Centers for Disease Control and Prevention
Model Performance Evaluation Program

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Report of Results
November 2013
Performance Evaluation Survey

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

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for November 2013 Survey

Purpose

The purpose of this report is to present the results of the U.S. Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP) for *Mycobacterium tuberculosis* complex drug susceptibility testing survey sent to participants in November 2013.

Report Content

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Introduction: Overview of MPEP Final Report

The Model Performance Evaluation Program (MPEP) is not a formal, graded proficiency testing program. It 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. This report includes results for a subset of laboratories performing 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 drug susceptibility testing (DST) results with those obtained by other participants using the same methods and drugs, by isolate. We encourage circulation of this report to personnel who are 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

The table below provides the anticipated results of the panels that were sent to participants in November 2013. Although CDC recommends broth-based methods for routine first-line DST of MTBC isolates, this table provides the results obtained by the reference agar proportion method, except in the case of pyrazinamide, where MGIT was the testing method.

Table 1. Expected Results for November 2013 Survey

	Conventional Results					Molecular Results
	First-Line Drugs				Second-Line Drugs	***rpoB*** Mutation
	INH	RMP	EMB	PZA	Expected Resistance	
2013F	S	S*	S	S		Asp516Tyr
2013G	S	R	S	S		His526Asp
2013H	R	S	S	S	ETO	Phe514Phe
2013I	S	S	S	S		wild-type
2013J	S	S*#	S	S		His526Leu

Note—S=susceptible, R=resistant

* Certain *rpoB* mutations have been noted to produce variable results when conventional DST methods are performed [2, 3]. These conventional DST results were obtained by agar proportion.

\# Less than 80% of reported results agreed with the expected result.

Abbreviations and Acronyms

AP	agar proportion – performed on Middlebrook 7H10 or 7H11
bp	base pair
CDC	U.S. Centers for Disease Control and Prevention
CLSI	Clinical Laboratory and Standards Institute
DNA	deoxyribonucleic acid
DST	drug susceptibility testing
ETO	ethionamide
HMO	Health Maintenance Organization
INH	isoniazid
MDR	multidrug resistant
MGIT	BACTEC MGIT 960 – Mycobacteria Growth Indicator Tube
MIC	minimum inhibitory concentration
MPEP	Model Performance Evaluation Program
MTBC	*Mycobacterium tuberculosis* complex
R	resistant
RMP	rifampin
RNA	ribonucleic acid
S	susceptible
Sensititre	Trek Diagnostic Systems Sensititre susceptibility panel
TB	tuberculosis
VersaTREK	VersaTREK Myco susceptibility kit
XDR	extensively drug resistant

Technical Notes

The following information pertains to all of the tables and figures for the 2013 MTBC isolates F, G, H, I, and J in this report.

- The source of data in all tables and figures is from the November 2013 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 included as part of the second-line table.
- Separate tables for molecular testing are included where data is of note; otherwise findings are reported in the summary.
- 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 90 (the number of participating laboratories). This report contains all results reported by participating laboratories.
- As a reference, a list of critical concentrations for antituberculous drugs, by method, can be found 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 31 laboratories reporting second-line drug results (with the exception of streptomycin), only 8 (26%) tested all three second-line injectable drugs and at least one fluoroquinolone needed to confidently define XDR TB. Second-line injectable drugs consist of amikacin, kanamycin, and capreomycin. Fluoroquinolones include ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin.

