

Centers for Disease Control and Prevention

***Model Performance Evaluation Program for
Mycobacterium tuberculosis and
Nontuberculous Mycobacteria Drug
Susceptibility Testing***

Report of Results for Performance Evaluation Survey Conducted During May 2010



MTB NTM DST Report for May 2010 Samples Survey

Purpose

The purpose of this report is to present the results of the Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program for *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria Drug Susceptibility Testing (MPEP MTB NTM DST) survey sent to participants in May 2010.

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Introduction: Analyses of the May 2010 *M. tuberculosis* and Nontuberculous Mycobacteria Drug Susceptibility Test Results Reported by Participating Laboratories

This report analyzes the laboratory demographic information and drug susceptibility testing results reported to the Centers for Disease Control and Prevention (CDC) by participating laboratories for the panel of five *Mycobacterium tuberculosis* complex (MTBC) isolates shipped in May 2010. Panels were sent to 99 participating laboratories. Of those, 96 laboratories participated in evaluation of the panels, yielding a response rate of 97%.

This aggregate report is prepared in a format that will allow laboratories to compare their results with those obtained by other participants for the same strain using the same method, drug, and drug concentrations. We encourage you to circulate this report to personnel who are involved with drug susceptibility testing, reporting, or interpreting 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 Laboratory and Standards Institute (CLSI), "Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes," M24-A (ISBN 1-56238-500-3).

Susceptibility Testing Results for the *M. tuberculosis* Isolates Panel Shipped May 17–18, 2010

The table below provides the results of the panel shipment that was sent to participants in May 2010. Although CDC recommends the broth-based methods for routine *M. tuberculosis* drug susceptibility testing, this report provides the results obtained by the reference agar proportion method, except in the case of pyrazinamide, where BACTEC™ MGIT™ 960 (MGIT™) was the reference method. Participants should use the equivalent critical concentrations for testing methods, as defined in CLSI M-24A standards, to determine their results.

Isolate ¹	Susceptibility Testing Results*
T	Resistant to RIF @ 1 µg/ml and to CIP and OFX @ 2 µg/ml
W	Resistant to RIF @ 1 µg/ml and to RBT @ 0.25 µg/ml
X	Resistant to INH @ 0.2 µg/ml
Y	<i>M. bovis</i> , resistant to PZA @ 100 µg/ml
Z	Susceptible to all first- and second-line drugs

*Drug Abbreviations

RIF: rifampin
 CIP: ciprofloxacin
 OFX: ofloxacin
 RBT: rifabutin
 INH: isoniazid
 PZA: pyrazinamide

¹ The letters U and V were excluded when designating isolates in order to prevent transcriptional errors.

Descriptive Information About Participant Laboratories

Primary Classification

This report contains the drug susceptibility testing results submitted to CDC by 96 laboratories in 41 states and Puerto Rico. There were no participants outside the United States.

The participants were asked to indicate the **primary classification** of their laboratory.

MPEP participants self classified as

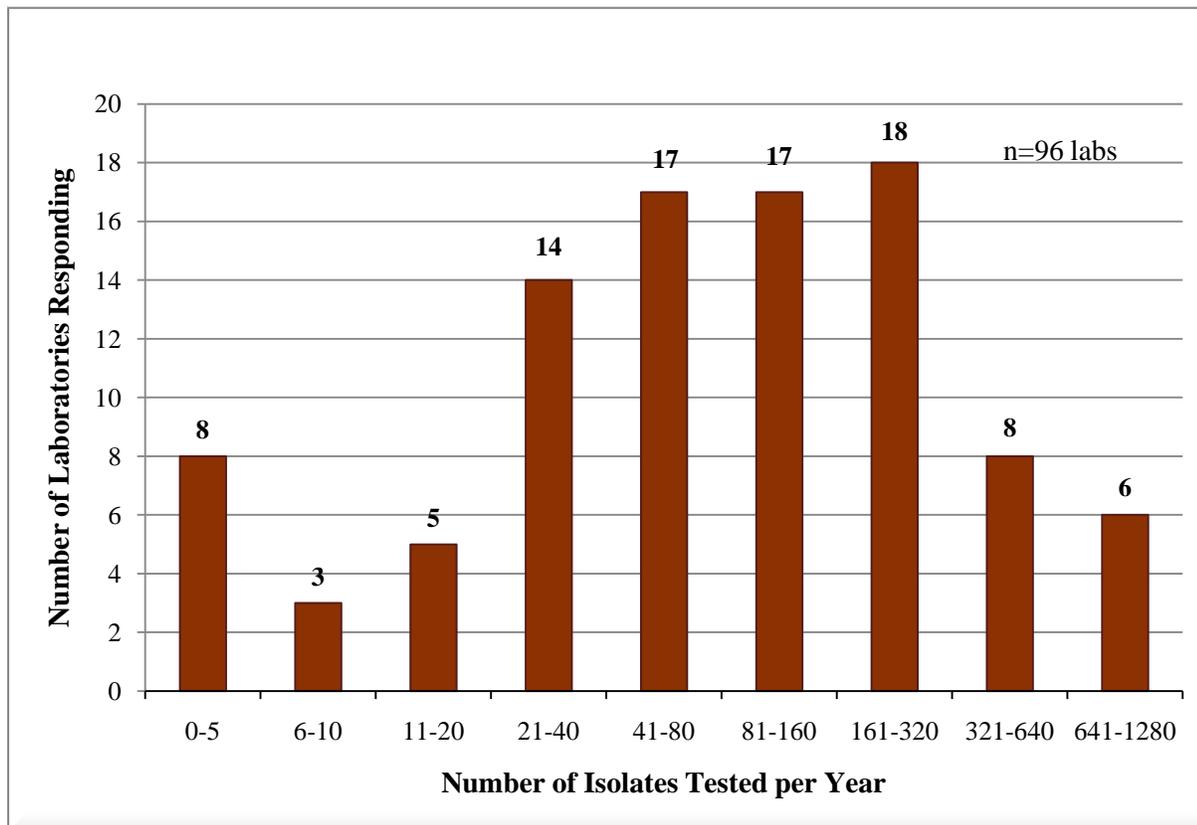
- 62 (64.6%): Health Department (city, county, state, regional, district, or national reference laboratory)
- 24 (25.0%): Hospital [city, county, district, community, state, regional, military, Veterans Administration, Federal government (other than military), privately-owned, university, HMO/PPO*-owned and operated, or religious-associated]
- 9 (9.4%): Independent [e.g., commercial, commercial manufacturer of reagents, HMO satellite clinic, reference laboratory (non-government affiliated)]
- 1 (1.0%): Other [Federal government research (nonmilitary)]

* HMO: health maintenance organization; PPO: preferred provider organization

Annual Number of MTBC Drug Susceptibility Tests Performed by Participants

Figure 1 shows the number of drug susceptibility tests performed on MTBC isolates by the 96 participants in one **calendar year**, January 1–December 31, 2009, excluding quality control isolates. The counts range from zero to 1,055. Sixteen (16) laboratories reported performing less than 21 drug susceptibility tests per year. Of these, two laboratories (one independent commercial laboratory and one health department laboratory not yet functional) reported zero susceptibility tests for the calendar year. To ensure testing proficiency, laboratories with low volumes may wish to consider referral of MTBC drug susceptibility testing to other facilities.

