



**Centers for Disease Control and Prevention  
Model Performance Evaluation Program**

***Mycobacterium tuberculosis*  
and  
Nontuberculous Mycobacteria Drug  
Susceptibility Testing Program**

**Report of Results  
for the Performance Evaluation Survey  
Conducted During November 2009**

**UNITED STATES DEPARTMENT OF HEALTH AND HUMAN  
SERVICES**

**Centers for Disease Control and Prevention  
Coordinating Center for Infectious Diseases  
Division of Laboratory Systems  
Atlanta, Georgia 30333**

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# *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria (NTM) Drug Susceptibility Testing Model Performance Evaluation Program

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# ***Mycobacterium tuberculosis* and Nontuberculous Mycobacteria (NTM) Drug Susceptibility Testing Model Performance Evaluation Program**

## **Overview**

This report analyzes the laboratory susceptibility testing results reported to the Centers for Disease Control and Prevention (CDC) by laboratories participating in the Model Performance Evaluation Program (MPEP) for *Mycobacterium tuberculosis* and nontuberculous mycobacteria (NTM) Drug Susceptibility Testing. Five (5) *Mycobacterium tuberculosis* complex (MTBC) isolates were shipped in November 2009 to United States (U.S.) laboratories. Drug susceptibility results were submitted by 98 (95.1%) of the 103 laboratories that received isolates in this shipment. All 98 laboratories reported results via the online data entry system.

## **Contents**

The five MPEP MTBC isolates in the November 2009 shipment were obtained by CDC as part of the annual World Health Organization (WHO) Supranational Reference Laboratory (SRL) Network 2008 proficiency panel and have been well characterized both phenotypically and genotypically.

The November 2009 shipment did not contain NTM isolates for performance evaluation. Laboratories that perform drug susceptibility testing on NTM are encouraged to follow the consensus report by the American Thoracic Society<sup>1</sup> and the Clinical and Laboratory Standards Institute (CLSI)<sup>2</sup> which provides recommendations for NTM test methods and clinically relevant drugs.

## **Laboratory types**

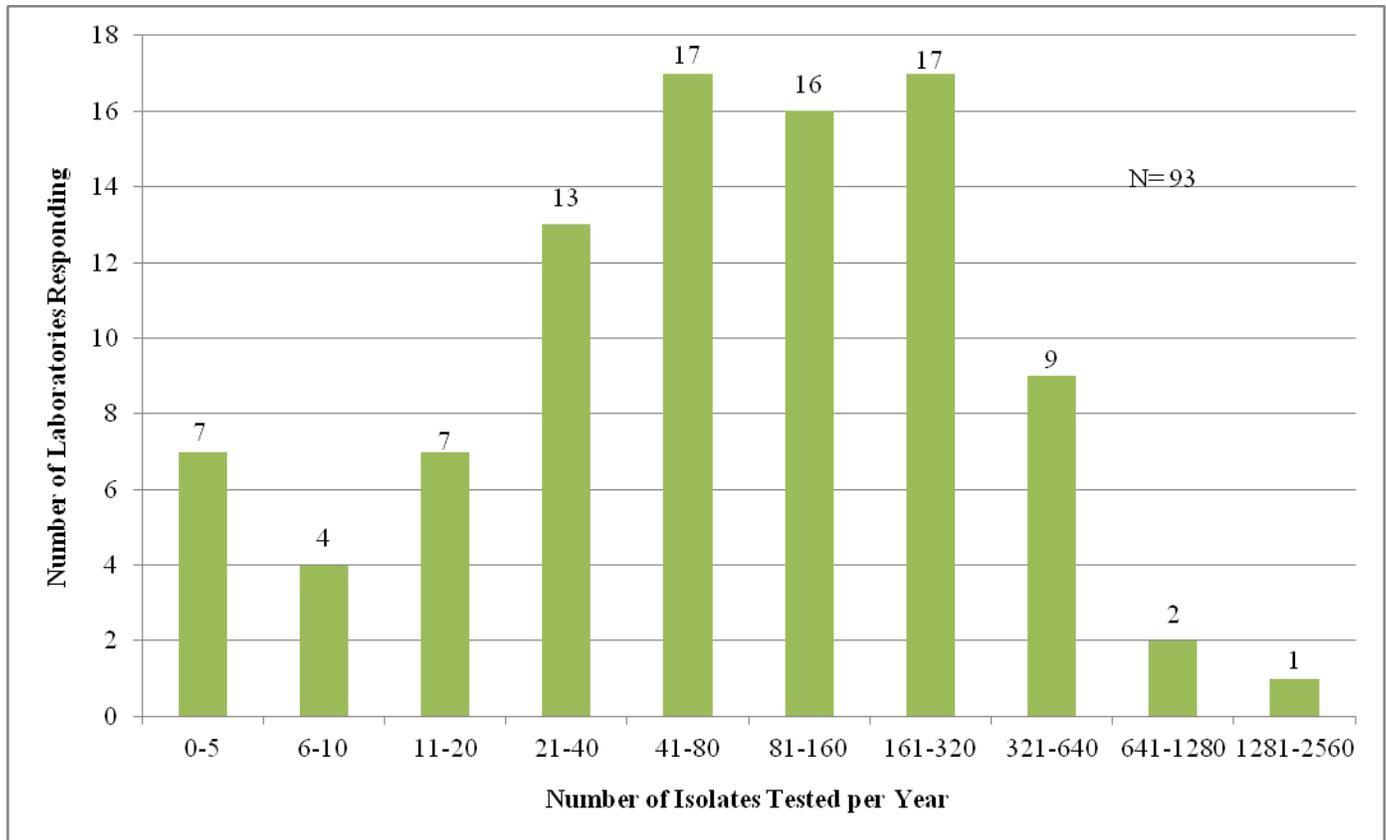
Each laboratory reported their primary classification. The 98 participant laboratories self identified as:

- 64 (65.31%) health departments;
- 25 (25.1%) hospitals; and
- 9 (9.18%) independent laboratories.