Descriptive Information about Participant Laboratories

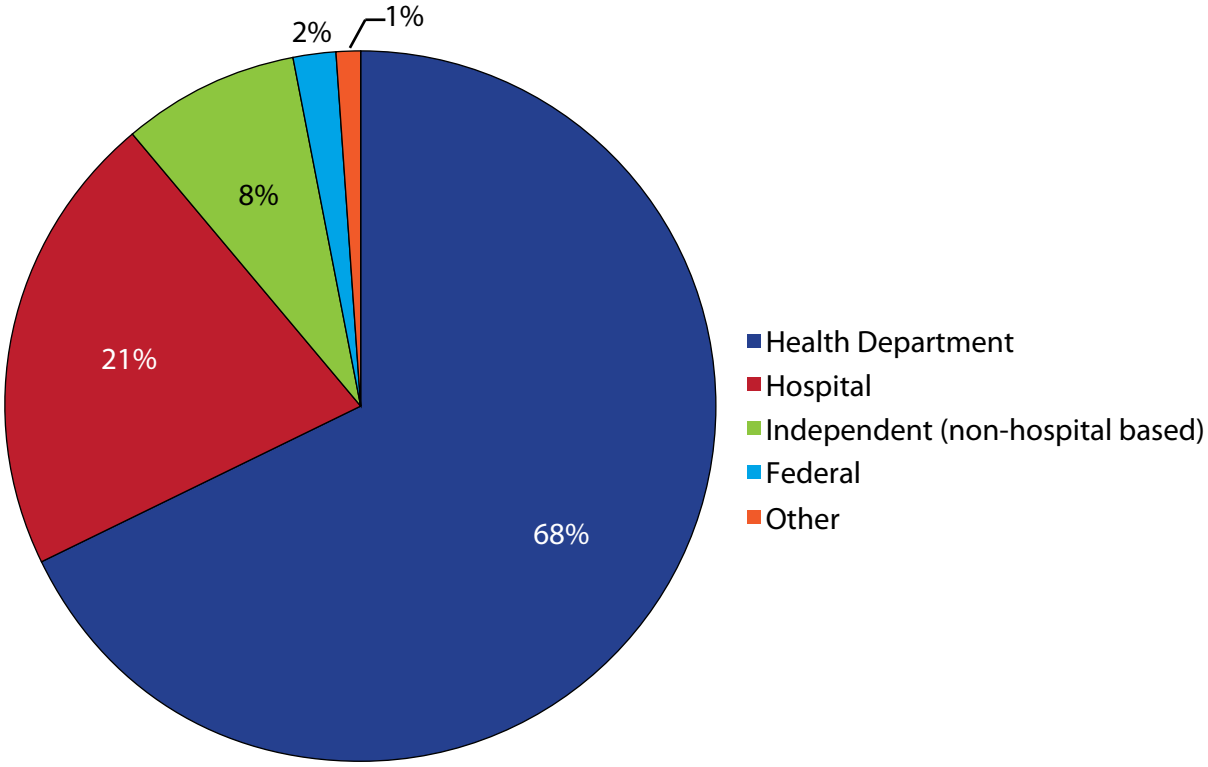
Primary Classification

This report contains the DST results submitted to CDC by survey participants at 90 laboratories in 42 states and 1 U.S. Territory.

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

- 61 (68%): Health department (city, country, state, regional, or district laboratory)
- 19 (21%): Hospital laboratory
- 7 (8%): Independent (e.g., commercial, commercial manufacturer of reagents, Health maintenance organization [HMO] satellite clinic, reference laboratory [non-governmental affiliated])
- 2 (2%): Federal government laboratory
- 1 (1%): Other (quality control manufacturer)

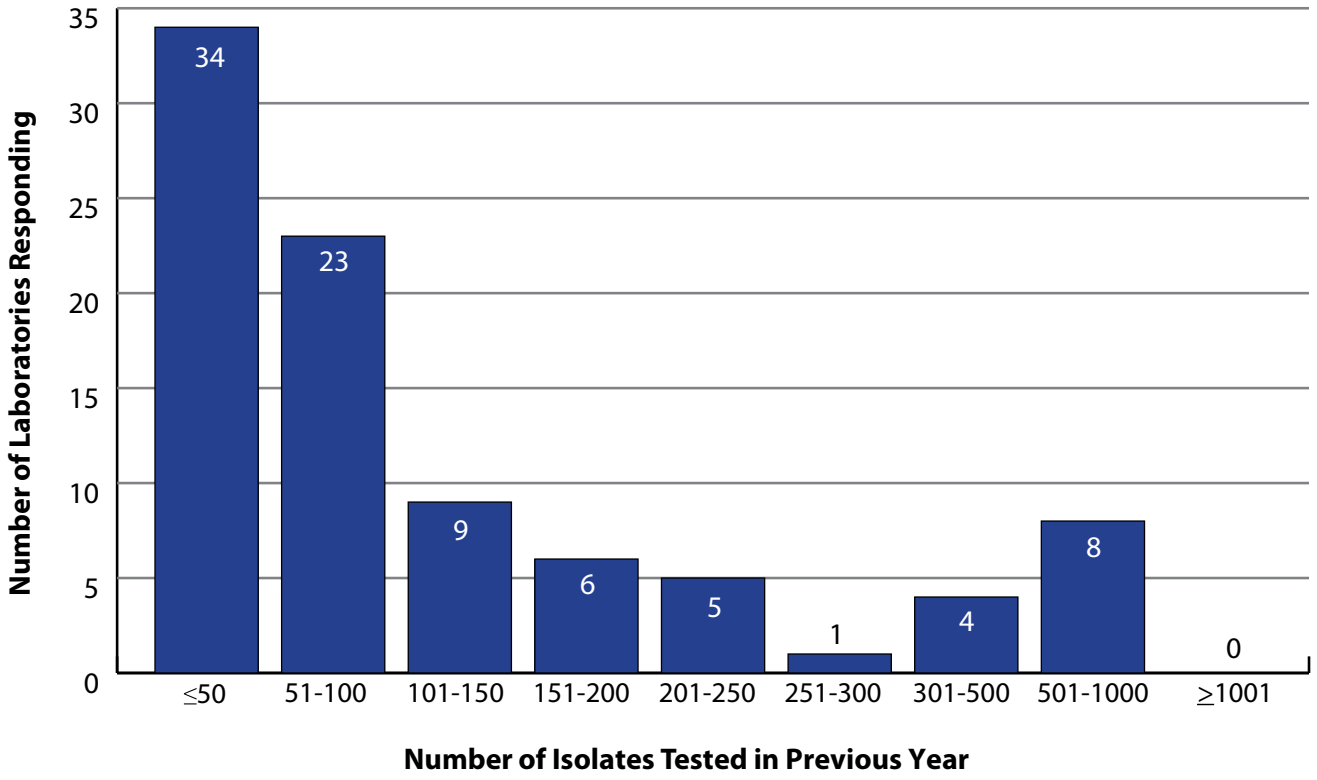
Figure 1. Primary Classification of Participating Laboratories (n=90)