Figure 1: Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in the 2009 Calendar Year



Transport Times and Condition of Cultures upon Arrival

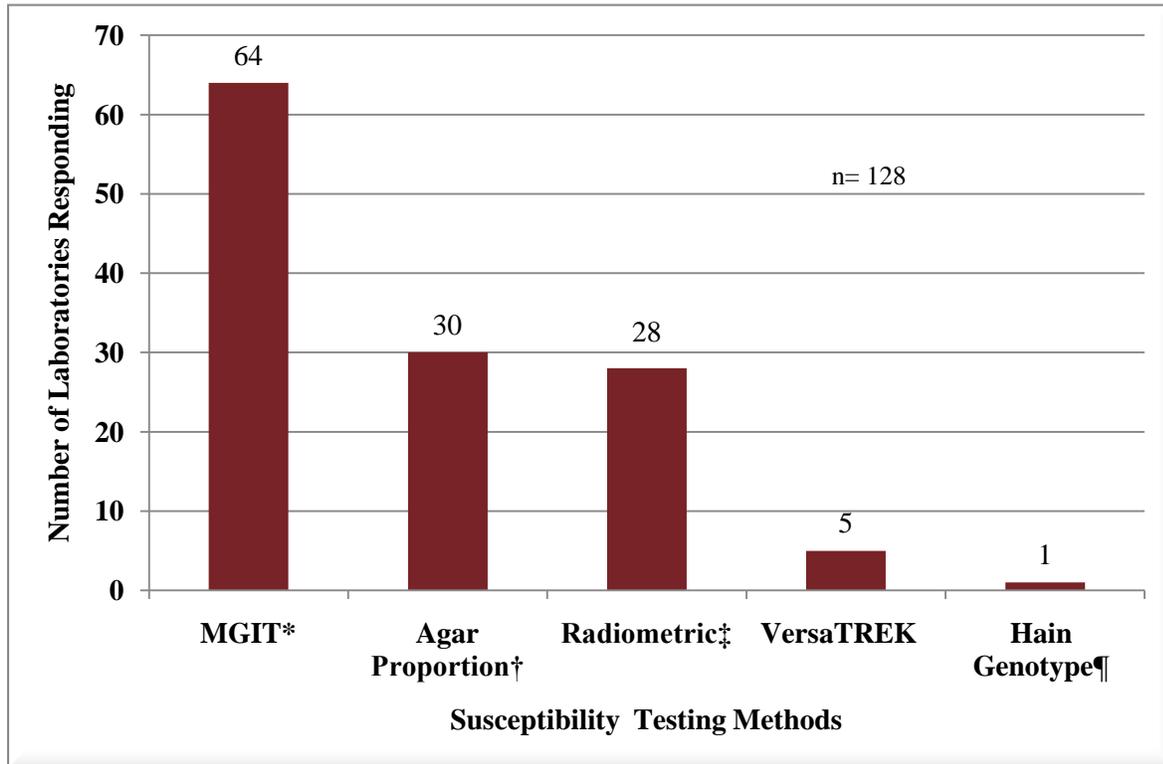
The laboratories were asked to report the date they received the panel of MTBC isolates in their laboratory in order to determine the amount of time required to receive the shipment. Eighty-four of the 96 laboratories (87.5%) reported having received the isolates within 24 hours of shipment; within 48 hours, an additional nine laboratories received the isolates. By the fourth day, all of the participants reported having received their MTBC panels.

While the strategy of the MTB NTM DST Program is to send only pure cultures and to have these cultures arrive in the laboratories in the required window of time to allow immediate susceptibility testing, a few cultures did not meet this standard. Of the eight laboratories reporting unsatisfactory cultures, five reported Isolate Y was either growing poorly or not at all. In all instances of unsatisfactory cultures, replacement cultures were sent to the laboratories. The MTB NTM DST will continue to strive to send pure cultures in satisfactory condition.

Laboratory Susceptibility Testing Procedures Used by Participants

Participants were asked to report all MTBC susceptibility testing methods that were used to test these isolates. Sixty-seven laboratories used only one method for testing, whereas 26 laboratories used two methods, and three laboratories used three methods. Figure 2 shows the reported susceptibility methods.

