Centers for Disease Control and Prevention

Model Performance Evaluation Program

Mycobacterium tuberculosis

Drug Susceptibility Testing Program

Report of Results

November 2012

Performance Evaluation Survey

United States Department of Health and Human Services

MTB DST Report for November 2012 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* drug susceptibility testing survey sent to participants in November 2012.
Report Content	The material in this report was developed and prepared by Cortney Stafford, MPH, MT (ASCP), Health Scientist, Laboratory Capacity Team, NCHHSTP, DTBE, LB Beverly Metchock, DrPH, D(ABMM), Team Lead, Reference Laboratory, NCHHSTP, DTBE, LB Acknowledged contributors: Lois Diem CDC/Atlanta, Mitch Yakrus CDC/Atlanta, Angela Starks CDC/Atlanta
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M. tuberculosis DST Report for November 2012 Samples Survey

Introduction: Overview of MPEP Final Report

This aggregate report is prepared in a format that will allow laboratories to compare their results with those obtained by other participants using the same methods, drug, and drug concentration, by isolate. We encourage circulation of this report to personnel who are involved with drug susceptibility testing (DST), reporting, or interpreting for *M. tuberculosis* complex isolates.

MPEP is not a formal, graded proficiency testing program. It is an educational, self-assessment tool for laboratories to monitor their ability to test for drug-resistant isolates of *M. tuberculosis* complex. This report includes results for a subset of laboratories performing DST for *M. tuberculosis* complex in the United States. MPEP is a voluntary program and the report reflects data received from those that have chosen to participate.

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-Second Edition," M24-A2 (ISBN 1-56238-746-4) [1].

Expected Susceptibility Testing Results

The table below provides the intended results of the panel shipment that was sent to participants in November 2012. Although CDC recommends broth-based methods for routine first-line drug susceptibility testing of *M. tuberculosis* complex, this table provides the results obtained by the reference agar proportion method, except in the case of pyrazinamide, where MGIT was the testing method.

	First-line Drugs				Second-line Drugs
	INH	**RMP**	**EMB**	**PZA**	**Resistant to:**
2012F	R	S	S	S	STR
2012G	S	S	S	S	none
2012H	S	S	S	S*	STR
2012I	S	R	R*	S*	none
2012J	S	S	S	S	none

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

Descriptive Information about Participant Laboratories

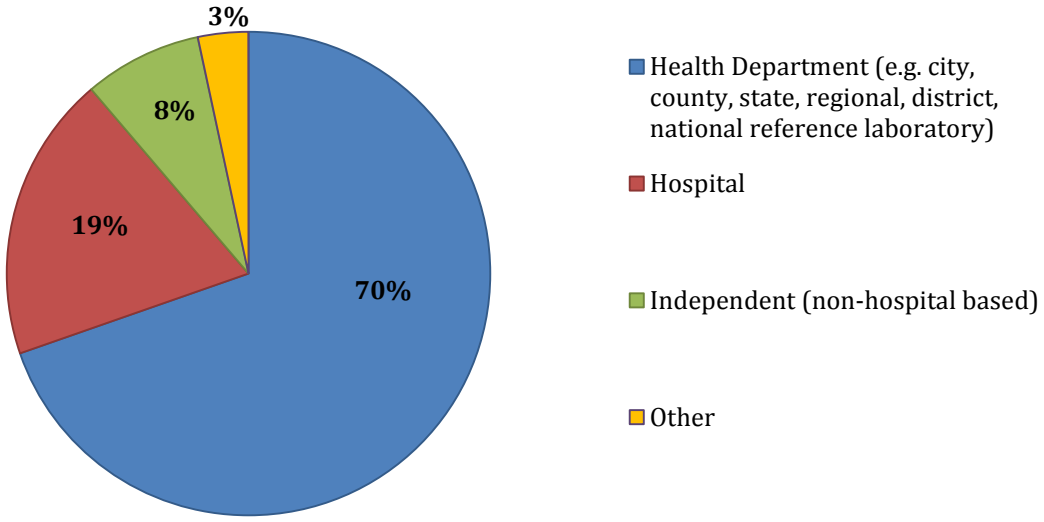
Primary Classification

This report contains the drug susceptibility testing results submitted to CDC by 89 laboratories in 43 states.

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

- 62 (70%): Health Department (city, country, state, regional, district, or national reference laboratory)
- 17 (19%): 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]
- 7 (8%): Independent [e.g., commercial, commercial manufacturer of reagents, HMO* satellite clinic, reference laboratory (non-governmental affiliated)]
- 3 (3%): Other [Federal government research (nonmilitary)].

