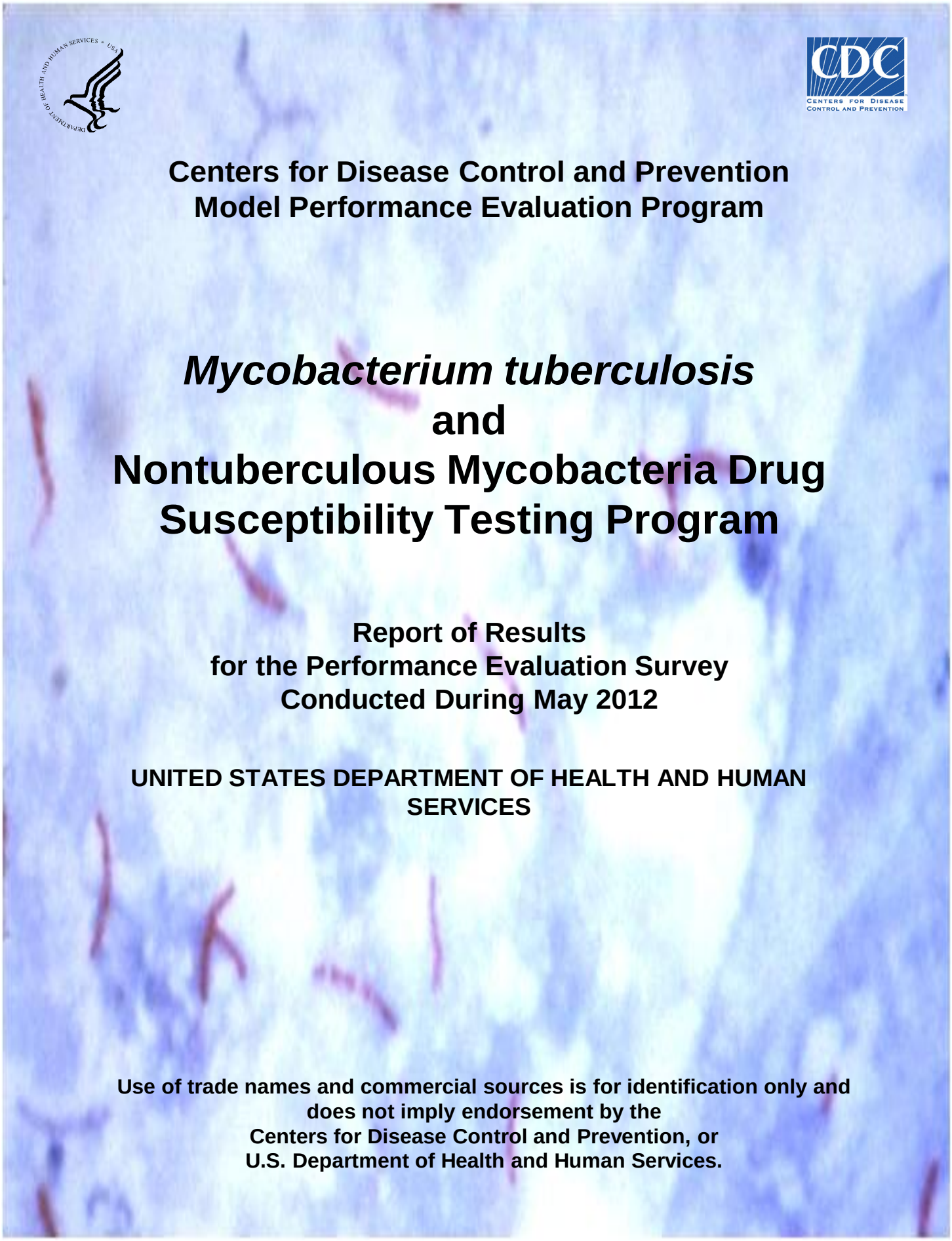

Centers for Disease Control and Prevention
Model Performance Evaluation Program

Mycobacterium tuberculosis
and
Nontuberculous Mycobacteria Drug Susceptibility Testing Program

Report of Results
for the Performance Evaluation Survey
Conducted During May 2012

UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES

MTB NTM DST Report for May 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* and Nontuberculous Mycobacteria Drug Susceptibility Testing (MPEP MTB NTM DST) survey sent to participants in May 2012.

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Use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services.