Figure 2: Susceptibility Testing Methods Reported by Participant Laboratories



* MGIT™ Mycobacteria Growth Indicator Tube

† Agar proportion using Middlebrook 7H10 or 7H11 medium

‡ Radiometric is BACTEC™ 460TB

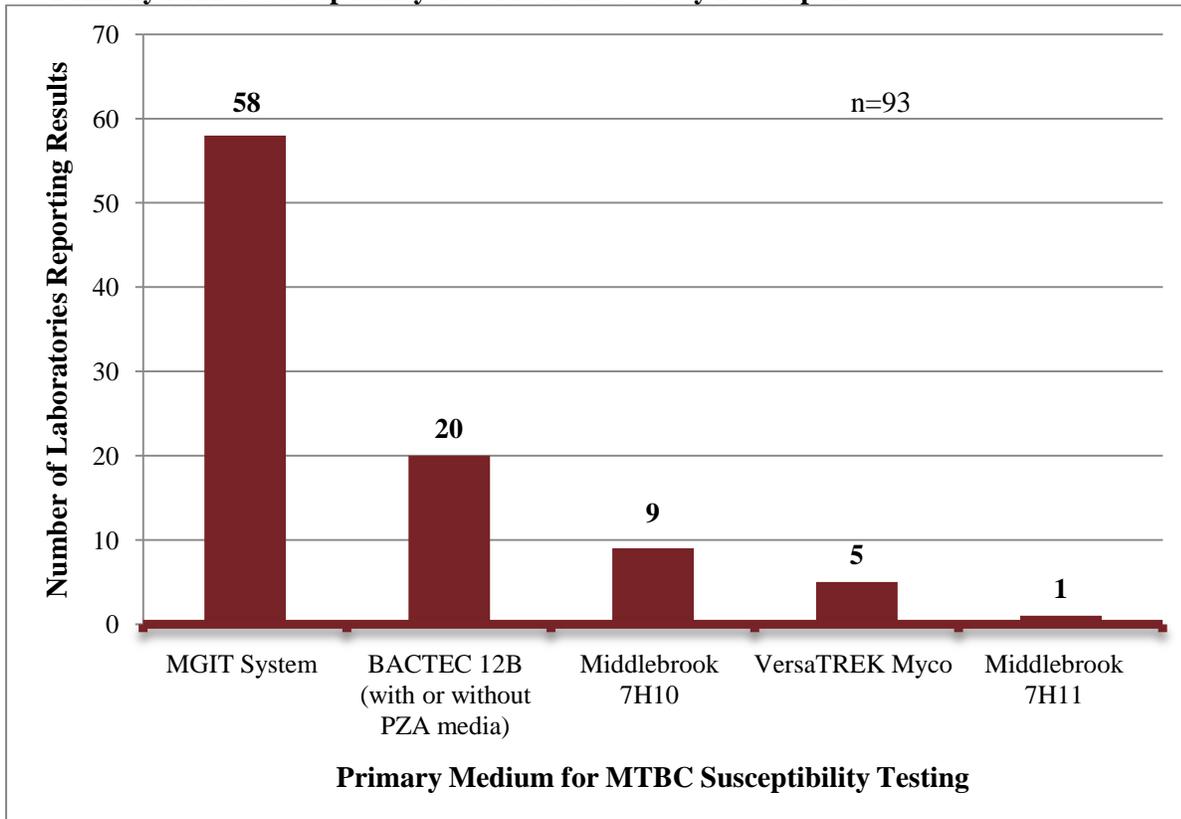
§ VersaTREK® Myco Susceptibility Kit

¶ Hain GenoType® MTBDR*plus* (detects MTBC and its resistance to rifampin and isoniazid) and Hain GenoType® MTBDR*sl* (a molecular genetics assay which allows the simultaneous detection of the MTBC and its resistance to fluoroquinolones, aminoglycosides, cyclic peptides and/or ethambutol).

The Primary MTBC Susceptibility Testing Media Used by Participants

Participants were asked to indicate the **primary** MTBC susceptibility test medium used by their laboratory for the isolates in the May 2010 shipment. Instructions were to select only one method as their primary method. Three participants incorrectly selected two methods for this question; therefore, their responses were not included in the analysis regarding primary methods. Figure 3 shows the responses submitted by the remaining 93 participants.

Figure 3: Primary MTBC Susceptibility Test Medium Used by Participants



Of the 64 laboratories that reported using MGIT™ to test the MTB NTM DST isolates, 58 indicated that the MGIT™ method is used as their primary method for susceptibility testing. Four laboratories using MGIT™ indicated they use 7H10 as their primary method.

(Note—2 answers were discounted because more than one primary method was selected.)

Of the 28 laboratories who reported using BACTEC™ 460TB (BACTEC™) to test the isolates, 20 used this as their primary method. Otherwise

- 5 used MGIT™ as their primary method, and
- 2 used AP as their primary method.

(Note—1 answer was discounted because more than one primary method was selected.)

Of the 30 laboratories who reported using AP to test the isolates, 10 used this as their primary method; otherwise

- 6 used BACTEC™ as their primary method;
- 10 used MGIT™ as their primary method; and
- 1 used VersaTREK®.

(Note—3 answers were discounted because more than one primary method was selected.)

All 5 laboratories who reported using VersaTREK® indicated that it is their primary method.

Rapid MTBC Susceptibility Tests Used by the Participants

The BACTEC™, MGIT™, and VersaTREK® systems are generally referred to as rapid methods. Ninety-one (91) laboratories reported that they used one or more rapid methods for drug susceptibility testing of the isolates. Of these labs, 58 (63.7%) responded that they purchased their antituberculosis drugs from the manufacturer of that rapid test method.

Participants Using Middlebrook 7H10 or 7H11 Media for Antituberculosis Drug Susceptibility Testing

Thirty (30) laboratories indicated they used agar proportion (Middlebrook medium) to perform drug susceptibility testing on the MTBC cultures in this shipment. When asked about the source of their media for any antituberculosis drug susceptibility testing, these 30 laboratories responded as follows (more than one answer could be selected):

- 4 (13.3%) purchased commercially-prepared media containing anti-tuberculosis drugs,
- 16 (53.3%) prepared media in-house with disks containing anti-tuberculosis drugs,
- 2 (6.7%) prepared media in-house by reconstituting and adding anti-tuberculosis drugs, and
- 11 (36.7%) indicated “Not Applicable.”

Nontuberculous Mycobacteria Drug Susceptibility Testing Capacity

To determine Nontuberculous Mycobacteria (NTM) drug susceptibility testing capacity, Questions 10 and 11 were added only for informational purposes. Question 10 asked if the laboratories performed susceptibility testing of NTM. All laboratory participants answered this question:

- 21 (21.9%) responded “Yes” and
- 75 (78.1%) responded “No.”

Question 11 asked participants that do not test NTM isolates in-house, if those isolates are referred to another laboratory for drug susceptibility testing. Forty-four (58.7%) of the 75 laboratories that do not test isolates in-house responded that they refer those isolates to another laboratory for drug susceptibility testing.

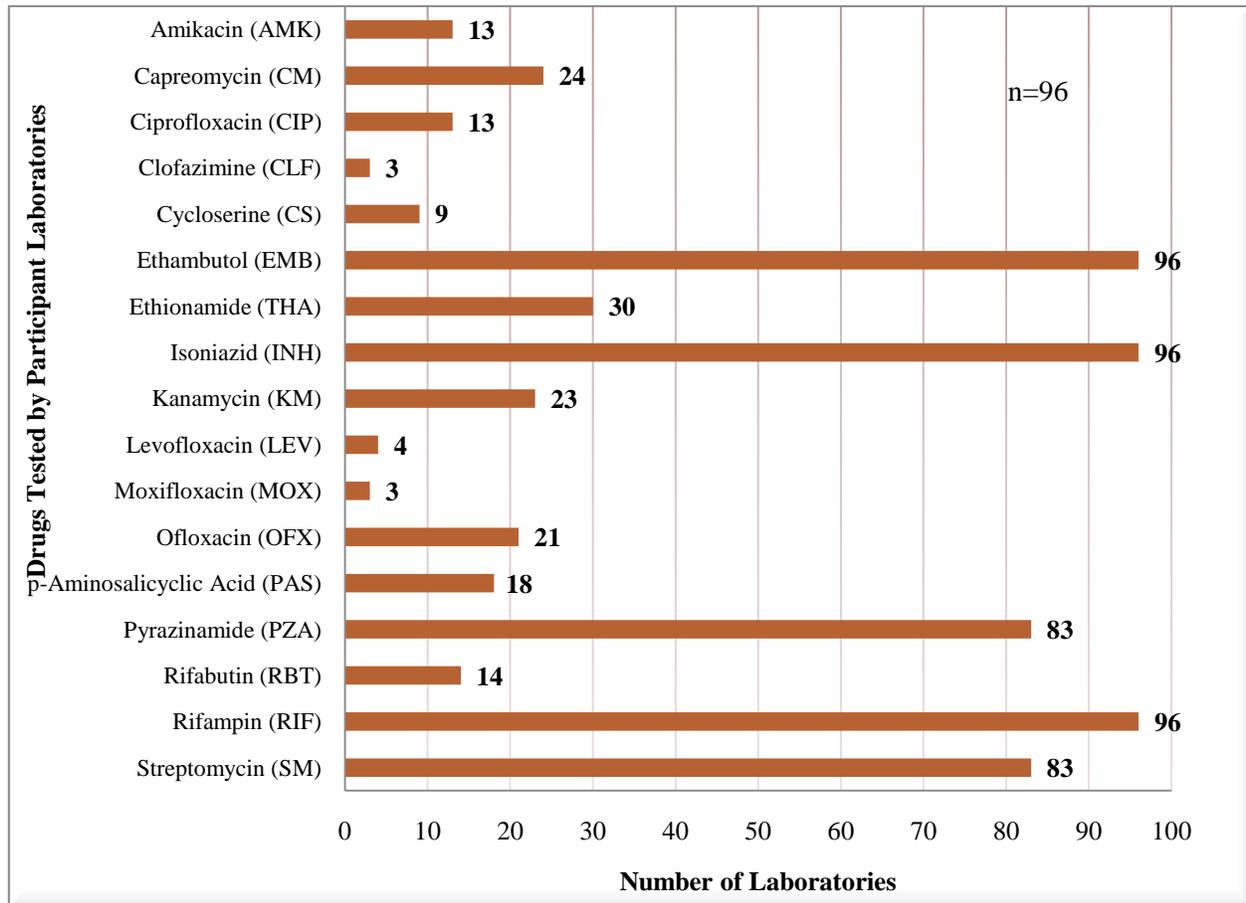
Antituberculous Drugs Used by Participants