Figure 1. Primary Classification of Participating Laboratories

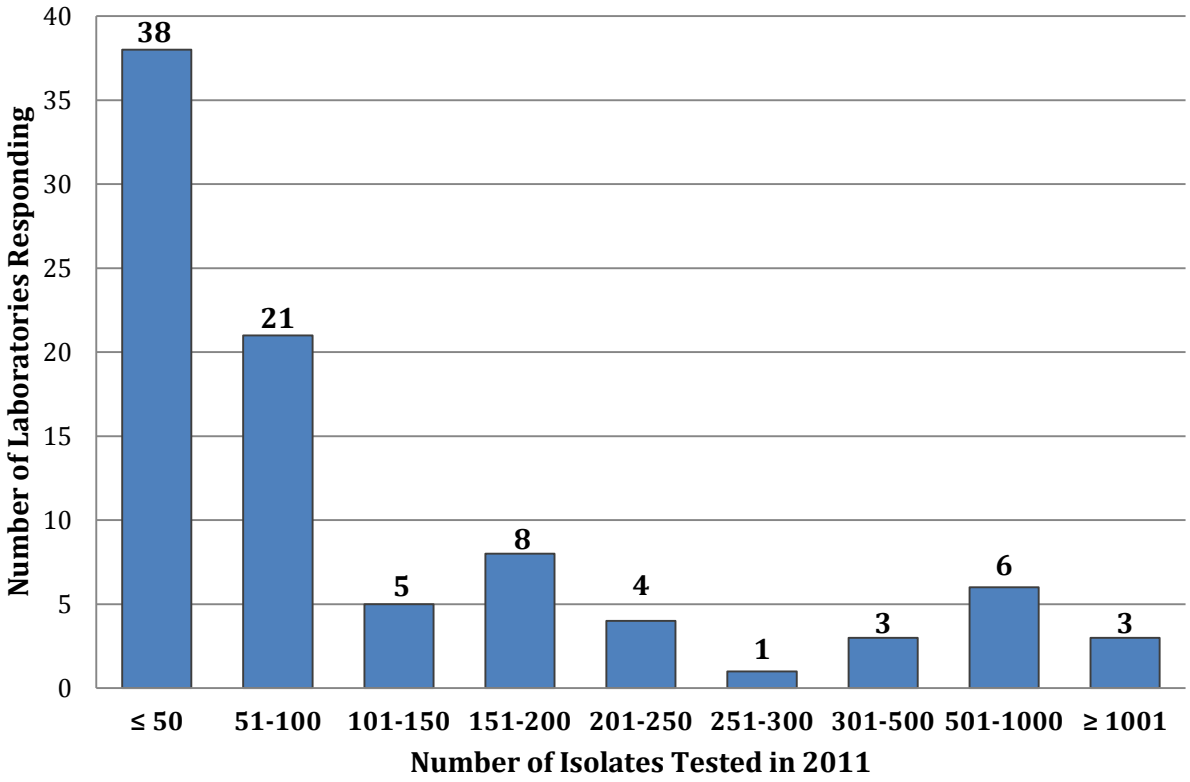


*HMO: Health maintenance organization; PPO: Preferred provider organization

Annual Number of *M. tuberculosis* Drug Susceptibility Tests Performed

Figure 2 shows the number of drug susceptibility tests performed on *M. tuberculosis* isolates by the 89 participants in one calendar year, January 1 through December 31, 2011 (excluding quality control isolates). The counts ranged from three to 1,536 tests. Thirty-eight (43%) laboratories reported performing less than or equal to 50 drug susceptibility tests per year. To ensure testing proficiency, these laboratories with low volumes are encouraged to consider referral of *M. tuberculosis* drug susceptibility testing [2].

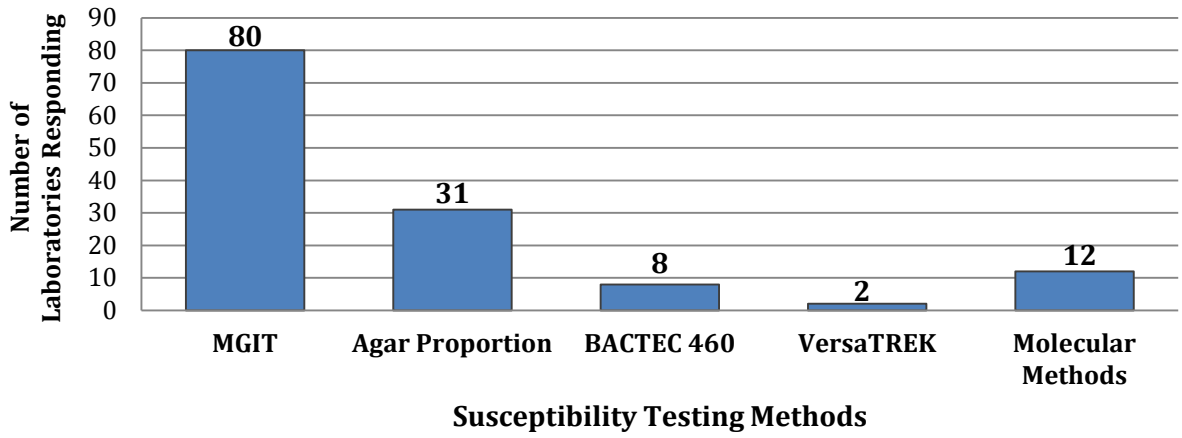
Figure 2. Annual Volume of *M. tuberculosis* Isolates Tested for Drug Susceptibility in 2011 (N=89)



Laboratory Susceptibility Testing Procedures Used

Participants were asked to report all drug susceptibility testing methods that were used for these isolates. Fifty-two (58%) laboratories used only one method. Thirty laboratories utilized two methods and 7 laboratories used three susceptibility methods. Molecular methods include: Laboratory Developed Tests (7 laboratories), Cepheid Xpert® MTB/RIF (4 laboratories), and Genotype® MTBDR*sl*/Genotype® MTBDR*plus* (1 laboratory).