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates subjected to DST by the 90 participants from the previous calendar year (excluding isolates used for quality control) is shown in Figure 2. The counts ranged from 0 to 948 tests. Participants at thirty-four (38%) laboratories reported testing less than or equal to 50 DST per year. Laboratories with low MTBC DST volumes are encouraged to consider referral of testing because of concerns about maintaining proficiency [4].

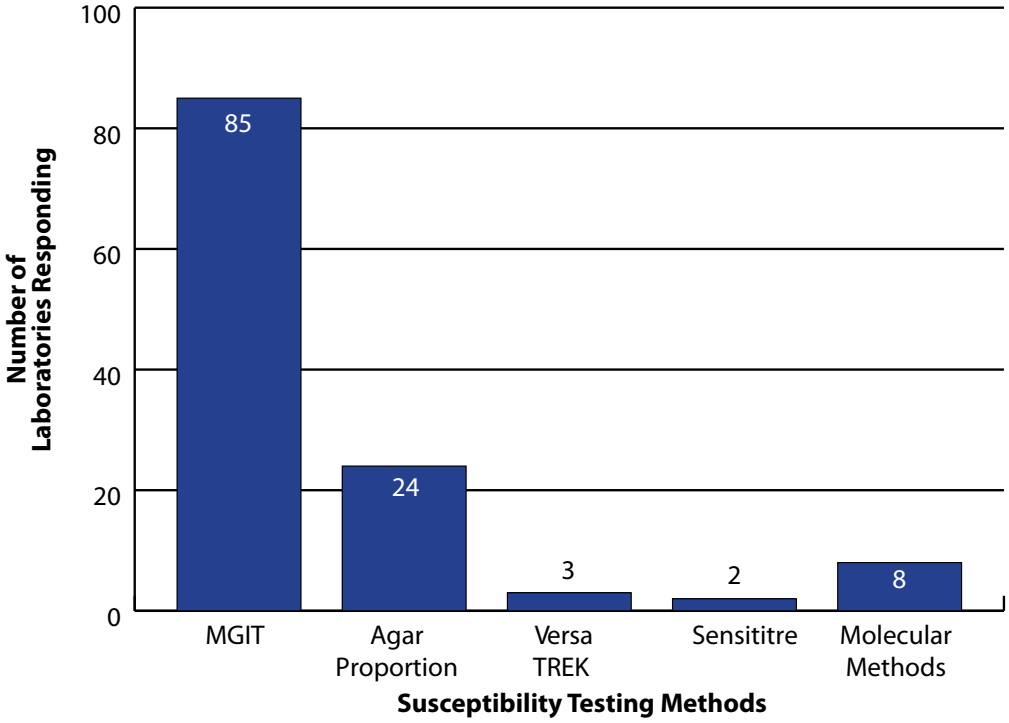
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in the Previous Calendar Year