All (96) participants tested three of the first-line drugs, Isoniazid (INH), Rifampin (RIF), and Ethambutol (EMB); 83 (86.5%) of these also tested Pyrozinamide (PZA).

CLSI recommends a full panel of first-line (primary) drugs (INH, RIF, EMB, and PZA), since it represents a combination of tests that provides the clinician with comprehensive information related to the four-drug therapy currently recommended for treatment of most patients in the United States with tuberculosis.

Figure 4 shows the number of laboratories testing each drug. The number at the right of the bars is the number of laboratories that tested the drug.

Figure 4: Antituberculous Drugs Used by 96 Participants



Note: Providing test results for all drugs that are reported to CDC by participants should not be construed as a recommendation or endorsement for testing particular drugs or drug concentrations with MTBC isolated from patients. It is assumed that some of the drugs are being tested for research purposes or potential use in the few referral institutions that may treat patients with MTBC isolates resistant to almost all standard drugs. Laboratories should not add drugs to their testing panel without consulting physicians with expertise in treating multidrug-resistant tuberculosis. Laboratories may contact their local tuberculosis control program for referrals to appropriate physicians.

Explanation of Tables 1 through 5

1. In the following tables, the shaded rows indicate critical concentrations for each test method. For each drug, the critical concentration is the lowest concentration that inhibits 95% of “wild-type” strains of MTBC organisms that have not been exposed to the drug, but that simultaneously does not inhibit strains of the *M. tuberculosis* considered resistant that are isolated from patients who are not responding to therapy.¹
2. The test results are listed in the appropriate (S, for susceptible or R, for resistant) columns with a corresponding total number of tests (Sum) column provided as a denominator for determining the level of consensus. This report contains all results reported by participating laboratories, including many drug concentrations with only one result.
3. Participants should note that the Clinical and Laboratory Standards Institute approved standard “Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes,” M24-A (ISBN 1-56238-500-3) CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA, 2003 recommends testing streptomycin as a second line drug and also adds ofloxacin and rifabutin to the list of recommended secondary drugs. For a complete list of drugs to be tested, consult the CLSI document M24-A.²
4. Concentrations are listed in micrograms per milliliter, µg/ml.
5. A concentration of 0.00 is entered for results associated with genetic testing [Hain GenoType[®] MTBDR*plus* Assay or Hain GenoType[®] MTBDRsl Kit (HAIN Lifescience, Germany)] for which no drug concentration is required.

Isolate T, *M. tuberculosis*–resistant to Rifampin at 1 µg/ml and to Ciprofloxacin and Ofloxacin at 2 µg/ml by Agar Proportion

Rifampin

Rifampin (RIF) 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 BACTEC™, MGIT™, and VersaTREK® of 2.0 µg/ml, 1.0 µg/ml, and 1.0 µg/ml, respectively. The mechanism of action of RIF is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase. More than 96% of RIF-resistant isolates contain a mutation in the 81-base pair (bp) central region of the *rpoB* gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase.² The activity of RIF in RIF-resistant isolates depends on both the mutation position and the type of amino acid change in the *rpoB* gene. Mutations in codons 531, 526, and 516 are among the most frequent mutations in RIF-resistant isolates and serve as predictors of RIF resistance. The most commonly encountered mutation, Ser531Leu, and some mutations in codons 526 and 516 have generally been reported to confer high-level resistance. However, a variety of mutations has been associated with low-level RIF resistance³, and includes the His526Leu mutation genetic testing by CDC revealed in Isolate T.

Of the 93 laboratories reporting RIF results for this isolate (some laboratories submitted results from more than one method) at the critical concentrations, this isolate was reported resistant by

- 65.2% (15/23) of the laboratories reporting AP results;
- 36.8% (7/19) of the laboratories reporting BACTEC™ results;
- 14.8% (9/61) of the laboratories reporting MGIT™ results; and
- 0% (0/5) of the laboratories reporting VersaTREK® results.

Interestingly, as shown above, 65.2% laboratories reporting AP results reported resistance to RIF, while only 14.8% of those using MGIT™ reported resistance. Three laboratories reported borderline results (1 reporting AP results and two reported BACTEC™ results). Seventeen laboratories reported results for more than one method; eight of these (47%) showed discordance between the methods. One laboratory reporting Hain GenoType® MTBDR*plus* genetic testing results reported resistance. One laboratory did not report a RIF result and two laboratories reported RIF results only at a concentration other than the critical concentration.

Isolate T was previously sent as a challenge in 2008 (Strain H) and similar results were obtained at that time. Low-level, but probably clinically significant RIF-resistance is linked to specific *rpoB* mutations and may be missed by standard growth-based methods, particularly the rapid, automated broth-based systems.⁴ It is possible that the critical concentration is too high to cover all clinically relevant resistance or that our methods may need modification (prolonged incubation, larger inoculum size) to detect the resistance of poorly growing strains. While the true frequency of these mutations is unknown, increased availability of genetic testing may help in

elucidating the problem.