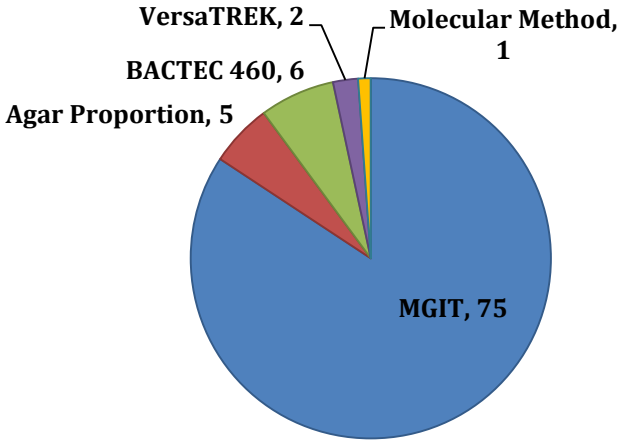
Figure 3. Susceptibility Testing Methods Reported (N=133)



Initial *M. tuberculosis* Susceptibility Testing Method Used by Participants

Participants were asked to indicate the initial *M. tuberculosis* susceptibility test method used by their laboratory for the isolates in the November 2012 shipment. Instructions were to select only one method as their initial method.

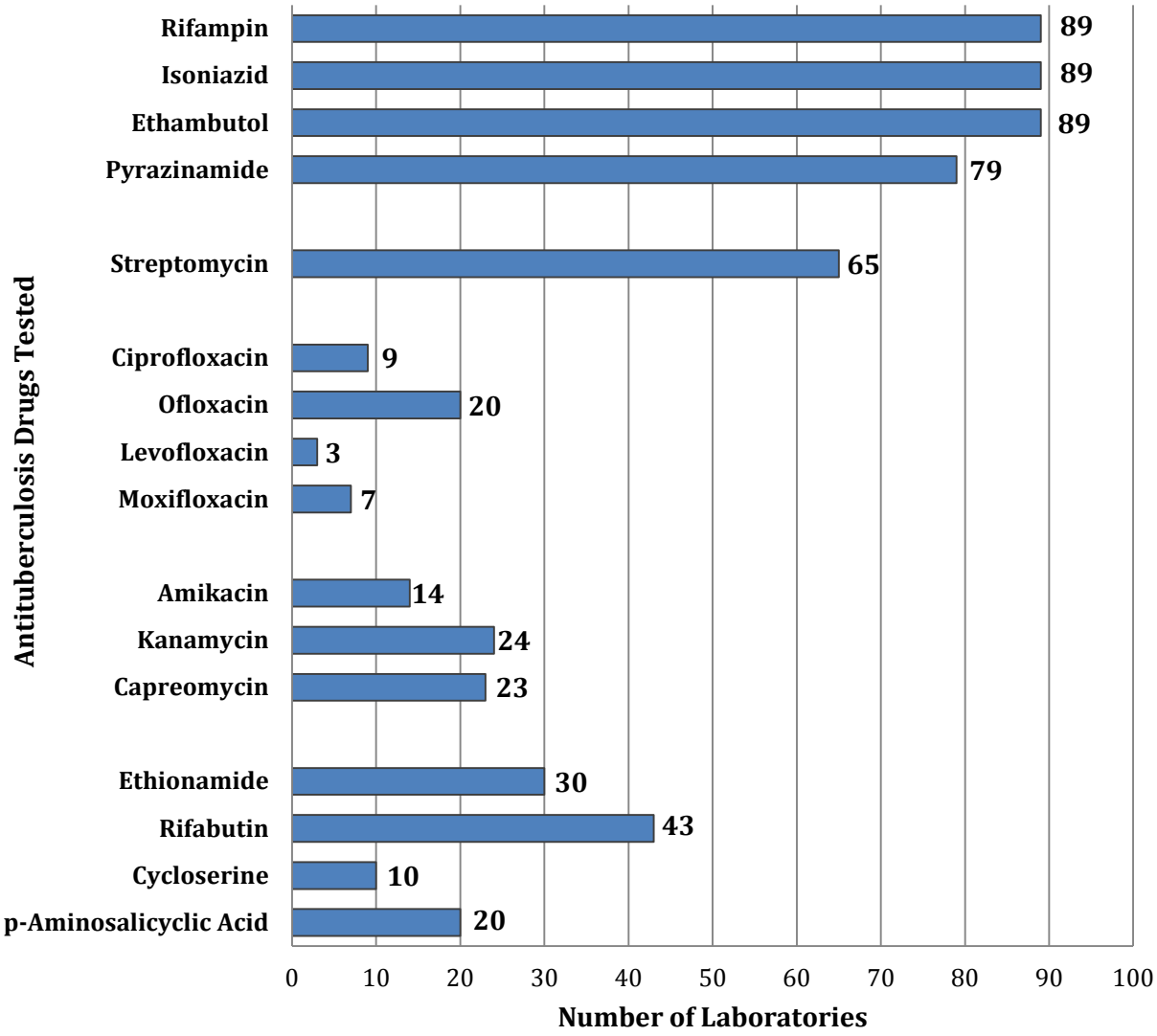
Figure 4. Initial *M. tuberculosis* Susceptibility Test Method Used (N=89)



Antituberculosis Drugs Tested

CLSI recommends a full panel of first-line drugs (isoniazid [INH], rifampin [RMP], 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 therapy currently recommended for treatment of most patients in the United States with tuberculosis. All participants reported results for three of the first-line drugs—INH, RMP, and EMB; 79 (89%) of the participants also reported results for PZA.