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Introduction: Analyses of the May 2012 *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[1] isolates shipped in May 2012. Panels were sent to 98 laboratories and 96 laboratories participated in evaluation of the panels.

Laboratories performed testing by using Agar Proportion 7H10 (AP 7H10); Agar Proportion 7H11 (AP 7H11) collectively called Agar Proportion methods (AP) when not mentioned individually; BACTEC™ 460 TB (BACTEC™); BACTEC™ MGIT™ 960 (MGIT™); VersaTREK® and molecular methods consist of Genotype MTBDR*sl*; Genotype MTBDR*plus*; Xpert MTB/RIF; and Laboratory Developed Tests.

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 strains using the same method, drug, and drug concentrations. We encourage circulation of this report to personnel who are involved with drug susceptibility testing, reporting, or interpreting for *M. tuberculosis* isolates.

CDC is neither recommending nor endorsing testing practices reported by participants. For approved standards, participants should refer to consensus documents published by the Clinical and Laboratory Standards Institute (CLSI), "Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard-Second Edition," M24-A2 (ISBN 1-56238-746-4).[1]

[1] Historically, the intent of the exercise was to assess performance using organisms that were of *Mycobacterium tuberculosis* Complex and were non-tuberculous mycobacteria. Over time, non-tuberculous mycobacteria have been dropped. Although it is possible that any of the eight species of *Mycobacterium tuberculosis* Complex could be present in the isolates selected, identification is not part of the panel selection nor the exercise and it is presumed *M. tuberculosis* is the dominant species represented. For these reasons and simplicity, we refer to *M. tuberculosis* throughout the report.

Susceptibility Testing Results for the *M. tuberculosis* Isolates Panel Shipped May 7, 2012

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

Isolate	Susceptibility Testing Results
2012A	Resistant to Rifampin (RIF) Resistant to Streptomycin (SM) Resistant to Kanamycin (KM)
2012B	Resistant to Isoniazid (INH) Resistant to Ethambutol (EMB) Resistant to Kanamycin (KM) Resistant to Capreomycin (CM) Resistant to Amikacin (AMK)
2012C	Resistant to Kanamycin (KM) Resistant to Capreomycin (CM) Resistant to Amikacin (AMK)
2012D	Resistant to Ofloxacin (OFX)
2012 E	Resistant to Streptomycin (SM)

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.

The participants were asked to indicate the **primary classification** of their laboratory.
MPEP participants self-classified as