MTBC DST Methods Used by Participants

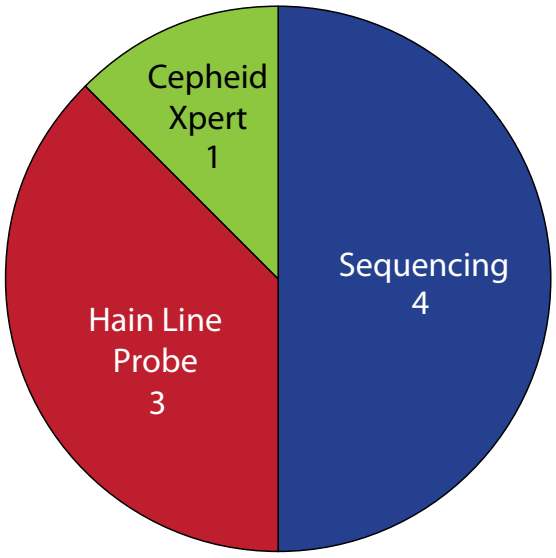
Participants were asked to report all DST methods that were used for these isolates. Sixty-two (69%) laboratories reported only one method, twenty-four laboratories reported two methods and four laboratories noted three susceptibility methods.

Figure 3. MTBC Susceptibility Test Method Used by Participants (n=122)



The breakdown of molecular methods reported is shown in Figure 4. The method used by half of the participants was DNA sequencing (50%), including pyrosequencing and Sanger sequencing. Three laboratories used the line probe assays Genotype MTBDR*plus* or Genotype MTBDR*sl* by Hain and only one laboratory reported results for the Cepheid Xpert MTB/RIF assay.

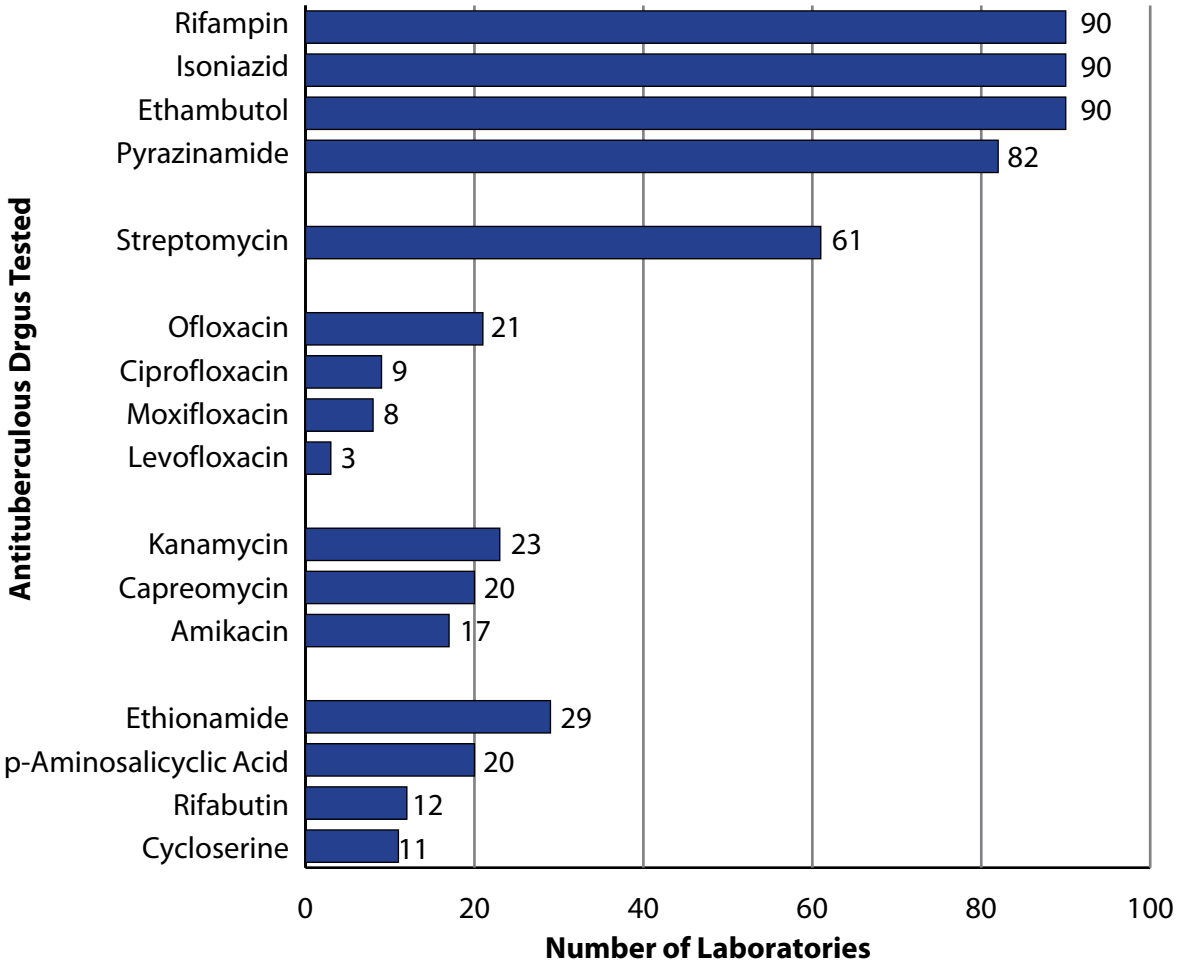
Figure 4. Molecular Method Used (n=8)