Figure 5. Antituberculosis Drugs Tested



Basic Information for Isolates F-J

This section provides the complete set of data in tabulated format for the *M. tuberculosis* isolates F, G, H, I, and J from the November 2012 survey. The following information pertains to all the tables.

- First-line and second-line drugs have been separated into individual tables for each isolate. Streptomycin is now included as part of the second-line table.
- Laboratories that use more than one DST method are encouraged to test isolates with each of those methods at the CLSI-recommended, or equivalent, critical concentrations. Also, some laboratories provided results for additional drug concentrations. Consequently, the number of results for some drugs may be greater than 89 (the number of participating laboratories). This report contains all results reported by participating laboratories, including drug concentrations with only one result.
- The tables indicate the number of reported results (S represents Susceptible and R represents Resistant) for each drug at the noted concentration.
- Separate tables for molecular testing are included where data is of note; otherwise findings are reported in the summary. If results are not provided for molecular tests, laboratories did not detect a mutation.
- A list of critical concentrations for antituberculosis drugs, by method, can be found at the end of this report.
- Of the 31 laboratories reporting second-line drug results (with the exception of streptomycin), only 9 (29%) tested the three second-line injectables and one fluoroquinolone needed to define XDR TB.

Isolate F

Expected Result: Resistant to isoniazid at 0.2 µg/ml and streptomycin at 2 µg/ml by agar proportion

Isoniazid

Isoniazid is the most widely used first-line antituberculosis drug. It is a cornerstone of all effective regimens for the treatment of TB disease and latent infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme KatG encoded by the *kat*G gene [3, 4]. 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 [3, 4]. The most common mechanism, mutations in *kat*G, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the *inh*A gene which are generally associated with low-level resistance. DNA sequence analysis of *inh*A and *kat*G of Isolate F revealed no mutations; this is known to occur in approximately 10-15% of isolates found to be INH resistant.

106 results were reported for INH for this isolate. As noted in the Basic Information, laboratories may provide results for more than one method. This isolate was reported **resistant** to INH at the critical concentration by method(s), as follows

- 96% (25/26) of the results when using AP;
- 100% (4/4) of the results when using BACTEC™ 460;
- 100% (74/74) of the results when using MGIT™;
- 100% (2/2) of the results when using VersaTREK®.

Five laboratories reported results for molecular methods; none reported INH resistance.

66 (94%) results were reported as susceptible at the higher concentrations of INH.

Streptomycin

78 results for SM were reported for this isolate. This isolate was reported as **resistant** to SM at these critical concentrations by method, as follows

- 89% (25/28) of the results when using AP;
- 100% (4/4) of the results when using BACTEC™ 460; and
- 98% (45/46) of the results when using MGIT™.

See Tables 1 and 2 for the complete results submitted by all participants for Isolate 2012F.

Table 1. Isolate F—Participant results for first-line drug susceptibility testing

Results by Method for First-Line Drugs													
		AP			BACTEC 460			MGIT			VersaTREK		
Drug	**Conc. (µg/ml)**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Rifampin	1.0	21	0	21	2	0	2	74	0	74	2	0	2
	2.0	1	0	1	4	0	4						
	5.0	3	0	3									
Isoniazid	0.1				0	4	4	0	74	74	0	2	2
	0.2	1	25	26				0	2	2			
	0.4				3	0	3	36	2	38	1	1	2
	1.0	26	1	27	1	0	1						
	5.0	6	0	6									
Ethambutol	2.5				4	0	4						
	5.0	16	0	16	2	0	2	75	0	75*	2	0	2
	7.5	2	0	2									
	8.0										2	0	2
	10.0	6	0	6									
Pyrazinamide	50.0				1	0	1						
	100.0				3	0	3	73	0	73**			
	200.0				1	0	1						
	300.0										1	0	1
	400.0				1	0	1						

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

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

Table 2. Isolate F—Participant results for second-line drug susceptibility testing