Fluoroquinolones

Although the fluoroquinolones are not approved by the United States Food and Drug Administration (U.S. FDA) for treating tuberculosis, they are commonly used to treat patients with drug-resistant tuberculosis or patients who are intolerant of some of the first line drugs.⁴

Fluoroquinolones inhibit DNA gyrase (topoisomerase II) and topoisomerase IV, enzymes responsible for maintaining chromosomes in an appropriate topological state for replication and transcription.³ In *M. tuberculosis*, *gyrA* and *gyrB* encode the A and B subunits of DNA gyrase, respectively. The conserved region known as the quinolone-resistance-determining region (QRDR) of *gyrA* and *gyrB* has been found to be the most important area involved in fluoroquinolone resistance. In fluoroquinolone resistant isolates, *gyrB* mutations are less common than *gyrA* mutations. Genetic testing at CDC detected an Asp94Gly mutation in the QRDR of *gyrA* in Isolate T.

Of the participant laboratories, 31.3% (30/96) tested at least one of the fluoroquinolones (CIP, LEV, MOX, and OFX):

- 13.5% (13/96) laboratories tested CIP;
- 4.2% (4/96) laboratories tested LEV;
- 3.1% (3/96) MOX; and
- 21.9% (21/96) OFX.

All participants reported this isolate to be resistant to all of the fluoroquinolones tested and at all concentrations tested.

See Table 1 for the complete results submitted by all participants for Isolate T.

Table 1: Participant results for *M. tuberculosis*, Isolate T-resistant to RIF at 1 µg/ml, CIP and OFX at 2 µg/ml by AP

DRUG	Conc.	Test Method													
		AP Results			BACTEC™ Results			MGIT™ Results			Other Tests Results*				
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum		
Isoniazid	0.00												1		1
Isoniazid	0.10				20		20	61	1	62			5		5
Isoniazid	0.20	19		19	1		1								
Isoniazid	0.40				7		7	23		23			5		5
Isoniazid	0.50	1		1											
Isoniazid	1.00	22		22	1		1	1		1					
Isoniazid	5.00	4		4											
Rifampin	0.00													1	1
Rifampin	1.00	8	15	23	1	2	3	52	9	61			5		5
Rifampin	2.00				12	7	19								
Rifampin	5.00	3		3	1		1								
Rifampin	10.00				1		1								
Pyrazinamide	100.00				18		18	60		60					
Pyrazinamide	300.00				1		1						5		5
Pyrazinamide	900.00				1		1								
Ethambutol	0.00												1		1
Ethambutol	2.50				20	1	21								
Ethambutol	5.00	20		20	1	1	2	62	1	63			5		5
Ethambutol	7.50	1		1	1		1	1		1					
Ethambutol	8.00												6		6
Ethambutol	10.00	9		9											
Streptomycin	1.00	1		1	1		1	50	1	51					
Streptomycin	2.00	22		22	20		20								
Streptomycin	4.00	1		1				10		10					
Streptomycin	10.00	18		18											
Ethionamide	5.00	17	1	18	2		2	1		1					
Ethionamide	10.00	4		4											
Kanamycin	0.00												1		1
Kanamycin	2.50				1		1								
Kanamycin	5.00	11		11	1		1								
Kanamycin	6.00	10		10											
Capreomycin	0.00												1		1
Capreomycin	2.50				1		1								
Capreomycin	3.00							1		1					
Capreomycin	5.00				2		2								
Capreomycin	10.00	16		16											
Cycloserine	25.00	1		1											
Cycloserine	30.00	6		6											
Cycloserine	50.00	1		1											
Cycloserine	60.00	1		1											

* VersaTREK® and /or Hain GenoType®

Table 1 continued: *M. tuberculosis*, Isolate T-resistant to RIF at 1 µg/ml, CIP and OFX at 2 µg/ml

DRUG	Conc.	Test Method														
		AP Results			BACTEC™ Results			MGIT™ Results			Other Tests Results*					
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum			
p-Aminosalicylic acid	2.00	14		14												
p-Aminosalicylic acid	4.00				1		1									
p-Aminosalicylic acid	8.00	2		2												
p-Aminosalicylic acid	10.00	4		4												
Amikacin	0.00											1				1
Amikacin	1.00	1		1												
Amikacin	1.50							1			1					
Amikacin	2.00	1		1	1		1									
Amikacin	4.00	3		3												
Amikacin	5.00	1		1	1		1									
Amikacin	6.00	4		4												
Amikacin	12.00	1		1												
Ofloxacin	0.00												1			1
Ofloxacin	1.00		3	3												
Ofloxacin	1.25						1	1								
Ofloxacin	2.00		15	15			3	3								
Ofloxacin	2.50						1	1								
Ofloxacin	4.00		1	1			1	1								
Ofloxacin	5.00						1	1								
Ofloxacin	8.00						1	1								
Clofazimine	1.00	2		2												
Rifabutin	0.05						1	1								
Rifabutin	0.25						1	1								
Rifabutin	0.50	4	2	6	3		3									
Rifabutin	1.00	3		3												
Rifabutin	2.00	7	1	8												
Ciprofloxacin	1.00		2	2			1	1								
Ciprofloxacin	1.25						1	1								
Ciprofloxacin	2.00		6	6			2	2								
Ciprofloxacin	2.50						1	1								
Ciprofloxacin	4.00						1	1								
Ciprofloxacin	5.00						1	1								
Levofloxacin	1.50								1	1						
Levofloxacin	2.00						2	2								
Levofloxacin	8.00						1	1								
Moxifloxacin	0.25								1	1						
Moxifloxacin	0.50		1	1												

* VersaTREK® and /or Hain GenoType®

Isolate W, *M. tuberculosis*–resistant to Rifampin at 1.0 µg/ml and to Rifabutin at 0.25 µg/ml by Agar Proportion

As noted in the previous section, mutations in codons 531, 526, and 516 are among the most frequent mutations in RIF-resistant isolates, and serve as predictors of RIF resistance. The mode of action and mechanism of resistance of Rifabutin (RBT) appears to be identical to those of RIF; however, approximately 30% of RIF-resistant MTB isolates are susceptible to RBT.⁵ This isolate contains the most common mutation in *rpoB*, Ser531Leu, which confers resistance to both RIF and RBT.

Rifampin

While all laboratories tested this isolate against RIF, 94 laboratories reported results at the critical concentrations. All reported the isolate as RIF-resistant.

Rifabutin

Fourteen laboratories tested RBT in at least one concentration. Of these, six participants tested RBT at the critical concentration of 0.5 µg/ml for AP; five of these six participants (83.3%) reported resistance.

See Table 2 for the complete results submitted by all participants for Isolate W.