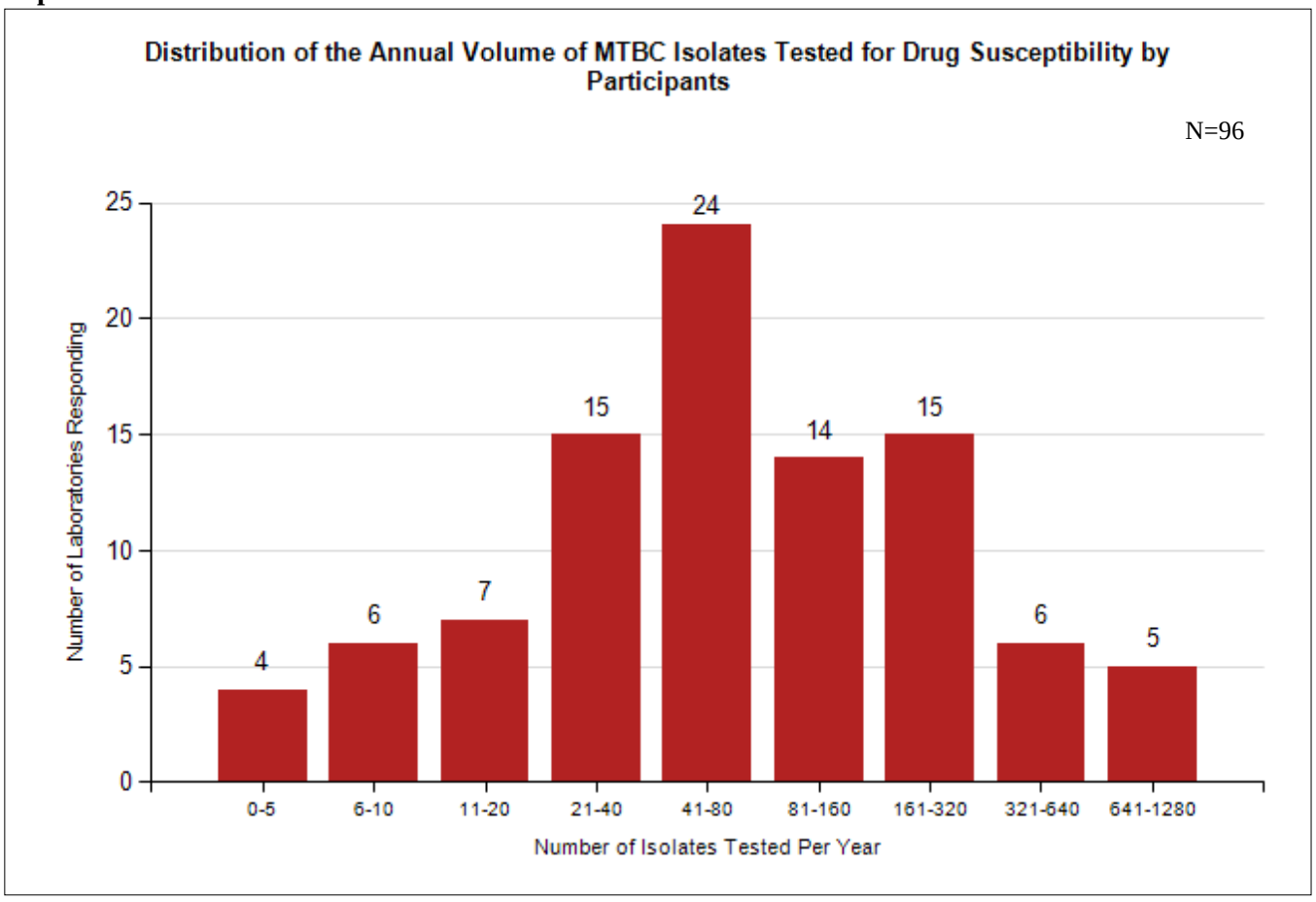
- 60 (62.5%): Health Department (city, county, state, regional, district, or national reference laboratory);
- 23 (24.0%): Hospital [city, county, district, community, state, regional, military, Veterans Administration, Federal government (other than military), privately-owned, university, HMO/PPO*-owned and operated, or religious-associated];
- 9 (9.4%): Independent [e.g., commercial, commercial manufacturer of reagents, HMO satellite clinic, reference laboratory (non-government affiliated)]; and
- 4 (4.2%): Other [Federal government research (nonmilitary)];

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

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

Figure 1 shows the number of drug susceptibility tests performed on *M. tuberculosis* isolates by the 96 participants in one **calendar year,** January 1–December 31, 2011, excluding quality control isolates. The counts range from four to 1,280. Seventeen (17) laboratories reported performing less than 21 drug susceptibility tests per year. To ensure testing proficiency, laboratories with low volumes are encouraged to consider referral of *M. tuberculosis* drug susceptibility testing.