Antituberculous Drugs Tested by Participants

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 antituberculous 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; Eighty-two (91%) of the participants also reported results for PZA.

Figure 5. Antituberculous Drugs Tested by Participants



Isolate 2013F

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

Rifampin

Rifampin (RMP) is a first-line drug for treatment of all forms of tuberculosis caused by organisms known or presumed to be susceptible to this drug. It is bactericidal for MTBC at the critical concentration of 1.0 µg/ml for AP (on Middlebrook 7H10 and 7H11 agars) and equivalent critical concentrations for both MGIT and VersaTREK of 1.0 µg/ml. The mechanism of action of RMP is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase [5]. More than 96% of RMP-resistant isolates contain a mutation in the 81-bp central region of the *rpoB* gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase. The activity of RMP in RMP-resistant isolates depends on both the mutation position and the type of amino acid change. Mutations in codons 531, 526, and 516 are among the most frequent mutations in RMP-resistant isolates and serve as predictors of RMP resistance [5, 6]. DNA sequence analysis of *rpoB* in Isolate 2013F revealed a G>T point mutation in codon 516 of *rpoB* resulting in aspartate being replaced by tyrosine (Asp516Tyr).

The most commonly encountered mutation in *rpoB*, Ser531Leu, and some mutations in codons 526 and 516 have generally been reported to confer high-level RMP resistance (i.e., minimum inhibitory concentration [MIC] is much higher than the critical concentration). However, some mutations have been associated with low-level, yet probably clinically relevant, RMP resistance, including the Asp516Tyr mutation [2, 3, 7]. Low level RMP resistance can be operationally defined as the presence of a mutation which increases the RMP MIC above the MIC seen in RMP-susceptible isolates that do not have a detectable mutation in *rpoB*. However, isolates with mutations conferring low-level RMP resistance may test as susceptible with growth-based drug susceptibility methods. The clinical impact of these *rpoB* mutations, sometimes referred to as "disputed" mutations, will depend on the frequency of their occurrence, which may vary from one setting to another [2, 8]. The diminished RMP activity suggests that clinical outcome in patients being treated with RMP-based standard therapy could be impacted. This is an area needing additional clinical studies.

CDC has recently recommended that RMP resistance detected by the Xpert MTB/RIF assay should be confirmed by DNA sequencing of genetic loci associated with RMP resistance (i.e., *rpoB*) [9]. The Xpert MTB/RIF assay may generate results that falsely report resistance when compared to growth-based methods due to the presence of silent mutations (nucleotide change but no change in amino acid) [2]. Sequencing of *rpoB* will allow for clarifying the result and understanding possible discordance between the molecular and growth-based testing results.

Among four methods, 105 results for RMP were reported for Isolate 2013F. This isolate was reported as **susceptible** to RMP by method, as follows

- 95% (19/20) of the results when using AP;
- 100% (80/80) of the results when using MGIT;
- 50% (1/2) of the results when using Sensititre; and
- 100% (3/3) of the results when using VersaTREK.

Six (86%) laboratories reporting molecular testing results for RMP detected a mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2013F are listed in Tables 2, 3, and 4.