## Isolates per calendar year

The distribution of the annual volume of MTBC susceptibility testing performed by participant laboratories is shown in **Figure 1**. The values on the top of the bars in the histogram indicate the number of laboratories within each group. Among the 98 participant laboratories, 93 (94.9%) reported their annual test volume. Eighteen laboratories (19.4%) reported that they performed less than 20 drug susceptibility tests per year. Laboratories with low testing volumes should consider referring drug susceptibility tests to other facilities or develop a system to ensure proficiency of testing.

**Figure 1: Distribution of Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participating Laboratories in Calendar Year 2009**



## Biosafety Levels of Participant Laboratories

The biosafety levels (BSL) reported by participant laboratories handling MTBC were:

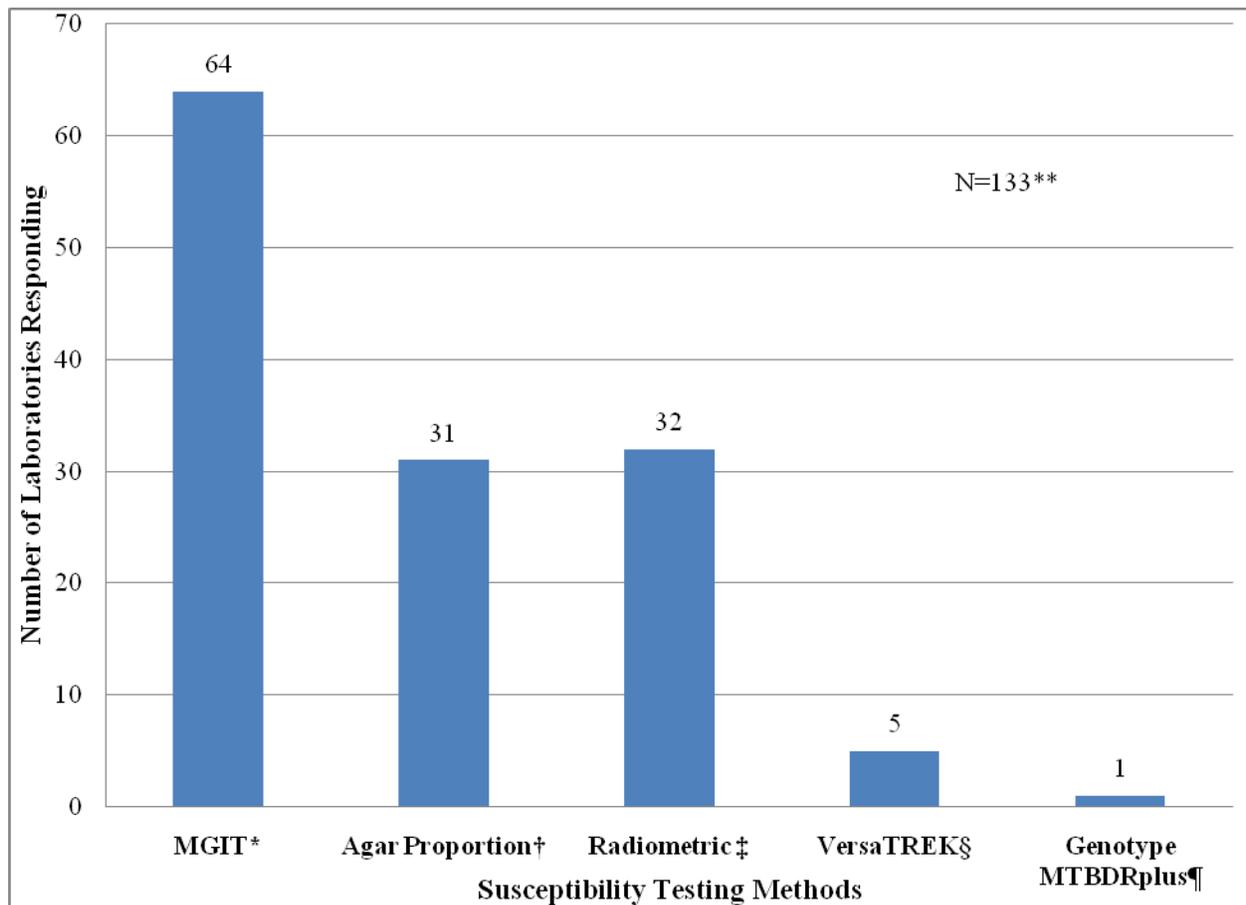
- BSL-3, 61 participants;
- BSL-2 (facilities with level 3 containment practices), 31 participants;
- BSL-2, 6 participants.

All laboratories are strongly encouraged to consult the CDC/NIH manual, *Biosafety in Microbiological and Biomedical Laboratories (5<sup>th</sup> Edition)*<sup>3</sup> recommendation and to determine their correct biosafety level. This manual can be accessed on the web at <http://www.cdc.gov/biosafety/publications/bmb15/>. This manual recommends the use of Biosafety Level 3 (BSL-3) practices when testing MTBC.

