. Isolate 2015E—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	26	0	26	73	0	73	5	0	5	2	0	2
Isoniazid–Low	8	16	24	4	68	72	2	3	5	0	2	2
Isoniazid–High	26	0	26	40	2	42	5	0	5	2	0	2
Ethambutol	26	0	26	73	0	73	5	0	5	2	0	2
Pyrazinamide				70	6	76				1	0	1

Note—S=susceptible, R=resistant

Table 16. Isolate 2015E—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	25	0	25	45	1	46	2	1	3*
Ofloxacin	15	0	15	4	0	4	1	1	2*
Ciprofloxacin	7	0	7	3	0	3			
Levofloxacin	1	0	1	3	0	3	1	0	1
Moxifloxacin	3	0	3	5	0	5	1	0	1*
Amikacin	12	0	12	5	0	5	4	0	4
Kanamycin	20	0	20	2	0	2	3	0	3
Capreomycin	17	0	17	6	0	6	1	0	1
Ethionamide	5	17	22#	1	5	6	1	3	4
Rifabutin	8	0	8	3	0	3	4	0	4
Cycloserine	9	0	9	1	0	1	4	0	4
p-Aminosalicylic acid	17	0	17	2	0	2	3	1	4

Note—S=susceptible, R=resistant

* In addition, one laboratory reported borderline for STR, OFL, and MOX by Sensititre and another laboratory reported borderline for MOX only by Sensititre.

\# In addition, one laboratory reported contamination for ETA by AP.

Table 17. Isolate 2015E—Participant Results for Molecular Testing

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

* Six laboratories noted the mutation detected was a silent mutation for INH

\# One laboratory noted the mutation detected was a silent mutation for EMB

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.

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