Table 2. Isolate 2013F—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	19	1	20*	80	0	80	1	1	2	3	0	3
Isoniazid-Low	21	0	21	80	0	80	2	0	2	3	0	3
Isoniazid-High	20	0	20	27	0	27	2	0	2	3	0	3
Ethambutol	20	0	20	80	0	80	2	0	2	3	0	3
Pyrazinamide				77	2	79#				1	0	1

Note—S=susceptible, R=resistant

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

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

Table 3. Isolate 2013F—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	50	0	50	2	0	2
Ofloxacin	13	0	13	4	0	4	0	1	1*
Ciprofloxacin	7	0	7	1	0	1			
Levofloxacin	2	0	2	1	0	1			
Moxifloxacin	2	0	2	4	0	4	1	0	1
Amikacin	10	0	10	5	0	5	2	0	2
Kanamycin	17	0	17	1	0	1	2	0	2
Capreomycin	15	0	15	4	0	4			
Ethionamide	18	0	18	6	0	6	2	0	2
Rifabutin	8	0	8	1	0	1	2	0	2
Cycloserine	8	0	8				2	0	2
p-Aminosalicylic acid	13	0	13	3	0	3	2	0	2

Note—S=susceptible, R=resistant

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

Table 4. Isolate 2013F—Participant results for molecular testing

	Molecular Testing		
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	6	1	7
Isoniazid	0	6	6
Ethambutol	0	1	1
Pyrazinamide	0	2	2
Ofloxacin	0	2	2
Ciprofloxacin	0	2	2
Levofloxacin	0	2	2
Moxifloxacin	0	2	2
Amikacin	0	2	2
Kanamycin	0	2	2
Capreomycin	0	2	2
Ethionamide	0	0	0
Rifabutin	1	0	1

Isolate 2013G

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

Rifampin

DNA sequence analysis of *rpoB* in Isolate 2013G revealed a C>G point mutation in codon 526 resulting in histidine being replaced by aspartate (His526Asp). Unlike the Asp516Tyr mutation detected in Isolate 2013F, isolates with His526Asp mutations consistently test as resistant to RMP in growth-based assays.

Of the 105 RMP results reported for Isolate 2013G, **resistance** was reported by

- 100% (23/23) of the results when using AP;
- 100% (77/77) of the results when using MGIT;
- 100% (2/2) of the results when using Sensititre; and
- 100% (3/3) of the results when using VersaTREK.

Eight (100%) laboratories reporting molecular testing results for RMP detected a mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2013G are listed in Tables 5, 6, and 7.

Table 5. Isolate 2013G—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	77	77#	0	2	2	0	3	3
Isoniazid–Low	21	1	22*	76	0	76#	2	0	2	3	0	3
Isoniazid–High	22	0	22	28	0	28	2	0	2	3	0	3
Ethambutol	22	0	22	75	1	76#	2	0	2	3	0	3
Pyrazinamide				80	0	80#				1	0	1

Note—S=susceptible, R=resistant

* In addition, one laboratory reported contamination for low-level INH by AP.

\# In addition, two laboratories reported no growth for RMP, INH, and EMB by MGIT, one laboratory reported no growth for RMP,INH, EMB, and PZA by MGIT, and one laboratory reported no growth for INH and EMB by MGIT.

Table 6. Isolate 2013G—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	11	10	21*	21	26	47#	2	0	2
Ofloxacin	14	0	14	4	0	4†	2	0	2
Ciprofloxacin	9	0	9	1	0	1			
Levofloxacin	2	0	2	1	0	1			
Moxifloxacin	2	0	2	4	0	4	1	0	1
Amikacin	10	0	10	6	0	6	2	0	2
Kanamycin	18	1	19	2	0	2	2	0	2
Capreomycin	16	0	16	5	0	5			
Ethionamide	20	0	20	8	0	8	2	0	2
Rifabutin	0	8	8	0	1	1	0	2	2
Cycloserine	9	0	9				2	0	2
p-Aminosalicylic acid	14	1	15	4	0	4	2	0	2

Note—S=susceptible, R=resistant

* In addition, one laboratory reported a borderline result for streptomycin by AP.

\# In addition, two laboratories reported a no growth for streptomycin by MGIT.

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

Table 7. Isolate 2013G—Participant results for molecular testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	8	0	8
Isoniazid	0	6	6
Ethambutol	0	1	1
Pyrazinamide	0	2	2
Ofloxacin	0	2	2
Ciprofloxacin	0	2	2
Levofloxacin	0	2	2
Moxifloxacin	0	2	2
Amikacin	0	2	2
Kanamycin	0	2	2
Capreomycin	0	2	2
Ethionamide	0	0	0
Rifabutin	1	0	1

Isolate 2013H

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

Isoniazid

Isoniazid (INH) is the most widely used first-line antituberculous drug. It 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 *katG* gene [5, 10]. The target of activated INH is enoyl-acyl-carrier protein reductase (*inhA*) which is required for mycolic acid biosynthesis. There are two described mechanisms that account for the majority of INH resistance [5, 6, 10]. The most common, mutations in *katG*, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the *inhA* gene which are generally associated with low-level resistance to INH and are less frequent than *katG* mutations. DNA sequence analysis of *inh*A and *katG* of Isolate 2013H revealed C-15T point mutation in the *inhA* locus; *katG* was wild-type (i.e., no mutations were detected).