Table 2: Participant results for *M. tuberculosis*, Isolate W-resistant to RIF at 1.0 µg /ml and RBT at 0.25 µg /ml by AP

DRUG	Conc.	Test Method												
		AP Results			BACTEC™ Results			MGIT™ Results			Other Results*			
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum	
Isoniazid	0.00											1		1
Isoniazid	0.10				20		20	62		62		5		5
Isoniazid	0.20	21		21	1		1							
Isoniazid	0.40				8		8	22		22		5		5
Isoniazid	0.50	1		1										
Isoniazid	1.00	24		24	1		1	1		1				
Isoniazid	5.00	4		4										
Rifampin	0.00											1		1
Rifampin	1.00		26	26		3	3		63	63		5		5
Rifampin	2.00					21	21							
Rifampin	5.00		2	2		1	1							
Rifampin	10.00					1	1							
Pyrazinamide	5.00							1		1				
Pyrazinamide	100.00				18		18	59	1	60		5		5
Pyrazinamide	300.00				1		1							
Ethambutol	0.00											1		1
Ethambutol	2.50				20	1	21							
Ethambutol	5.00	21		21	2		2	62	1	63		5		5
Ethambutol	7.50	2		2	1		1	1		1				
Ethambutol	8.00											6		6
Ethambutol	10.00	10		10										
Streptomycin	1.00	1		1	1		1	51		51				
Streptomycin	2.00	24		24	20		20							
Streptomycin	4.00	1		1				9		9				
Streptomycin	10.00	18		18										
Ethionamide	1.25				1		1							
Ethionamide	2.50				1		1							
Ethionamide	5.00	21		21	3		3	1		1				
Ethionamide	10.00	4		4										
Kanamycin	0.00											1		1
Kanamycin	2.50				1		1							
Kanamycin	5.00	11		11	1		1							
Kanamycin	6.00	11		11										
Capreomycin	0.00											1		1
Capreomycin	2.50				1		1							
Capreomycin	3.00							1		1				
Capreomycin	5.00				4		4							
Capreomycin	10.00	18		18										

* VersaTREK® and /or Hain GenoType®

Table 2 continued: *M. tuberculosis*, Isolate W-resistant to RIF at 1.0 µg/ml and RBT at 0.25 µg/ml

DRUG	Conc.	Test Method												
		AP Results			BACTEC™ Results			MGIT™ Results			Other Tests Results*			
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum	
Cycloserine	25.00	1		1										
Cycloserine	30.00	7		7										
Cycloserine	50.00	1		1										
Cycloserine	60.00	1		1										
p-Aminosalicylic acid	2.00	15		15										
p-Aminosalicylic acid	4.00				1		1							
p-Aminosalicylic acid	8.00	2		2										
p-Aminosalicylic acid	10.00	4		4										
Amikacin	0.00										1		1	
Amikacin	1.00	1		1										
Amikacin	1.50							1		1				
Amikacin	2.00	1		1	1		1							
Amikacin	2.50				1		1							
Amikacin	4.00	3		3										
Amikacin	5.00	1		1	1		1							
Amikacin	6.00	4		4										
Amikacin	12.00	1		1										
Ofloxacin	0.00										1		1	
Ofloxacin	1.00	3		3	1		1							
Ofloxacin	1.25				1		1							
Ofloxacin	2.00	15		15	4		4							
Ofloxacin	4.00	1		1	1		1							
Ofloxacin	8.00				1		1							
Clofazimine	0.50				1		1							
Clofazimine	1.00	2		2										
Rifabutin	0.05						1	1						
Rifabutin	0.25						1	1						
Rifabutin	0.50	1	5	6			3	3						
Rifabutin	1.00	1	2	3			1	1						
Rifabutin	2.00	4	4	8										
Ciprofloxacin	1.00	2		2	2		2							
Ciprofloxacin	1.25				1		1							
Ciprofloxacin	2.00	7		7	2		2							
Ciprofloxacin	4.00				1		1							
Levofloxacin	1.50							1		1				
Levofloxacin	2.00				3		3							
Levofloxacin	8.00				1		1							
Moxifloxacin	0.25							1		1				
Moxifloxacin	0.50	1		1										
Moxifloxacin	1.00	1		1										

Isolate X, *M. tuberculosis*–resistant to Isoniazid at 0.2 µg/ml by Agar Proportion

Isoniazid (INH) is the most widely used first-line anti-TB drug. It is the cornerstone of all effective regimens for the treatment of TB disease and latent infection. There are two described mechanisms that account for the majority of INH resistance. The most common method involved is mutation within the *katG* gene, which encodes a catalase- peroxidase enzyme required for the activation of INH. Mutations in *katG* are 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 encodes enoyl-acyl carrier protein reductase, which is required for mycolic acid biosynthesis. Mutations in *inhA* are generally associated with low-level resistance to INH and are less frequent than *katG* mutations.³ Isolate X did not have a mutation at either the *katG* or *inhA* loci suggesting an unknown mechanism of resistance.

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 BACTEC™, MGIT™, and VersaTREK® are 0.1 µg/ml and 0.4 µg/ml. All participants tested this isolate with at least one concentration, but six laboratories did not test at the critical concentration (three reporting AP results and three reporting BACTEC™ results).

At the critical concentration for INH (0.2 µg/ml for AP; 0.1 µg/ml for BACTEC™, MGIT™, and VersaTREK®), resistance was reported by

- 86.4% (19/22) of the participant laboratories reporting AP results;
- 78.9% (15/19) of the participant laboratories reporting BACTEC™ results;
- 90.3% (56/62) of the participant laboratories reporting MGIT™ results;
- 100% (5/5) of the participant laboratories reporting VersaTREK® results;

The laboratory using Hain GenoType® MTBDR*plus* also reported INH resistance.

At the higher concentrations of INH (1.0 µg/ml for AP and 0.4 µg/ml for BACTEC™, MGIT™, and VersaTREK®), susceptibility was reported by

- 100% (25/25) of the participants using AP;
- 100% (8/8) of the participants using BACTEC™;
- 96.6% (28/29) participants using MGIT™; and
- 100% (5/5) of the participants using VersaTREK®.

See Table 3 for the complete results submitted by all participants for Isolate X.

Table 3: Participant results for *M. tuberculosis*, Isolate X-resistant to INH at 0.2 µg/ml by AP

DRUG	Conc.	Test Method												
		AP Results			BACTEC™ Results			MGIT™ Results			OTHER TEST Results*			
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum	
Isoniazid	0.00											1		1
Isoniazid	0.10				4	15	19	6	56	62		5		5
Isoniazid	0.20	3	19	22	2		2							
Isoniazid	0.40				8		8	28	1	29	5			5
Isoniazid	0.50		1	1										
Isoniazid	1.00	25		25	2		2		1	1				
Isoniazid	5.00	4		4	1		1							
Rifampin	0.00											1		1
Rifampin	1.00	23		23	3		3	63		63	5			5
Rifampin	2.00				20		20							
Rifampin	5.00	2		2										
Pyrazinamide	5.00							1		1				
Pyrazinamide	100.00				17		17	57	2	59				
Pyrazinamide	300.00				1		1				5			5
Ethambutol	0.00											1		1
Ethambutol	2.50				21		21							
Ethambutol	5.00	20		20	2		2	63		63	5			5
Ethambutol	7.50	2		2	1		1	1		1				
Ethambutol	8.00										6			6
Ethambutol	10.00	10		10										
Streptomycin	1.00	1		1	1		1	51		51				
Streptomycin	2.00	23		23	19		19							
Streptomycin	4.00	1		1				9		9				
Streptomycin	10.00	18		18										
Ethionamide	2.50				1		1							
Ethionamide	5.00	9	10	19	1	1	2		1	1				
Ethionamide	10.00	3	1	4										
Kanamycin	0.00											1		1
Kanamycin	2.50				1		1							
Kanamycin	5.00	10		10										
Kanamycin	6.00	11		11										
Capreomycin	0.00											1		1
Capreomycin	2.50				1		1							
Capreomycin	3.00							1		1				
Capreomycin	5.00				4		4							
Capreomycin	10.00	17		17										
Cycloserine	25.00	1		1										
Cycloserine	30.00	7		7										
Cycloserine	50.00	1		1										
Cycloserine	60.00	1		1										