Results by Method for Second-Line Drugs										
		AP			BACTEC 460			MGIT		
Drug	**Conc. (µg/ml)**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Streptomycin	1.0							1	45	46
	2.0	3	25	28	0	4	4			
	4.0							10	2	12*
	6.0				0	1	1			
	10.0	24	0	24						
Ofloxacin	1.0	4	0	4						
	1.5							1	0	1
	2.0	15	0	15				2	0	2
	4.0	2	0	2						
Ciprofloxacin	1.0							3	0	3
	2.0	7	0	7						
	4.0	1	0	1						
Levofloxacin	1.0	2	0	2						
	2.0	1	0	1				1	0	1
	4.0	1	0	1						
Moxifloxacin	0.13							1	0	1
	0.25							2	0	2
	0.5	2	0	2						
	1.0	3	0	3						
	2.0	1	0	1						
	4.0	1	0	1						
Amikacin	2.0	1	0	1						
	4.0	4	0	4				1	0	1
	5.0	1	0	1						
	6.0	6	0	6						
	12.0	1	0	1						
Kanamycin	5.0	10	0	10						
	6.0	12	0	12						
Capreomycin	1.25				1	0	1			
	3.0							3	0	3
	10.0	19	0	19						
Ethionamide	1.25				0	2	2			
	2.5				1	0	1			
	5.0	15	7	22				1	0	1
	10.0	3	1	4						
Rifabutin	0.12	1	0	1						
	0.25	1	0	1	1	0	1			
	0.5	7	0	7						
	1.0	2	0	2	1	0	1			
	2.0	7	0	7						
Cycloserine	30.0	8	0	8						
	60.0	2	0	2						
p-Aminosalicylic acid	2.0	17	0	17						
	8.0	2	0	2						
	10.0	4	0	4						

* In addition, one laboratory reported borderline for streptomycin by MGIT.

Isolate G

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

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

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

See Tables 3 and 4 for the complete results submitted by all participants for Isolate 2012G.

Table 3. Isolate G—Participant results for first-line drug susceptibility testing

Results by Method for First-Line Drugs													
		AP			BACTEC 460			MGIT			VersaTREK		
Drug	**Conc. (µg/ml)**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Rifampin	1.0	21	0	21	2	0	2	76	0	76	2	0	2
	2.0				4	0	4						
	5.0	2	0	2									
Isoniazid	0.1				4	0	4	74	0	74	2	0	2
	0.2	19	1	20				2	0	2			
	0.4				3	0	3	24	0	24	2	0	2
	1.0	20	0	20	1	0	1						
	5.0	4	0	4									
Ethambutol	2.5				4	0	4						
	5.0	16	1	17	2	0	2	74	2	76	2	0	2
	7.5	2	0	2									
	8.0										2	0	2
	10.0	6	0	6									
Pyrazinamide	50.0				1	0	1						
	100.0				3	0	3	73	0	73			
	200.0				1	0	1						
	300.0										1	0	1
	400.0				1	0	1						

Table 4. Isolate G—Participant results for second-line drug susceptibility testing

Results by Method for Second-Line Drugs										
		AP			BACTEC 460			MGIT		
Drug	**Conc. (µg/ml)**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Streptomycin	1.0							41	0	41
	2.0	21	0	21	6	0	6			
	4.0	2	0	2				7	0	7
	6.0									
	10.0	18	0	18						
Ofloxacin	1.0	3	0	3						
	1.5							1	0	1
	2.0	15	0	15	2	0	2			
	4.0	2	0	2						
Ciprofloxacin	1.0							2	0	2
	2.0	5	0	5						
	4.0	1	0	1						
Levofloxacin	1.0	2	0	2						
	2.0	1	0	1						
	4.0	1	0	1						
Moxifloxacin	0.25							1	0	1
	1.0	3	0	3						
	2.0	1	0	1						
	4.0	1	0	1						
Amikacin	2.0	1	0	1						
	4.0	4	0	4				1	0	1
	5.0	1	0	1						
	6.0	5	0	5						
	12.0	1	0	1						
Kanamycin	5.0	9	0	9						
	6.0	9	0	9						
Capreomycin	3.0							2	0	2
	10.0	15	1	16						
Ethionamide	1.25				1	0	1			
	2.5				1	0	1			
	5.0	16	0	16				1	0	1
	10.0	4	0	4						
Rifabutin	0.12	1	0	1						
	0.25	1	0	1						
	0.5	7	0	7						
	1.0	2	0	2						
	2.0	7	0	7						
Cycloserine	30.0	6	0	6						
	60.0	1	0	1						
p-Aminosalicylic acid	2.0	12	0	12						
	8.0	2	0	2						
	10.0	3	0	3						

Isolate H

Expected Result: Resistant to streptomycin at 2 µg/ml and 10 µg/ml by agar proportion

Streptomycin

77 SM results were reported for this isolate. This isolate was reported **resistant** to SM at the critical concentration by method, as follows

- 100% (26/26) of the results when using AP;
- 100% (8/8) of the results when using BACTEC™460; and
- 98% (42/43) of the results when using MGIT™.

Pyrazinamide

This isolate was expected to be susceptible to PZA. However, of those testing PZA, **resistance** was reported by

- 0% (0/3) of the results when using BACTEC™460;
- 22% (16/74) of the results when using MGIT™; and
- 100% (1/1) of the results when using VersaTREK®.

Four laboratories reported results for molecular methods; none reported PZA resistance.

Issues with false resistance to PZA have been reported [5], and as indicated by these results, remains a potential concern.

See Tables 5 and 6 for the complete results submitted by all participants for Isolate 2012H.