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

For Isolate 2013H, 109 INH results were reported. This isolate was reported **resistant** to INH at the critical concentration by method, as follows

- 96% (23/24) of the results when using AP;
- 100% (80/80) of the results when using MGIT;
- 100% (2/2) of the results when using Sensititre; and
- 100% (3/3) of the results when using VersaTREK.

Sixty-seven (99%) results were reported as **susceptible** at the higher concentrations of INH.

Six (86%) laboratories using molecular methods for INH reported that a mutation was detected.

Ethionamide

Ethionamide (ETO) is a structural analog of INH. Both drugs target *inhA*, an enzyme involved in mycolic acid biosynthesis [11]. Resistance to INH and ETO and can occur by mutations in the promoter region of the *inhA* gene which are generally associated with low-level resistance to INH. Mutations in *ethA* also confer resistance to ETO, without concomitant resistance to INH [11]. A point mutation (C-15T) was detected in the *inhA* locus for Isolate 2013H.

Of the 29 results reported for ETO for Isolate 2013H, **resistance** was reported by

- 84% (16/19) of the results when using AP;
- 75% (6/8) of the results when using MGIT; and
- 100% (2/2) of the results when using Sensititre.

Rifampin

DNA sequence analysis of *rpoB* in Isolate 2013H revealed a C>T point mutation in codon 514 of the *rpoB* locus. However, this mutation does not result in an amino acid change; phenylalanine remains phenylalanine (Phe514Phe). This synonymous (i.e., 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 will 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 108 RMP results reported for Isolate 2013H, **susceptible** was reported by

- 100% (23/23) of the results when using AP;
- 98% (78/80) of the results when using MGIT;
- 100% (2/2) of the results when using Sensititre; and
- 100% (3/3) of the results when using VersaTREK.

Five (71%) laboratories reporting molecular testing results for RMP detected a mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2013H are listed in Tables 8, 9, and 10.

Table 8. Isolate 2013H—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	78	2	80	2	0	2	3	0	3
Isoniazid–Low	1	23	24	0	80	80	0	2	2	0	3	3
Isoniazid–High	23	0	23	39	1	40*	2	0	2	3	0	3
Ethambutol	22	0	22	79	1	80	2	0	2	3	0	3
Pyrazinamide				80	1	81				1	0	1

Note—S=susceptible, R=resistant

* In addition, one laboratory reported no growth for high-level INH by MGIT.

Table 9. Isolate 2013H—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	11	11	22	6	43	49	0	1	1#
Ofloxacin	13	1	14	5	0	5	0	1	1†
Ciprofloxacin	8	1	9	1	0	1			
Levofloxacin	2	0	2	1	0	1			
Moxifloxacin	2	0	2	4	0	4	0	0	0†
Amikacin	10	0	10	6	0	6	2	0	2
Kanamycin	19	0	19	2	0	2	2	0	2
Capreomycin	16	0	16	5	0	5			
Ethionamide	3	16	19*	2	6	8	0	2	2
Rifabutin	8	0	8	1	0	1	2	0	2
Cycloserine	8	1	9				2	0	2
p-Aminosalicylic acid	15	0	15	3	1	4	2	0	2

Note—S=susceptible, R=resistant

* In addition, one laboratory reported contamination for ETO by AP.

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

† In addition, one laboratory reported borderline for ofloxacin and moxifloxacin by Sensititre

Table 10. Isolate 2013H—Participant results for molecular testing

	Molecular Testing		
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	5	2	7
Isoniazid	6	1	7
Ethambutol	0	1	1
Pyrazinamide	0	2	2
Ofloxacin	0	2	2
Ciprofloxacin	0	2	2
Levofloxacin	0	2	2
Moxifloxacin	0	2	2
Amikacin	0	2	2
Kanamycin	0	2	2
Capreomycin	0	2	2
Ethionamide	0	0	0
Rifabutin	1	0	1

Isolate 2013I

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

This isolate is susceptible to all first- and second-line drugs.

Most (99%) laboratories reported this isolate susceptible to all drugs tested by all methods.

No laboratories reported the detection of a mutation for any drug using molecular methods.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2013I are listed in Tables 11, 12, and 13.