## Laboratory Susceptibility Testing Methods

The types of test methods reported as being used for drug susceptibility testing by participating laboratories are shown in **Figure 2**. Some laboratories reported using more than one method. Therefore, the N value is greater than the total number of participants. The MGIT method (BACTEC Mycobacteria Growth Indicator tube, Becton Dickinson) was used by 65.31% of the participant laboratories. Other test methods reported included agar proportion (AP), BACTEC 460TB, VersaTREK Myco TB System, and Hain GenoType MTBDR*plus* (Hain Lifescience GmbH, Germany). The GenoType MTBDR*plus* test is a deoxyribonucleic acid (DNA) strip assay which uses polymerase chain reaction (PCR) and hybridization to detect genetic mutations in the genes that confer isoniazid (INH) and rifampin (RIF) resistance.<sup>4</sup> [Note: This assay is not approved by the U.S. Food and Drug Administration (FDA)]

**Figure 2: Methods reported to be used by Participant Laboratories for MTBC Susceptibility Testing**



\*MGIT, Mycobacteria Growth Indicator Tube

†Agar proportion using Middlebrook 7H10 or 7H11 medium

‡Radiometric is BACTEC 460TB

§Versa TREK Myco Susceptibility Kit

¶Hain GenoType MTBDR*plus*

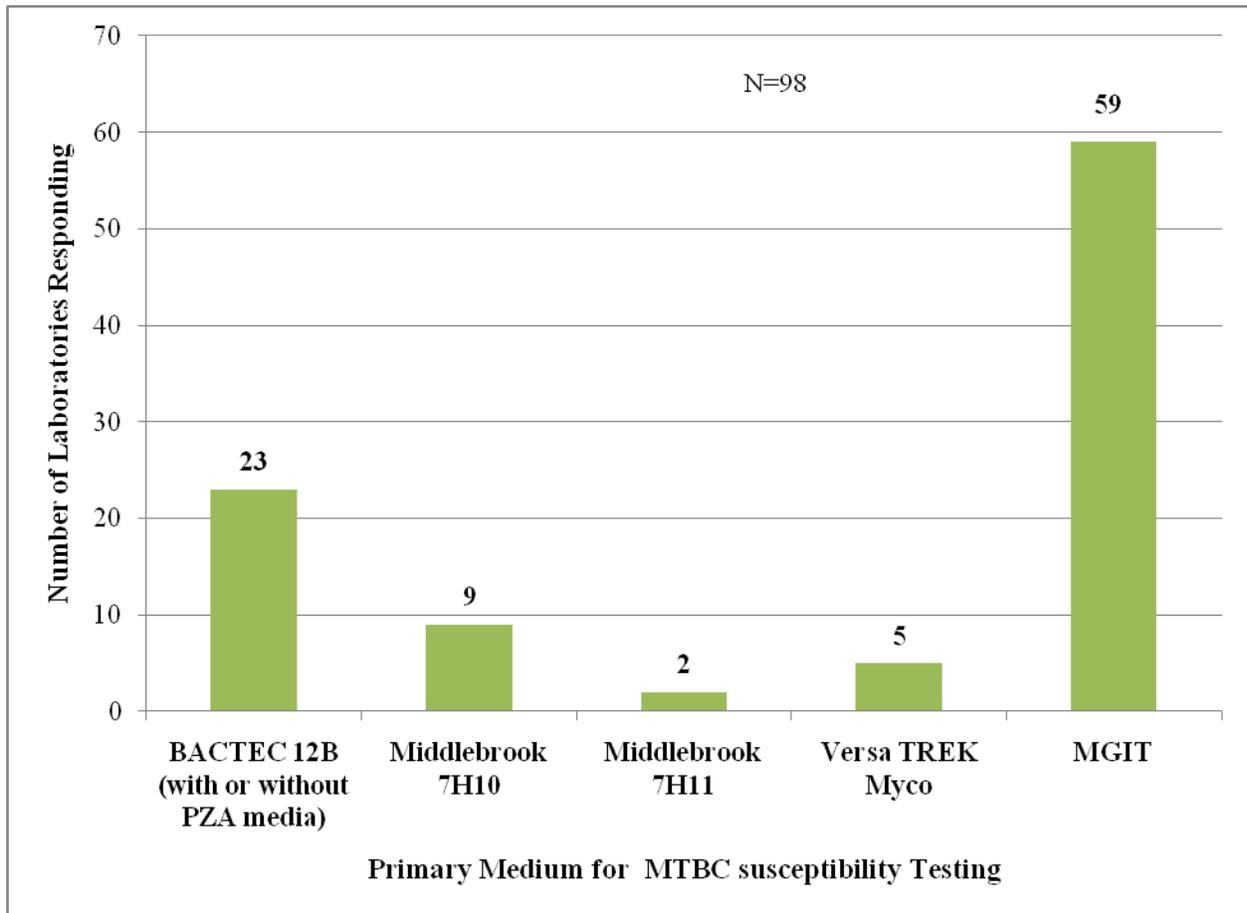
\*\* Some laboratories reported using more than one method

Many participant laboratories reported results for more than one method in this survey. Based on the reported drug susceptibility testing results, 21 laboratories used two methods and two laboratories used three methods for INH and RIF testing.

## The primary MTBC susceptibility test medium

Figure 3 shows the primary media used by the 98 participants in this shipment. Eighty-seven laboratories (88.8 %) reported using a broth-based system as their primary testing method.

**Figure 3: Primary MTBC Susceptibility Test Medium Used by Participants**



## Rapid Test Methods

The BACTEC 460TB, BACTEC MGIT 960, and VersaTREK systems are generically referred to as rapid broth methods.

Of the laboratories that indicated a rapid susceptibility test method was used for MTBC testing:

- 59 indicated they purchased the antituberculous drugs from the manufacturer;
- 7 indicated they did not purchase the antituberculous drugs from the manufacturer.

There was no text field associated with this question. Therefore, it is unknown where the respondents purchased the antituberculous drugs.