Table 5. Isolate H—Participant results for first-line drug susceptibility testing

Results by Method for First-Line Drugs													
		AP			BACTEC 460			MGIT			VersaTREK		
Drug	**Conc. (µg/ml)**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Rifampin	1.0	22	0	22*	2	0	2	75	1	76	2	0	2
	2.0				4	0	4						
	5.0	3	0	3									
Isoniazid	0.1				4	0	4	74	0	74	2	0	2
	0.2	22	0	22				2	0	2			
	0.4				3	0	3	24	0	24	2	0	2
	1.0	22	0	22	1	0	1						
	5.0	5	0	5									
Ethambutol	2.5				4	0	4						
	5.0	17	0	17	2	0	2	76	0	76	2	0	2
	7.5	2	0	2									
	8.0										2	0	2
	10.0	6	0	6									
Pyrazinamide	50.0				1	0	1						
	100.0				3	0	3	57	16	73**			
	200.0				1	0	1						
	300.0										0	1	1
	400.0				1	0	1						

* In addition, one laboratory reported borderline for rifampin by agar proportion.
**In addition, one laboratory reported borderline for pyrazinamide by MGIT.

Table 6. Isolate H—Participant results for second-line drug susceptibility testing

Results by Method for Second-Line Drugs										
		AP			BACTEC 460			MGIT		
Drug	**Conc. (µg/ml)**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Streptomycin	1.0							1	42	43
	2.0	0	26	26	0	8	8			
	4.0	0	5	5				0	12	12
	6.0				0	1	1			
	10.0	0	23	23						
Ofloxacin	1.0	4	0	4						
	1.5							1	0	1
	2.0	15	0	15						
	4.0	2	0	2						
Ciprofloxacin	1.0							3	0	3
	2.0	5	0	5						
	4.0	1	0	1						
Levofloxacin	1.0	2	0	2						
	2.0	1	0	1						
	4.0	1	0	1						
Moxifloxacin	0.25							1	0	1
	1.0	3	0	3						
	2.0	1	0	1						
	4.0	1	0	1						
Amikacin	1.5							1	0	1
	2.0	1	0	1						
	4.0	4	0	4				1	0	1
	5.0	1	0	1						
	6.0	5	0	5						
	12.0	1	0	1						
Kanamycin	5.0	10	0	10						
	6.0	10	0	10						
Capreomycin	3.0							2	0	2
	10.0	16	0	16						
Ethionamide	1.25				1	0	1			
	2.5				1	0	1			
	5.0	13	2	15*				1	0	1
	10.0	4	0	4						
Rifabutin	0.12	1	0	1						
	0.25	1	0	1						
	0.5	7	0	7						
	1.0	2	0	2						
	2.0	7	0	7						
Cycloserine	30.0	8	0	8						
	60.0	2	0	2						
p-Aminosalicylic acid	2.0	14	0	14						
	8.0	2	0	2						
	10.0	3	0	3						

***** In addition, one laboratory reported borderline for ethionamide by agar proportion.

Isolate I

Expected Result: Resistant to rifampin at 1 µg/ml and ethambutol at 5 µg/ml by agar proportion

Rifampin

Rifampin 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 *M. tuberculosis* at the critical concentration of 1.0 µg/ml for AP (on Middlebrook 7H10 and 7H11 agars) and equivalent critical concentrations for BACTEC™ 460, MGIT™, and VersaTREK® of 2.0 µg/ml, 1.0 µg/ml, and 1.0 µg/ml, respectively. The mechanism of action of RMP is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase. More than 96% of RMP-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 [3, 4]. The activity of RMP in RMP-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 RMP-resistant isolates and serve as predictors of RMP resistance. DNA sequence analysis of *rpoB* in Isolate 2012I revealed a C>T point mutation in the *rpoB* locus resulting in histidine being replaced by tyrosine at codon 526 (His526Tyr).

Of the 105 results reported for RMP for this isolate, **resistance** was reported by

- 100% (24/24) of the results when using AP;
- 100% (3/3) of the results when using BACTEC™ 460;
- 100% (76/76) of the results when using MGIT™; and
- 100% (2/2) of the results when using VersaTREK®

Ten (100%) laboratories using molecular methods reported this isolate as RMP resistant.

Rifabutin

Nine laboratories tested RBT at the critical concentration of 0.5 µg/ml by AP; 100% reported resistance.

Ethambutol

Ethambutol (EMB) is an important first-line drug for the treatment of tuberculosis and is used in combination with INH, RMP, and PZA to prevent emergence of drug resistance. EMB is a

bacteriostatic agent that is active against growing bacilli and has no effect on non-replicating bacilli [3, 4]. EMB targets the arabinosyl transferases (*emb*CAB operon), thereby inhibiting the biosynthesis of the cell wall components arabinogalactan and lipoarabinomannan [6].

Culture–based methods for EMB susceptibility testing are problematic [7, 8]. Sequence analysis of EMB-resistant clinical isolates has shown that EMB resistance is associated primarily with missense mutations within the EMB resistance determining region of the gene *emb*B at codons 306, 406, and 497[6,9]. DNA sequence analysis of *emb*B of Isolate I did not reveal an *embB* mutation.

104 EMB results were reported for this isolate. This isolate was reported **resistant** to EMB by method, as follows

- 9.1% (2/22) of the results when using AP;
- 0% (0/4) of the results when using BACTEC™ 460;
- 5.3% (4/76) of the results when using MGIT™; and
- 0% (0/2) of the results when using VersaTREK®.