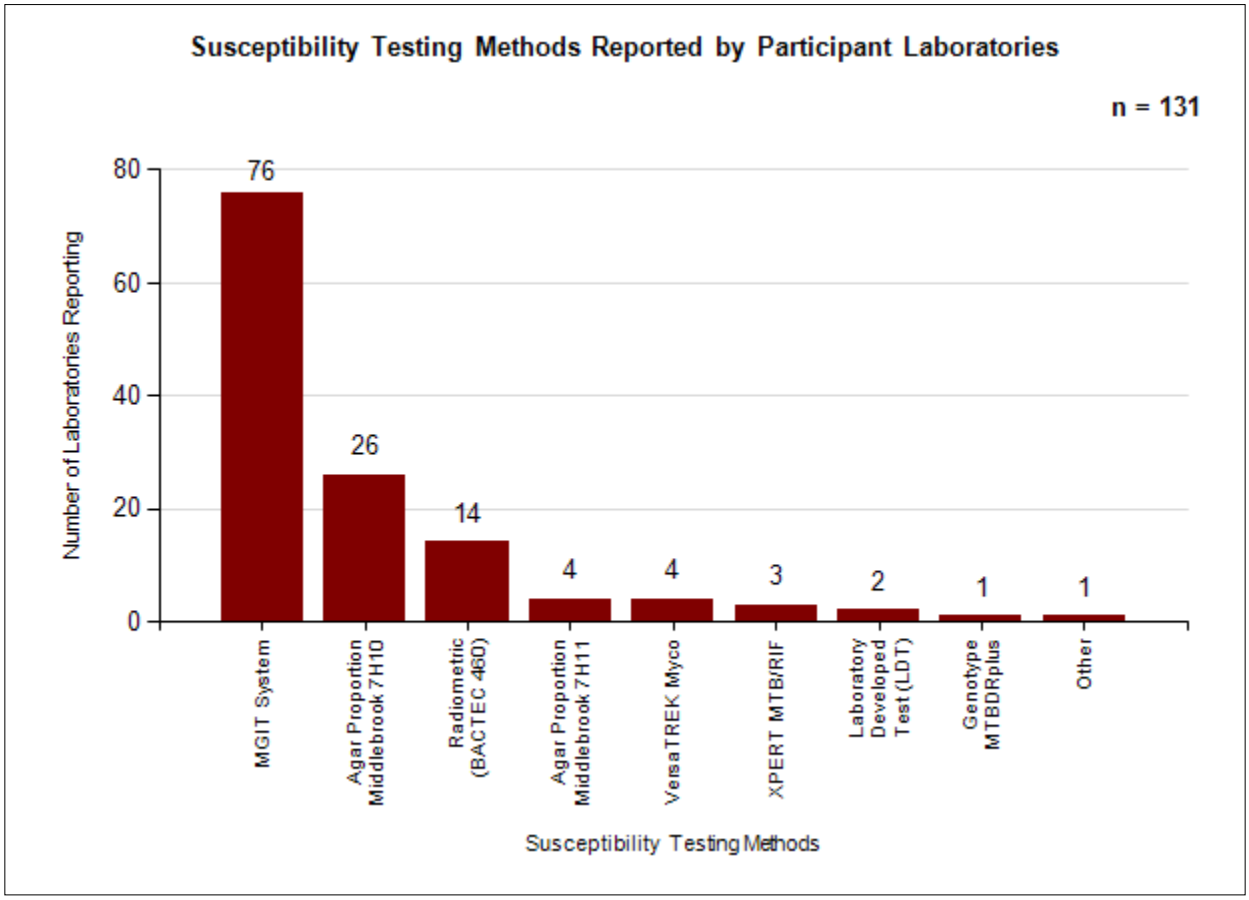
Figure 1: Distribution of the Annual Volume of *M. tuberculosis* Isolates Tested for Drug Susceptibility by Participants in the 2011 Calendar Year



Laboratory Susceptibility Testing Procedures Used by Participants

Participants were asked to report all *M. tuberculosis* susceptibility testing methods that were used to test these isolates. Sixty-five laboratories used only one method for testing, whereas 27 laboratories used two methods, and four laboratories used three methods. Figure 2 shows the reported susceptibility methods.