## Agar proportion susceptibility testing methods

Of the 31 laboratories that reported using Middlebrook 7H10 or 7H11 media for Agar proportion susceptibility testing:

- 2 purchased commercially-prepared media containing antituberculous drugs;
- 10 only used media they prepared in-house with disks containing antituberculous drugs;
- 8 only used media they prepared in-house by reconstituting and adding antituberculous drugs;
- 11 used media they prepared in-house by both techniques: with disks containing antituberculous drugs; and by reconstituting and adding antituberculosis drugs.

## Laboratories Performing Susceptibility Testing on NTM

This program has, in previous shipments, sent isolates of NTM to participants for susceptibility testing. The shipments in April and November 2009 did not contain NTM isolates. To determine if it is appropriate to add NTM isolates to future panel shipments, we asked laboratories if they performed NTM susceptibilities on-site.

Of the 98 participant laboratories responding:

- 23 (23.5%) perform NTM susceptibility testing in their laboratories;
- 75 (76.5%) do not perform NTM susceptibility testing on-site.

We also asked if the laboratories referred isolates of NTM that they did not test in-house to another laboratory for susceptibility testing. Of the respondents:

- 65 (66.3%) reported sending NTM isolates to another laboratory for susceptibility testing;
- 17 (17.3%) did not send NTM to another laboratory; and
- 16 (16.3%) laboratories reported “not applicable”.

## Antituberculous Drugs Used in the United States

The table below lists the first-line and second-line drugs used in the United States (U.S.).

**Table 1: Antituberculosis Drugs<sup>5</sup>**

<b>First-line drugs</b>	<b>Second-line Drugs</b>
Isoniazid (INH) Rifampin (RIF) Pyrazinamide (PZA) Ethambutol (EMB) Rifabutin (RBT) Rifapentine (RPT)	Streptomycin (SM) Cycloserine (CS) p-Aminosalicylic acid (PAS) (not available in the U.S.) Ethionamide (ETH) Amikacin (AMK)/Kanamycin (KM)* Capreomycin (CPM) Levofloxacin (LEV)* Moxifloxacin (MOX)*
	*Not approved by the FDA for treatment of tuberculosis.

Other drugs tested and reported by participants are:

- Azithromycin (AZM)
- Ciprofloxacin (CPR)
- Clarithromycin (CLM)
- Clofazimine (CLM)
- Ofloxacin (OFX)

These drugs are not approved by the FDA for treatment of tuberculosis.

## Antituberculous Drugs Used by Participants

Of the 98 participant laboratories,

- 83 (84.7%) reported testing all four first-line drugs (INH, RIF, EMB, and PZA).
- 15 (15.3%) tested INH, RIF, and EMB; but not PZA. (To ensure that clinicians are provided with comprehensive information regarding this multidrug regimen, initial isolates from all patients should be tested for susceptibility to all four agents.)

In addition to first-line drugs mentioned above,

- 53 (54.1%) laboratories tested SM as the only second-line drug. It is unclear as to whether SM is part of the routine first-line drug panel in these laboratories. Laboratories should note that current recommendations do not include SM as a first-line drug.<sup>6</sup>
- 34 (34.7%) tested at least one second-line drug (other than SM).
- 30 (30.6%) tested at least one injectable drug (KM, AMK, CM) and one fluoroquinolone; and
- 10 (10.2%) tested all 3 injectable drugs and a fluoroquinolone.
- 11 (11.2%) did not test any second-line drugs, including SM.

*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 patient isolates for the *M. tuberculosis* complex. It is assumed that some of the drugs are being tested for research purpose or potential use in the few referral institutions that may treat patients with *M. tuberculosis* isolates resistant to almost all standard drugs. Laboratories should not add drugs to their testing regiment without consulting physicians having expertise in treating multidrug-resistant tuberculosis. Laboratories may contact their local TB control program for referrals of physicians with experience and expertise in treating multidrug-resistant tuberculosis.

## MTBC Isolate Susceptibility Testing Results

### Isolate O, *M. tuberculosis*-Rifampin resistant at 1.0µg/ml

Rifampin (RIF) is an important first-line drug for the treatment of TB. It is bactericidal for MTBC with a critical concentration of 1.0µg/ml for AP (on Middlebrook 7H10 and 7H11 agars) and equivalent critical concentrations for BACTEC TB460, MGIT, and VersaTREK of 2.0µg/ml, 1.0µg/ml, and 1.0µg/ml, respectively.

Of the 98 laboratories that tested this isolate against RIF:

- Resistance at the critical concentration was reported by 100% (57/57) of the laboratories that use the AP, BACTEC, and/or VersaTREK methods.
- The one laboratory that performed Hain GenoType MTBDR<sub>plus</sub> testing reported RIF resistant.
- One of 63 laboratories using the MGIT reported RIF as susceptible.

Isolate O is also resistant to rifabutin (RBT). Of the 11 laboratories that tested this isolate against RBT, one laboratory that performed AP testing found it to be susceptible.

Mutations in a defined region of an 81 base pair (bp) region of the *rpoB* gene are found in about 96% of RIF-resistant MTBC isolates.<sup>6</sup> Molecular analysis of the 81 bp region in isolate O shows a nucleotide change (CAC>TAC) with the corresponding amino acid change (His526Tyr). This genotypic change is associated with phenotypic RIF resistance.