Pyrazinamide

As previously noted, the occurrence of false resistance to PZA in MGIT™ has been established [5]; 33 (47%) laboratories testing PZA by MGIT™ reported resistance for Isolate I.

Isoniazid

Six (8%) laboratories reported INH resistance at the critical concentration of 0.1 µg/ml by MGIT™ although resistance was not expected.

See Tables 7, 8, and 9 for the complete results submitted by all participants for Isolate 2012I.

Table 7. Isolate I—Participant results for first-line drug susceptibility testing

Results by Method for First-Line Drugs													
		AP			BACTEC 460			MGIT			VersaTREK		
Drug	**Conc. (µg/ml)**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Rifampin	1.0	0	24	24*	0	3	3	0	76	76	0	2	2
	2.0				0	3	3						
	5.0	0	4	4									
	10.0	0	1	1									
Isoniazid	0.1				4	0	4	67	6	73**	2	0	2
	0.2	24	0	24				2	0	2			
	0.4				3	0	3	25	0	25**	2	0	2
	1.0	23	0	23	1	0	1						
	5.0	5	0	5									
Ethambutol	2.5				4	0	4						
	5.0	18	2	20	2	0	2	72	4	76	2	0	2
	7.5	2	0	2									
	8.0										2	0	2
	10.0	9	0	9									
Pyrazinamide	50.0				1	0	1						
	100.0				3	0	3	37	33	70#			
	200.0				1	0	1						
	300.0										0	1	1
	400.0				1	0	1						

*In addition, one laboratory reported borderline for rifampin by agar proportion.
**In addition, one laboratory reported borderline for isoniazid at 0.1 and 0.4 by MGIT
#In addition, one laboratory reported borderline for pyrazinamide by MGIT

Table 8. Isolate I—Participant results for second-line drug susceptibility testing

Results by Method for Second-Line Drugs										
		AP			BACTEC 460			MGIT		
Drug	**Conc. (µg/ml)**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Streptomycin	1.0							41	3	44*
	2.0	23	0	23*	6	0	6			
	4.0	2	0	2				8	0	8
	10.0	20	0	20						
Ofloxacin	1.0	3	1	4						
	1.5							1	0	1
	2.0	16	0	16				3	0	3
	4.0	2	0	2						
Ciprofloxacin	2.0	6	0	6				3	0	3
	4.0	1	0	1						
Levofloxacin	1.0	2	0	2						
	2.0	1	0	1				1	0	1
	4.0	1	0	1						
Moxifloxacin	0.13							1	0	1
	0.25							2	0	2
	1.0	3	0	3						
	2.0	1	0	1						
	4.0	1	0	1						
	5.0	2	0	2						
Amikacin	2.0	1	0	1						
	4.0	4	0	4				1	0	1
	5.0	1	0	1						
	6.0	6	0	6						
	12.0	1	0	1						
Kanamycin	5.0	10	0	10						
	6.0	12	0	12						
Capreomycin	2.5							1	0	1
	10.0	18	0	18						
Ethionamide	2.5				1	0	1			
	5.0	19	0	19				3	0	3
	10.0	4	0	4						
Rifabutin	0.05	0	1	1						
	0.12	0	1	1						
	0.25	0	2	2						
	0.5	0	8	8	0	1	1			
	1.0	0	2	2				0	1	1
	2.0	0	7	7						
Cycloserine	30.0	8	0	8						
	60.0	2	0	2						
p-Aminosalicylic acid	2.0	17	0	17						
	8.0	2	0	2						
	10.0	4	0	4						

*In addition, one laboratory reported borderline for streptomycin by agar proportion and MGIT

Table 9. Isolate I—Participant results for molecular testing

	Molecular Testing		
Drug	**Mutation Detected**	**Mutation Not Detected**	**Total**
Rifampin	10	0	10
Isoniazid	0	4	4
Ethambutol	0	1	1
Pyrazinamide	1	1	2
Ethionamide	0	1	1
Rifabutin	1	0	1
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

Isolate J

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

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

Most (99.7%) results reported for this isolate indicated it was susceptible to all drugs tested by all methods.

See Tables 10 and 11 for the complete results submitted by all participants for Isolate 2011J.

Table 10. Isolate J—Participant results for first-line drug susceptibility testing

Results by Method for First-Line Drugs													
		AP			BACTEC 460			MGIT			VersaTREK		
Drug	**Conc. (µg/ml)**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Rifampin	1.0	21	0	21	3	0	3	76	0	76	2	0	2
	2.0				3	0	3						
	5.0	2	0	2									
Isoniazid	0.1				4	0	4	73	0	73	2	0	2
	0.2	20	0	20				2	1	3			
	0.4				3	0	3	24	0	24	2	0	2
	1.0	20	0	20	1	0	1						
	5.0	5	0	5									
Ethambutol	2.5				3	0	3						
	5.0	15	0	15	3	0	3	73	1	74*	2	0	2
	7.5	2	0	2									
	8.0										2	0	2
	10.0	6	0	6									
Pyrazinamide	50.0				1	0	1						
	100.0				4	0	4	74	0	74			
	200.0				1	0	1						
	300.0										1	0	1
	400.0				1	0	1						