Table 3 continued: *M. tuberculosis*, Isolate X-resistant to isoniazid at 0.2 µg/ml

DRUG	Conc.	Test Method													
		AP Results			BACTEC™ Results			MGIT™ Results			Other Tests Results*				
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum		
p-Aminosalicylic acid	2.00	15		15											
p-Aminosalicylic acid	4.00				1		1								
p-Aminosalicylic acid	8.00	2		2											
p-Aminosalicylic acid	10.00	4		4											
Amikacin	0.00											1		1	
Amikacin	1.00	1		1											
Amikacin	1.50							1		1					
Amikacin	2.00	1		1	1		1								
Amikacin	2.50				1		1								
Amikacin	4.00	3		3											
Amikacin	5.00	1		1	1		1								
Amikacin	6.00	4		4											
Amikacin	12.00	1		1											
Ofloxacin	0.00											1		1	
Ofloxacin	1.00	3		3	1		1								
Ofloxacin	1.25				1		1								
Ofloxacin	2.00	14		14	3		3								
Ofloxacin	4.00	1		1	1		1								
Ofloxacin	8.00				1		1								
Clofazimine	0.50				1		1								
Clofazimine	1.00	2		2											
Rifabutin	0.05				1		1								
Rifabutin	0.50	6		6											
Rifabutin	1.00	3		3	1		1								
Rifabutin	2.00	7		7											
Ciprofloxacin	1.00	2		2	2		2								
Ciprofloxacin	1.25				1		1								
Ciprofloxacin	2.00	7		7	2		2								
Ciprofloxacin	4.00				1		1								
Levofloxacin	1.50							1		1					
Levofloxacin	2.00				3		3								
Levofloxacin	8.00				1		1								
Moxifloxacin	0.25							1		1					
Moxifloxacin	0.50	1		1											
Moxifloxacin	1.00	1		1											

* VersaTREK® and /or Hain GenoType®

Isolate Y, *M. bovis*–resistant to Pyrazinamide at 100 µg/ml

Pyrazinamide (PZA) is an important first-line drug for treatment of tuberculosis and is used with INH and RIF. Its role is to shorten TB treatment from the previous 9-12 months to 6 months because it kills a population of persistent bacilli in acidic pH environment in the lesions that is not killed by other drugs.³ PZA is a prodrug that requires conversion of its active form, pyrazinoic acid, by the pyrazinamidase encoded by the *pncA* gene of *M. tuberculosis*. PZA-resistant *M. tuberculosis* strains lose pyrazinamidase activity and resistance to PZA is usually caused by diverse nucleotide changes scattered throughout the *pncA* gene. (All mechanisms of resistance are still unknown.) *M. bovis* has a natural resistance to PZA. Unlike *M. tuberculosis*, resistance to PZA by *M. bovis* is caused by a characteristic single point mutation resulting in a change of C (cytosine) to G (guanine) at nucleotide position 169 of the *pncA* gene which causes the replacement of histidine (CAC) with aspartic acid (GAC) in amino acid position 57 in *M. bovis* PZase. This substitution causes defective PZase activity and confers natural PZA resistance in *M. bovis* strains, including BCG substrains.^{6,7} This isolate was confirmed by CDC confirmed this isolate has this mutation (CAC>GAC).

Of the participants reporting PZA results (83), resistance was reported by

- 94.4% (17/18) of the laboratories reporting BACTEC™ results;
- 100% (60/60) of the participants reporting MGIT™ results; and,
- 100% (5/5) of the laboratories reporting VersaTREK® results.

See Table 4 for the complete results submitted by all participants for Isolate Y.

Table 4: Participant results for *M. bovis*, Isolate Y-resistant to PZA at 100 µg/ml

DRUG	Conc.	Test Method											
		AP Results			BACTEC™ Results			MGIT™ Results			Other Test Results*		
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00											1	1
Isoniazid	0.10				18	2	20	62		62		5	5
Isoniazid	0.20	19	1	20	1		1						
Isoniazid	0.40				8		8	23		23		5	5
Isoniazid	0.50	1		1									
Isoniazid	1.00	22	1	23	1		1	1		1			
Isoniazid	5.00	4		4									
Rifampin	0.00											1	1
Rifampin	1.00	22		22	3		3	62	1	63		5	5
Rifampin	2.00				20		20						
Rifampin	5.00	2		2									
Pyrazinamide	5.00								1	1			
Pyrazinamide	100.00				1	17	18	60		60			
Pyrazinamide	300.00				1		1					5	5
Ethambutol	0.00											1	1
Ethambutol	2.50				20	1	21						
Ethambutol	5.00	20		20	2		2	63		63		5	5
Ethambutol	7.50	1		1	1		1	1		1			
Ethambutol	8.00											6	6
Ethambutol	10.00	10		10									
Streptomycin	1.00	1		1	1		1	50	1	51			
Streptomycin	2.00	22		22	19		19						
Streptomycin	4.00	1		1				10		10			
Streptomycin	10.00	18		18									
Ethionamide	2.50					1	1						
Ethionamide	5.00	16	3	19	2		2	1		1			
Ethionamide	10.00	4		4									
Kanamycin	0.00											1	1
Kanamycin	5.00	10		10									
Kanamycin	6.00	11		11									
Capreomycin	0.00											1	1
Capreomycin	3.00							1		1			
Capreomycin	5.00				3		3						
Capreomycin	10.00	17		17									
Cycloserine	25.00	1		1									
Cycloserine	30.00	7		7									
Cycloserine	50.00	1		1									
Cycloserine	60.00	1		1									