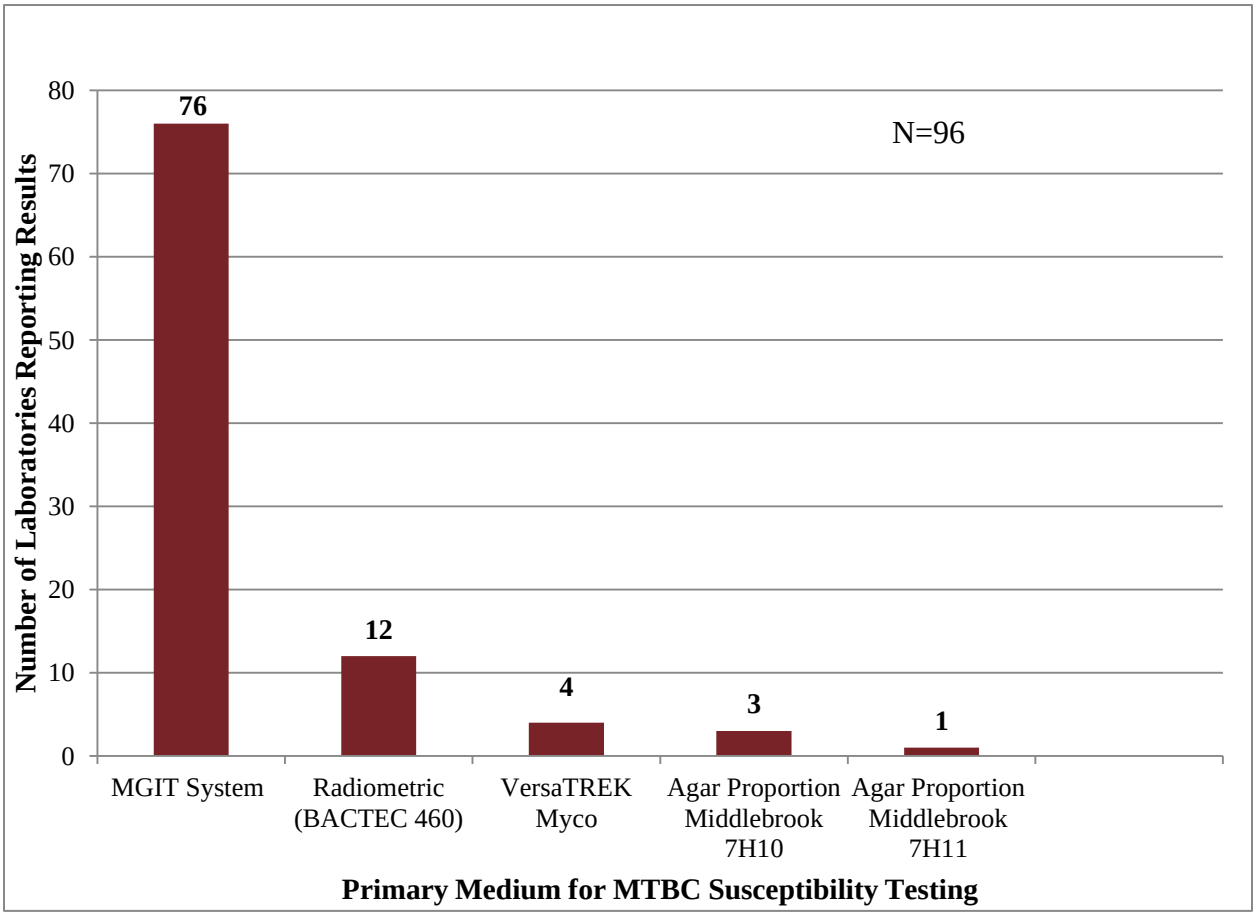
Figure 2: Susceptibility Testing Methods Reported by Participant Laboratories



The Primary *M. tuberculosis* Susceptibility Testing Media Used by Participants

Participants were asked to indicate the **primary** *M. tuberculosis* susceptibility test medium used by their laboratory for the isolates in the May 2012 shipment. Instructions were to select only one method as their primary method. Figure 3 shows the responses submitted by the 96 participants.

Figure 3: Primary *M. tuberculosis* Susceptibility Test Medium Used by Participants



Of the 76 laboratories that reported using MGIT™ as one of their methods for testing the MTB NTM DST isolates,

- 76 indicated that the MGIT™ method was their primary method for susceptibility testing; and
- 2 laboratories also indicated Agar proportion (AP) was their primary method using AP 7H10.

Of the 26 laboratories who reported using AP 7H10 as a method for testing the isolates,

- 3 laboratories used this as their primary method;
- 20 laboratories also indicated using MGIT™ as their primary method;
- 3 laboratories also indicated using BACTEC™ as their primary method; and
- 1 laboratory used VersaTREK® as their primary method.

Of the 14 laboratories who reported using BACTEC™ 460TB as one of their methods for testing the isolates,

- 12 used this as their primary method;
- 3 also used MGIT™ as their primary method.

Of the 4 laboratories who reported using VersaTREK® as a method for testing the isolates.

- 4 laboratories indicated this as their primary method.

Of the 4 laboratories who reported using AP 7H11 as a method for testing the isolates,