**Table 2**, on page 12, contains all results reported by all laboratories for isolate O.

**Table 2: Participant Results for Isolate O, *M. tuberculosis*-resistant to rifampin at 1.0µg/ml and rifabutin (shaded rows indicate critical concentrations for test method)**

DRUG	Conc	Test Method											
		AP Results			BACTEC Results			MGIT Results			VersaTREK Results		
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.10	1		1	24		24	61	1	62	5		5
Isoniazid	0.20	25		25	1		1						
Isoniazid	0.40				9		9	26		26	5		5
Isoniazid	1.00	25		25	1		1	1		1			
Isoniazid	2.00				1		1						
Isoniazid	5.00	3		3									
Rifampin	1.00		27	27		3	3	1	62	63		5	5
Rifampin	2.00					25	25						
Rifampin	5.00		3	3									
Rifampin	5.00					2	2						
Rifampin	10.00					1	1						
Pyrazinamide	100.00				18	1	19	59		59			
Pyrazinamide	300.00				1		1				3	2	5
Pyrazinamide	900.00				1		1						
Ethambutol	2.50				22		22						
Ethambutol	5.00	22		22	4		4	63	1	64	5		5
Ethambutol	7.50	2		2	3		3	1		1			
Ethambutol	8.00										5		5
Ethambutol	10.00	12		12									
Streptomycin	1.00							46	5	51			
Streptomycin	2.00	25	1	26	23		23						
Streptomycin	4.00	1		1				11		11			
Streptomycin	6.00				3		3						
Streptomycin	10.00	21		21									
Ethionamide	2.50				2		2						
Ethionamide	5.00	23		23	3		3	1		1			
Ethionamide	10.00	3		3									
Kanamycin	2.50				1		1						
Kanamycin	5.00	9		9	1		1						
Kanamycin	6.00	13		13									
Capreomycin	2.50				2		2						
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	2		2									
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	5		5									

**Table 2. continued: Results for Isolate O, *M. tuberculosis*, resistant to Rifampin at 1.0µg/ml and Rifabutin**

DRUG	Conc	Test Method														
		AP Results			BACTEC Results			MGIT Results			VersaTREK Results					
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum			
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	2		2												
Amikacin	5.00	1		1	1		1									
Amikacin	6.00	7		7												
Amikacin	8.00				1		1									
Amikacin	12.00	1		1												
Ofloxacin	1.00	3		3	1		1									
Ofloxacin	1.25				1		1									
Ofloxacin	2.00	14		14	4		4									
Ofloxacin	4.00	1		1	1		1									
Ofloxacin	8.00				1		1									
Clarithromycin	3.00		1	1												
Clofazimine	0.06				1		1									
Clofazimine	0.12				1		1									
Clofazimine	0.25				1		1									
Clofazimine	0.50				2		2									
Clofazimine	1.00	2		2												
Rifabutin	0.25						1	1								
Rifabutin	0.50		5	5			2	2								
Rifabutin	1.00		3	3			1	1								
Rifabutin	2.00	1	5	6												
Ciprofloxacin	1.00	2		2	1		1									
Ciprofloxacin	1.25				1		1									
Ciprofloxacin	2.00	7		7	2		2									
Ciprofloxacin	2.50				1		1									
Ciprofloxacin	4.00				1		1									
Levofloxacin	1.00	1		1												
Levofloxacin	1.50								1		1					
Levofloxacin	2.00				4		4									
Levofloxacin	8.00				1		1									
Moxifloxacin	0.50	1		1												
Moxifloxacin	1.00	2		2												
Azithromycin	3.00		1	1												

## Isolate P, *M. tuberculosis*-Isoniazid resistant at 2.0µg/ml and 1.0µg/ml; and Ethambutol resistant at 5.0µg/ml

### Isoniazid

Isoniazid is the most widely used first-line TB drug with a critical concentration of 0.2µg/ml for AP (on Middlebrook 7H10 and 7H11 agars) and equivalent critical concentrations for BACTEC TB460, MGIT, and VersaTREK of 0.1µg/ml, 0.1µg/ml, and 0.4µg/ml, respectively. It is recommended that, in addition to the critical concentration, a higher concentration of INH be tested, especially when resistance to the critical concentration is detected. Results from this higher concentration may provide information on the level of INH resistance and in turn, this information may be useful to the clinician for making treatment decisions.

Of the 98 laboratories that tested this isolate against INH:

- 33 tested INH at the critical concentration only;
- 63 tested at the critical concentration and the higher concentration;
- 2 did not test at the critical concentration, but did test the higher concentration.

It is recommended that if only one concentration is tested, it should be the lower, critical concentration of INH.

Results for INH:

- 49 (100%) of the participants using AP and BACTEC methods reported INH resistance.
- Of the 62 laboratories reporting MGIT results, one laboratory reported susceptible at the critical concentration of 0.1µg/ml.
- 5 (100%) of the participants using VersaTREK method reported INH resistance.  
The one participant using Hain Genotype method reported INH resistance.

Mutation in *katG* is the main mechanism of INH resistance and is associated with high-level resistance. Resistance to INH can also occur by mutations in *inhA* or its promoter region. These mutations are associated with low-level resistance. Other mechanisms of INH resistance are less frequent. Molecular analysis of the *inhA* (promoter region) and *katG* (Ser315 codon) loci in isolate P showed a Ser315Thr mutation in *katG*, and *inhA* was wild-type.

### Ethambutol

Ethambutol is a first-line drug that is used in combination with INH, RIF, and PZA to prevent emergence of drug resistance. Critical concentrations for testing are 5.0µg/ml, 2.5µg/ml, 5.0µg/ml, and 5.0µg/ml for AP, BACTEC, MGIT, and VersaTREK, respectively.

Resistance at the critical concentrations was reported by 97.7% (42/43) of participants using AP and BACTEC.

Of the 63 laboratories testing at the critical concentration using MGIT:

- 26 reported susceptible;
- 37 detected resistance.

False susceptibility was reported by 41.3% (26/63) of the MGIT users.