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

Table 11. Isolate J—Participant results for second-line drug susceptibility testing

Results by Method for Second-Line Drugs										
		AP			BACTEC 460			MGIT		
Drug	**Conc. (µg/ml)**	**S**	**R**	**Total**	**S**	**R**	**Total**	**S**	**R**	**Total**
Streptomycin	1.0							43	0	43
	2.0	21	0	21	5	0	5			
	4.0	2	0	2				5	0	5
	10.0	18	0	18						
Ofloxacin	1.0	3	0	3						
	1.5							1	0	1
	2.0	15	0	15						
	4.0	2	0	2						
Ciprofloxacin	1.0							2	0	2
	2.0	5	0	5						
	4.0	1	0	1						
Levofloxacin	1.0	2	0	2						
	2.0	1	0	1						
	4.0	1	0	1						
Moxifloxacin	0.25							1	0	1
	1.0	3	0	3						
	2.0	1	0	1						
	4.0	1	0	1						
Amikacin	2.0	1	0	1						
	4.0	4	0	4				1	0	1
	5.0	1	0	1						
	6.0	5	0	5						
	12.0	1	0	1						
Kanamycin	5.0	9	0	9						
	6.0	8	0	8						
Capreomycin	10.0	16	0	16						
Ethionamide	1.25	1	0	1						
	2.5				1	0	1			
	5.0	15	0	15				2	0	2
	10.0	4	0	4						
Rifabutin	0.12	1	0	1						
	0.25	1	0	1						
	0.5	7	0	7						
	1.0	2	0	2						
	2.0	7	0	7						
Cycloserine	30.0	6	0	6						
	60.0	1	0	1						
p-Aminosalicylic acid	2.0	12	0	12						
	8.0	2	0	2						
	10.0	3	0	3						

Glossary

AP	agar proportion – performed on Middlebrook 7H10 or 7H11
BACTEC™ 460	BACTEC™ 460TB – a radiometric broth based DST method
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
HMO	Health Maintenance Organization
MDR	multidrug-resistant
MGIT™	BACTEC™ MGIT™ 960 – Mycobacteria Growth Indicator Tube
MPEP	Model Performance Evaluation Program
MTBC	*Mycobacterium tuberculosis* complex
PPO	Preferred Provider Organization
RNA	ribonucleic acid
TB	Tuberculosis
VersaTREK®	VersaTREK® Myco susceptibility kit
XDR	extensively drug-resistant
RMP	rifampin
INH	isoniazid
EMB	ethambutol
PZA	pyrazinamide
RBT	rifabutin
SM	streptomycin

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

	BACTEC 460	MGIT	VersaTREK
First-line Drugs			
Isoniazid	0.1 (and 0.4*)	0.1 (and 0.4*)	0.1 (and 0.4*)
Rifampin	2.0	1.0	1.0
Ethambutol	2.5 (and 7.5*)	5.0	5.0 (and 8.0*)
Pyrazinamide	100.0	100.0	300.0
Second-line Drug			
Streptomycin	2.0 (and 6.0*)	1.0 (and 4.0*)	

NOTE: Critical concentrations as indicated in applicable manufacturer package inserts

*The higher concentration of INH, EMB, and SM should be tested after resistance at the critical concentration is detected.

References

1. Clinical and Laboratory Standards Institute, *Susceptibility Testing of Mycobacteria, Nocardia, and Other Aerobic Actinomycetes; Approved Standard–2nd ed. M24–A2.*, 2011, CLSI: Wayne, PA.
2. Association of Public Health Laboratories (APHL). 2007. *TB Drug Susceptibility Testing*; *Expert Panel Meeting Summary Report*. Association of Public Health Laboratories. Washington, D.C.
3. Almeida Da Silva, P.E. and J.C. Palomino, *Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs.* J Antimicrob Chemother 2011. 66(7): p. 1417-30.
4. Zhang, Y. and W.W. Yew, *Mechanisms of drug resistance in Mycobacterium tuberculosis.* Int J Tuberc Lung Dis, 2009. 13(11): p. 1320-30.
5. Chedore, P., et al., *Potential for erroneous results indicating resistance when using the Bactec MGIT 960 system for testing susceptibility of Mycobacterium tuberculosis to pyrazinamide.* J Clin Microbiol, 2010. 48(1): p. 300-1.
6. Starks, A.M., et al., *Mutations at embB Codon 306 Are an Important Molecular Indicator of Ethambutol Resistance in Mycobacterium tuberculosis.* Antimicrob Agents Chemother, 2009. 53(3): p. 1061-1066.
7. Madison B., et al., "Multicenter evaluation of ethambutol susceptibility testing of Mycobacterium tuberculosis by agar proportion and radiometric methods." *J. Clin. Microbiol.* 2002. 40 (11): p. 3976-3979.
8. Angra, P.K., et al., *Performance of tuberculosis drug susceptibility testing in U.S. Laboratories from 1994 to 2008.* J Clin Microbiol, 2012. 50(4): p. 1233-9.
9. Campbell, P.J., et al., *Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis.* Antimicrob Agents Chemother, 2011. 55(5): p. 2032-41.