Table 11. Isolate 2013I—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	78	1	79	2	0	2	3	0	3
Isoniazid–Low	21	0	21	79	0	79	2	0	2	3	0	3
Isoniazid–High	20	0	20	28	0	28	2	0	2	3	0	3
Ethambutol	20	0	20	78	0	78	2	0	2	3	0	3
Pyrazinamide				79	1	80				1	0	1

Note—S=susceptible, R=resistant

Table 12. Isolate 2013I—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	48	0	48	2	0	2
Ofloxacin	12	0	12	4	0	4	1	0	1*
Ciprofloxacin	7	0	7	1	0	1			
Levofloxacin	2	0	2	1	0	1			
Moxifloxacin	2	0	2	3	0	3	0	0	0*
Amikacin	10	0	10	5	0	5	2	0	2
Kanamycin	17	0	17	1	0	1	2	0	2
Capreomycin	15	0	15	4	0	4			
Ethionamide	18	0	18	5	1	6	2	0	2
Rifabutin	8	0	8	1	0	1	2	0	2
Cycloserine	8	0	8				1	0	1
p-Aminosalicylic acid	13	0	13	3	0	3	1	0	1

Note—S=susceptible, R=resistant

* In addition, one laboratory reported borderline for ofloxacin and moxifloxacin by Sensititre

Table 13. Isolate 2013I—Participant results for molecular testing

Molecular Testing			
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	0	7	7
Isoniazid	0	6	6
Ethambutol	0	1	1
Pyrazinamide	0	2	2
Ofloxacin	0	2	2
Ciprofloxacin	0	2	2
Levofloxacin	0	2	2
Moxifloxacin	0	2	2
Amikacin	0	2	2
Kanamycin	0	2	2
Capreomycin	0	2	2
Ethionamide	0	0	0
Rifabutin	0	1	1

Isolate 2013J

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

Rifampin

DNA sequence analysis of *rpoB* in Isolate 2013J revealed an A>T point mutation in codon 526 resulting in histidine being replaced by leucine (His526Leu). Like the Asp516Tyr mutation detected in Isolate 2013F, isolates with His526Leu mutations are associated with low-level RMP resistance and often test as susceptible in growth-based assays [3, 7].

For Isolate 2013J, 99 RMP results were reported. The isolate was reported **susceptible** to RMP by method, as follows

- 17% (3/18) of the results when using AP;
- 50% (38/76) of the results when using MGIT;
- 50% (1/2) of the results when using Sensititre; and
- 66% (2/3) of the results when using VersaTREK.

Eight laboratories reported results for molecular methods; 100% reported that a mutation was detected for RMP.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2013J are listed in Tables 14, 15, and 16.

Table 14. Isolate 2013J—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	3	15	18*	38	38	76#	1	1	2	2	1	3
Isoniazid–Low	21	0	21	74	1	75#	2	0	2	3	0	3
Isoniazid–High	20	0	20	30	0	30	2	0	2	3	0	3
Ethambutol	19	1	20	74	2	76#	2	0	2	3	0	3
Pyrazinamide				79	0	79				1	0	1

Note—S=susceptible, R=resistant

* In addition, two laboratories reported borderline for RMP by AP.

In addition, five laboratories reported no growth for RMP, low-level INH, and EMB by MGIT.

Table 15. Isolate 2013J—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	46	0	46*	2	0	2
Ofloxacin	13	0	13	4	0	4	2	0	2
Ciprofloxacin	7	0	7	1	0	1			
Levofloxacin	2	0	2	1	0	1			
Moxifloxacin	2	0	2	4	0	4	2	0	2
Amikacin	10	0	10	6	0	6	2	0	2
Kanamycin	17	0	17	2	0	2	2	0	2
Capreomycin	14	1	15	5	0	5			
Ethionamide	18	0	18	8	0	8	2	0	2
Rifabutin	7	1	8	2	0	2	2	0	2
Cycloserine	8	0	8				2	0	2
p-Aminosalicylic acid	13	0	13	4	0	4	2	0	2

Note—S=susceptible, R=resistant

* In addition, one laboratory reported no growth for streptomycin by MGIT.

Table 16. Isolate 2013J—Participant results for molecular testing

Molecular Testing			
Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	8	0	8
Isoniazid	0	6	6
Ethambutol	0	1	1
Pyrazinamide	0	2	2
Ofloxacin	0	2	2
Ciprofloxacin	0	2	2
Levofloxacin	0	2	2
Moxifloxacin	0	2	2
Amikacin	0	2	2
Kanamycin	0	2	2
Capreomycin	0	2	2
Ethionamide	0	0	0
Rifabutin	1	0	1

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.

References

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