**Table 3**, on page 15 contains all susceptibility testing results reported by all participants.

**Table 3: Participant Results for culture P, *M. tuberculosis*, resistant to isoniazid at 0.2µg/ml and to ethambutol at 5.0µg/ml**

DRUG	Conc	Test Method											
		AP Results			BACTEC Results			MGIT Results			VersaTREK Results		
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.10		1	1		24	24	1	61	62		5	5
Isoniazid	0.20		25	25		2	2						
Isoniazid	0.40					9	9	1	32	33		5	5
Isoniazid	1.00		26	26		2	2		1	1			
Isoniazid	2.00					2	2						
Isoniazid	5.00	1	2	3	1		1						
Rifampin	1.00	27		27	3		3	62	1	63	5		5
Rifampin	2.00				24		24						
Rifampin	5.00	3		3									
Rifampin	5.00				1		1						
Pyrazinamide	100.00				20		20	51	8	59			
Pyrazinamide	300.00				1		1				2	3	5
Pyrazinamide	900.00				1		1						
Ethambutol	2.50				1	21	22						
Ethambutol	5.00		21	21		5	5	26	37	63	1	4	5
Ethambutol	7.50		2	2		4	4	1		1			
Ethambutol	8.00										5		5
Ethambutol	10.00	7	6	13		1	1						
Streptomycin	1.00							50	1	51			
Streptomycin	2.00	26		26	23		23						
Streptomycin	4.00	1		1				10	1	11			
Streptomycin	6.00				3		3						
Streptomycin	10.00	21		21									
Ethionamide	1.25					1	1						
Ethionamide	2.50				1	1	2						
Ethionamide	5.00	22		22	2		2	1		1			
Ethionamide	10.00	3		3									
Kanamycin	2.50				1		1						
Kanamycin	5.00	9		9	2		2						
Kanamycin	6.00	13		13									
Capreomycin	1.25				1		1						
Capreomycin	2.50				2		2						
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	2		2									
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	5		5									

**Table 3 continued: Participant Results for isolate P, *M. tuberculosis*, resistant to isoniazid at 0.2µg/ml and to ethambutol at 5.0µg/ml**

DRUG	Conc	Test Method														
		AP Results			BACTEC Results			MGIT Results			VersaTREK Results					
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum			
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	2		2												
Amikacin	5.00	1		1	1		1									
Amikacin	6.00	6		6												
Amikacin	8.00				1		1									
Amikacin	12.00	1		1												
Ofloxacin	1.00	3		3	1		1									
Ofloxacin	1.25				1		1									
Ofloxacin	2.00	14		14	5		5									
Ofloxacin	4.00	1		1	1		1									
Ofloxacin	8.00				1		1									
Clofazimine	0.06				1		1									
Clofazimine	0.12				1		1									
Clofazimine	0.25				1		1									
Clofazimine	0.50				3		3									
Clofazimine	1.00	2		2												
Rifabutin	0.50	5		5	1		1									
Rifabutin	1.00	3		3	1		1									
Rifabutin	2.00	6		6												
Ciprofloxacin	1.00	2		2	1		1									
Ciprofloxacin	2.00	7		7	2		2									
Ciprofloxacin	2.50				1		1									
Ciprofloxacin	4.00				1		1									
Levofloxacin	1.00	1		1												
Levofloxacin	1.50							1		1						
Levofloxacin	2.00				4		4									
Levofloxacin	8.00				1		1									
Moxifloxacin	0.50	1		1												
Moxifloxacin	1.00	2		2												

## Isolate Q, *M. tuberculosis*-fully susceptible to first-line drugs; resistant to kanamycin at 5.0µg/ml, capreomycin at 10.0µg/ml, and to amikacin at 5.0µg/ml First-line drugs

For the first-line drugs (INH, RIF, PZA, EMB):

- There was 100% agreement by laboratories that used BACTEC.
- One laboratory using AP reported resistance to EMB at 5.0µg/ml.
- Two laboratories using MGIT reported resistance:
  - 1 reported resistance to INH at 0.1µg/ml;
  - 1 reported resistance to EMB at 5.0µg/ml.

### Capreomycin

Seventeen laboratories tested CPM:

- 15 laboratories used AP as their test method,
- 1 used BACTEC TB460, and
- 1 used MGIT.

All were reported as resistant.

### Kanamycin

Nineteen laboratories tested KM: All 19 used AP as their test method; and 18 reported as resistant.

### Amikacin

Twelve laboratories reported results for AMK (2 laboratories tested AMK and >1 concentration):

- 10 laboratories used AP as their test method,
- 1 used BACTEC TB460, and
- 1 used MGIT.

All were reported as resistant.

Three laboratories tested AMK, CPM, or KM for strains O and P. This was probably reflex testing of second-line drugs due to the presence of first-line drug resistance. It would be prudent for laboratories to test all MPEP isolates against all challenge isolates in order to evaluate proficiency of detecting second-line drug resistance. (NOTE: The challenge isolate panel will never contain MDR-TB or RIF-R TB that also has resistance to injectable drugs and/or fluoroquinolone.)