Table 4 continued: *M. bovis*, Isolate Y–resistant to pyrazinamide at 100 µg/ml

DRUG	Conc.	Test Method												
		AP Results			BACTEC™ Results			MGIT™ Results			Other Tests Results*			
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum	
p-Aminosalicylic acid	2.00	15		15										
p-Aminosalicylic acid	8.00	2		2										
p-Aminosalicylic acid	10.00	4		4										
Amikacin	0.00											1		1
Amikacin	1.00	1		1										
Amikacin	1.50							1			1			
Amikacin	2.00	1		1	1		1							
Amikacin	2.50				1		1							
Amikacin	4.00	3		3										
Amikacin	5.00	1		1										
Amikacin	6.00	4		4										
Amikacin	12.00	1		1										
Ofloxacin	0.00											1		1
Ofloxacin	1.00	3		3	1		1							
Ofloxacin	2.00	14		14	3		3							
Ofloxacin	4.00	1		1	1		1							
Ofloxacin	8.00				1		1							
Clofazimine	0.50				1		1							
Clofazimine	1.00	2		2										
Rifabutin	0.50	6		6										
Rifabutin	1.00	3		3	1		1							
Rifabutin	2.00	7		7										
Ciprofloxacin	1.00	2		2	2		2							
Ciprofloxacin	2.00	7		7	1		1							
Ciprofloxacin	4.00				1		1							
Levofloxacin	1.50							1			1			
Levofloxacin	2.00				3		3							
Levofloxacin	8.00				1		1							
Moxifloxacin	0.25							1			1			
Moxifloxacin	0.50	1		1										
Moxifloxacin	1.00	1		1										

* VersaTREK® and /or Hain GenoType®

Isolate Z, *M. tuberculosis*–susceptible to All First- and Second-line Drugs by Agar Proportion

This isolate is susceptible to all of the first-line [(INH, RIF, EMB, and PZA), the other rifamycins (rifabutin and rifapentine)³ are also considered first-line drugs in special situations] and second-line drugs (cycloserine, ethionamide, streptomycin, amikacin, kanamycin, capreomycin, p-aminosalicylic acid, and fluoroquinolones).

Among the participants, only 3 different laboratories reported one instance of resistance:

- 1 laboratory using AP reported resistance to INH at 0.2 µg/ml;
- 1 laboratory using MGIT™ reported resistant to PZA at 100 µg/ml; and,
- 1 laboratory using VersaTREK® reported resistance to RIF at 1.0 µg/ml.

Almost all of the participants (99.6%) reported this isolate susceptible to all drugs tested by all methods.

See Table 5 for the complete results submitted by all participants for Isolate Z.

Table 5: Participant results for *M. tuberculosis*, Isolate Z–susceptible to all first- and second-line drugs by AP

DRUG	Conc.	Test Method											
		AP Results			BACTEC™ Results			MGIT™ Results			Other Tests Results*		
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00										1		1
Isoniazid	0.10				20		20	62		62	5		5
Isoniazid	0.20	17	1	18	1		1						
Isoniazid	0.40				7		7	23		23	5		5
Isoniazid	0.50	1		1									
Isoniazid	1.00	21		21	1		1	1		1			
Isoniazid	5.00	4		4									
Rifampin	0.00										1		1
Rifampin	1.00	21		21	3		3	63		63	4	1	5
Rifampin	2.00				20		20						
Rifampin	5.00	2		2									
Pyrazinamide	5.00							1		1			
Pyrazinamide	100.00				18		18	58	1	59			
Pyrazinamide	300.00				1		1				5		5
Ethambutol	0.00										1		1
Ethambutol	2.50				21		21						
Ethambutol	5.00	19		19	2		2	63		63	5		5
Ethambutol	7.50	1		1	1		1	1		1			
Ethambutol	8.00										6		6
Ethambutol	10.00	9		9									
Streptomycin	1.00	1		1	1		1	51		51			
Streptomycin	2.00	21		21	19		19						
Streptomycin	4.00	1		1				10		10			
Streptomycin	10.00	18		18									
Ethionamide	5.00	18		18	1		1	1		1			
Ethionamide	10.00	4		4									
Kanamycin	0.00										1		1
Kanamycin	5.00	10		10									
Kanamycin	6.00	10		10									
Capreomycin	0.00										1		1
Capreomycin	3.00							1		1			
Capreomycin	5.00				1		1						
Capreomycin	10.00	15		15									
Cycloserine	25.00	1		1									
Cycloserine	30.00	6		6									
Cycloserine	50.00	1		1									
Cycloserine	60.00	1		1									

* VersaTREK® and/or Hain GenoType®

Table 5 continued: Isolate Z, *M. tuberculosis*–susceptible to all first-and second-line drugs

DRUG	Conc.	Test Method												
		AP Results			BACTEC™ Results			MGIT™ Results			Other Tests*			
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum	
p-Aminosalicylic acid	2.00	14		14										
p-Aminosalicylic acid	8.00	2		2										
p-Aminosalicylic acid	10.00	4		4										
Amikacin	0.00											1		1
Amikacin	1.00	1		1										
Amikacin	1.50							1			1			
Amikacin	2.00	1		1	1		1							
Amikacin	4.00	3		3										
Amikacin	5.00	1		1										
Amikacin	6.00	4		4										
Amikacin	12.00	1		1										
Ofloxacin	0.00											1		1
Ofloxacin	1.00	3		3										
Ofloxacin	2.00	14		14	2		2							
Ofloxacin	4.00	1		1	1		1							
Ofloxacin	8.00				1		1							
Clofazimine	1.00	2		2										
Rifabutin	0.50	6		6										
Rifabutin	1.00	3		3										
Rifabutin	2.00	7		7										
Ciprofloxacin	1.00	2		2	1		1							
Ciprofloxacin	2.00	6		6	1		1							
Ciprofloxacin	4.00				1		1							
Levofloxacin	1.50							1			1			
Levofloxacin	2.00				2		2							
Levofloxacin	8.00				1		1							
Moxifloxacin	0.25							1			1			
Moxifloxacin	0.50	1		1										

* VersaTREK® and /or Hain GenoType®

Abbreviations Used in This Report

AMK	amikacin
AP	agar proportion
BACTEC™	BACTEC™ 460TB
bp	base pair
BSL	Biosafety Level
CDC	Centers for Disease Control and Prevention (CDC)
CIP	ciprofloxacin
CLF	clofazimine
CLSI	Clinical Laboratory and Standards Institute
CM	capreomycin
CS	cycloserine
DNA	deoxyribonucleic acid
DST	Drug Susceptibility Testing
EMB	ethambutol
HMO	Health Maintenance Organization
INH	isoniazid
KM	kanamycin
LEV	levofloxacin
MGIT™	BACTEC™ MGIT™ 960 (Mycobacteria Growth Indicator Tube)
MOX	moxifloxacin
MPEP MTB NTM DST	Model Performance Evaluation Program for <i>Mycobacterium tuberculosis</i> and Nontuberculous Mycobacteria Drug Susceptibility Testing
MTB	<i>Mycobacterium tuberculosis</i>
MTBC	<i>Mycobacterium tuberculosis</i> complex
NIH	National Institutes of Health
NTM	Nontuberculous Mycobacteria
OFX	ofloxacin
PAS	p-aminosalicylic acid
PPO	Preferred Provider Organization
PZA	pyrazinamide
QRDR	quinolone-resistance-determining region
RBT	rifabutin
RIF	rifampin
RNA	ribonucleic acid
SM	streptomycin
THA	ethionamide
VersaTREK®	VersaTREK® Myco Susceptibility Kit

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