**Table 4**, on page 18 contains all susceptibility results for isolate Q reported by all participants.

**Table 4: Participant Results for isolate Q, *M. tuberculosis*, susceptible to first-line drugs; resistant to kanamycin at 5.0µg/ml, capreomycin at 10.0µg/ml and to amikacin at 5.0µg/ml**

DRUG	Conc	Test Method											
		AP Results			BACTEC Results			MGIT Results			VersaTREK Results		
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.10	1		1	24		24	60	1	61	5		5
Isoniazid	0.20	21		21	1		1						
Isoniazid	0.40				7		7	26		26	5		5
Isoniazid	1.00	22		22	1		1	1		1			
Isoniazid	5.00	3		3									
Rifampin	0.00										1		1
Rifampin	1.00	23		23	3		3	63		63	5		5
Rifampin	2.00				24		24						
Rifampin	5.00	2		2									
Pyrazinamide	100.00				20		20	59		59			
Pyrazinamide	300.00				1		1				3	2	5
Pyrazinamide	900.00				1		1						
Ethambutol	2.50				22		22						
Ethambutol	5.00	18	1	19	4		4	63	1	64	5		5
Ethambutol	7.50	1		1	3		3	1		1			
Ethambutol	8.00										5		5
Ethambutol	10.00	11		11									
Streptomycin	1.00							51		51			
Streptomycin	2.00	22		22	22		22						
Streptomycin	4.00	1		1				11		11			
Streptomycin	6.00				3		3						
Streptomycin	10.00	19		19									
Ethionamide	5.00	18		18	1		1	1		1			
Ethionamide	10.00	3		3									
Kanamycin	5.00		7	7									
Kanamycin	6.00	1	11	12									
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									
p-Aminosalicylic acid	2.00	12		12									
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	4		4									
Amikacin	1.00		1	1									
Amikacin	1.50								1	1			
Amikacin	2.00		1	1		1	1						
Amikacin	4.00		2	2									
Amikacin	5.00		1	1									
Amikacin	6.00		6	6									
Amikacin	8.00					1	1						
Amikacin	12.00		1	1									

**Table 4 continued: Participant Results for isolate Q, *M. tuberculosis*, susceptible to first-line drugs; resistant to kanamycin at 5.0µg/ml, capreomycin at 10.0µg/ml and to amikacin at 5.0µg/ml**

DRUG	Conc	Test Method												
		AP Results			BACTEC Results			MGIT Results			VersaTREK Results			
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum	
Ofloxacin	1.00	2		2										
Ofloxacin	2.00	13		13	2		2							
Ofloxacin	4.00	1		1	1		1							
Ofloxacin	8.00				1		1							
Clofazimine	0.06					1	1							
Clofazimine	0.12					1	1							
Clofazimine	0.25					1	1							
Clofazimine	0.50					1	1							
Clofazimine	1.00	2		2										
Rifabutin	0.50	5		5										
Rifabutin	1.00	3		3										
Rifabutin	2.00	5		5										
Ciprofloxacin	1.00	1		1										
Ciprofloxacin	2.00	7		7	1		1							
Ciprofloxacin	4.00				1		1							
Levofloxacin	1.00	1		1										
Levofloxacin	1.50							1		1				
Levofloxacin	2.00					2	2							
Levofloxacin	8.00					1	1							
Moxifloxacin	0.50	1		1										
Moxifloxacin	1.00	2		2										

## Isolate R, *M. tuberculosis*-fully susceptible to first-line and second-line drugs

### First-line drugs

All participants tested this isolate against INH, RIF, and EMB; 83 laboratories tested PZA. There was 100% agreement among laboratories that used the AP, BACTEC, and VersaTREK methods.

Of the 64 laboratories that used MGIT, there was 96.9% (62/64) agreement among laboratories. Two different laboratories reported resistance to EMB at 5.0µg/ml.

### Second-line drugs

Of the laboratories that tested second-line drugs [AMK(15), CM(25), CS(9), LEV(6), MOX(3), PAS(17), SM(84) and ETH(33)], there was 100% agreement.

**Table 5** on page 21 contains all susceptibility results for isolate R reported by all participants.

**Table 5: Isolate R, *M. tuberculosis* susceptibility to first-line and second-line drugs**

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	0.10	1		1	24		24	62		62	5		5
Isoniazid	0.20	20		20	1		1						
Isoniazid	0.40				7		7	26		26	5		5
Isoniazid	1.00	21		21	1		1	1		1			
Isoniazid	5.00	3		3									
Rifampin	1.00	22		22	3		3	63		63	5		5
Rifampin	2.00				24		24						
Rifampin	5.00	2		2									
Pyrazinamide	100.00				20		20	59		59			
Pyrazinamide	300.00				1		1				5		5
Pyrazinamide	900.00				1		1						
Ethambutol	2.50				21		21						
Ethambutol	5.00	18		18	4		4	62	2	64	5		5
Ethambutol	7.50	1		1	3		3	1		1			
Ethambutol	8.00										5		5
Ethambutol	10.00	10		10									
Streptomycin	1.00							50		50			
Streptomycin	2.00	21		21	22		22						
Streptomycin	4.00	1		1				10		10			
Streptomycin	6.00				3		3						
Streptomycin	10.00	19		19									
Ethionamide	5.00	16		16	1		1	1		1			
Ethionamide	10.00	3		3									
Kanamycin	5.00	8		8									
Kanamycin	6.00	11		11									
Capreomycin	3.00							1		1			
Capreomycin	5.00				1		1						
Capreomycin	10.00	15		15									
Cycloserine	25.00	1		1									
Cycloserine	30.00	5		5									
Cycloserine	50.00	1		1									
Cycloserine	60.00	1		1									
p-Aminosalicylic acid	2.00	12		12									
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	4		4									
Amikacin	1.00	1		1									
Amikacin	1.50							1		1			
Amikacin	2.00	1		1	1		1						
Amikacin	4.00	2		2									
Amikacin	5.00	1		1									
Amikacin	6.00	6		6									
Amikacin	8.00				1		1						
Amikacin	12.00	1		1									

**Table 5 continued: Isolate R, *M. tuberculosis* susceptibility to first-line and second-line drugs**

DRUG	Conc	Test Method												
		AP Results			BACTEC Results			MGIT Results			VersaTREK Results			
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum	
Ofloxacin	1.00	2		2										
Ofloxacin	2.00	13		13	2		2							
Ofloxacin	4.00	1		1	1		1							
Ofloxacin	8.00				1		1							
Clofazimine	0.06					1	1							
Clofazimine	0.12				1		1							
Clofazimine	0.25				1		1							
Clofazimine	0.50				1		1							
Clofazimine	1.00	2		2										
Rifabutin	0.50	5		5										
Rifabutin	1.00	3		3										
Rifabutin	2.00	5		5										
Ciprofloxacin	1.00	1		1										
Ciprofloxacin	2.00	6		6	1		1							
Ciprofloxacin	4.00				1		1							
Levofloxacin	1.00	1		1										
Levofloxacin	1.50							1		1				
Levofloxacin	2.00				2		2							
Levofloxacin	8.00				1		1							
Moxifloxacin	0.50	1		1										
Moxifloxacin	1.00	2		2										

## Isolate S, *M. tuberculosis* -fully susceptible to the first-line; resistant to ofloxacin at 2.0µg/ml and resistant to ciprofloxacin at 2.0µg/ml

All laboratories using AP, BACTEC, and/or VersaTREK reported this isolate to be fully susceptible to the first-line drugs at all concentrations tested. However, of the 62 laboratories using MGIT:

- Three reported resistance to INH at 0.1µg/ml;
- One reported resistance to INH at 0.4µg/ml; and
- One reported resistance to PZA at 100µg/ml.

All laboratories that tested ofloxacin and ciprofloxacin reported resistance.

**Table 6** on page 24 contains the susceptibility results submitted by all participants.

**Table 6: Isolate S, *M. tuberculosis* fully susceptibility to first-line drugs; resistant to ofloxacin at 2.0µg/ml and resistant to ciprofloxacin at 2.0µg/ml**

DRUG	Conc	Test Method											
		AP Results			BACTEC Results			MGIT Results			VersaTREK Results		
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.10	1		1	24		24	59	3	62	5		5
Isoniazid	0.20	20		20	1		1						
Isoniazid	0.40				7		7	25	1	26	5		5
Isoniazid	1.00	21		21	1		1	1		1			
Isoniazid	5.00	3		3									
Rifampin	0.00										1		1
Rifampin	1.00	22		22	3		3	63		63	5		5
Rifampin	2.00				24		24						
Rifampin	5.00	2		2									
Pyrazinamide	100.00				19	1	20	58	1	59			
Pyrazinamide	300.00				1		1				5		5
Pyrazinamide	900.00				1		1						
Ethambutol	2.50				22		22						
Ethambutol	5.00	18		18	4		4	64		64	5		5
Ethambutol	7.50	1		1	3		3	1		1			
Ethambutol	8.00										5		5
Ethambutol	10.00	10		10									
Streptomycin	1.00							51		51			
Streptomycin	2.00	21		21	22		22						
Streptomycin	4.00	1		1				11		11			
Streptomycin	6.00				3		3						
Streptomycin	10.00	19		19									
Ethionamide	5.00	18		18	1		1	1		1			
Ethionamide	10.00	3		3									
Kanamycin	5.00	8		8									
Kanamycin	6.00	11		11									
Capreomycin	3.00							1		1			
Capreomycin	5.00				1		1						
Capreomycin	10.00	15		15									
Cycloserine	25.00	1		1									
Cycloserine	30.00	5		5									
Cycloserine	50.00	1		1									
Cycloserine	60.00	1		1									
p-Aminosalicylic acid	2.00	12		12									
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	4		4									
Amikacin	1.00	1		1									
Amikacin	1.50							1		1			
Amikacin	2.00	1		1	1		1						
Amikacin	4.00	2		2									
Amikacin	5.00	1		1									
Amikacin	6.00	6		6									
Amikacin	8.00				1		1						
Amikacin	12.00	1		1									

**Table 6 continued:** Isolate S, *M. tuberculosis* fully susceptibility to first-line drugs; resistant to ofloxacin at 2.0µg/ml and resistant to ciprofloxacin at 2.0µg/ml

DRUG	Conc	Test Method														
		AP Results			BACTEC Results			MGIT Results			VersaTREK Results					
		S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum			
Ofloxacin	1.00		2	2												
Ofloxacin	2.00		13	13		2	2									
Ofloxacin	4.00		1	1		1	1									
Ofloxacin	8.00					1	1									
Clofazimine	0.06					1	1									
Clofazimine	0.12					1	1									
Clofazimine	0.25					1	1									
Clofazimine	0.50					1		1								
Clofazimine	1.00	2		2												
Rifabutin	0.50	5		5												
Rifabutin	1.00	3		3												
Rifabutin	2.00	5		5												
Ciprofloxacin	1.00		1	1												
Ciprofloxacin	2.00		6	6		1	1									
Ciprofloxacin	4.00					1	1									
Levofloxacin	1.00		1	1												
Levofloxacin	1.50							1	1							
Levofloxacin	2.00					2	2									
Levofloxacin	8.00					1		1								
Moxifloxacin	0.50		1	1												
Moxifloxacin	1.00		2	2												

## References

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## Acknowledgements

Special thanks to the following persons for assisting with the production of this report: Sandra Neal, M.S., CDC/Atlanta, Beverly Metchock, PhD, CDC/Atlanta, Jagdeep Bedi, CDC contractor (Northrop Grumman); Charlene Smith, M.S., CDC/Atlanta; Suzette Brown, M.S.CDC/Atlanta; Pamela Robinson, CDC/Atlanta, Sharon Granade, M.S., CDC/Atlanta.