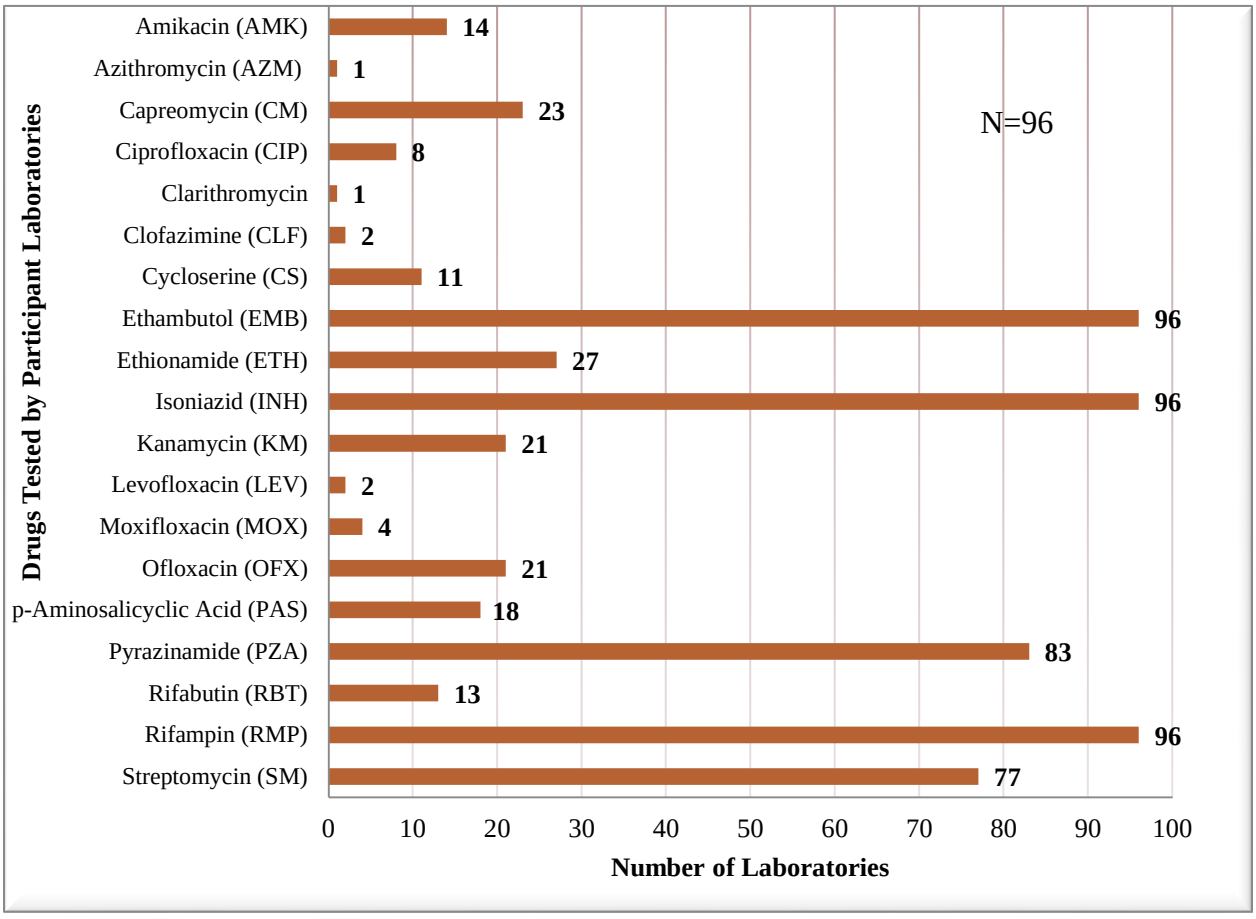
- 1 used this as their primary method;
- 2 used MGIT™ as their primary method; and
- 1 used BACTEC™ as their primary method.

Antituberculous Drugs Used by Participants

CLSI recommends a full panel of first-line (primary) 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; 83 (86.5%) of the participants also reported results for PZA.

Figure 4 shows the number of laboratories reporting results for each drug. The number at the right of each bar represents the number of laboratories that reported at least one result for the drug.

Figure 4: Antituberculous Drugs Used by 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 *M. tuberculosis* 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 *M. tuberculosis* isolates resistant to almost all standard drugs. According to CLSI, "Second-line drugs may be tested simultaneously if mutations associated with INH and RMP resistance have been detected by molecular assays, or if epidemiological situations support the practice and resources are available. Second-line drugs, both traditional and newer agents, should be tested for isolates resistant to RMP or any two of the primary drugs. Isolates with mono-resistance to the critical concentration of INH also should be tested for susceptibility to second-line agents if the clinician is planning to include a fluoroquinolone in the treatment regimen. 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 physician experts in the treatment and care of tuberculosis".

Tabulated data

This section provides the complete set of data in tabulated format for the *M .tuberculosis* isolates 2012A, 2012B, 2012C, 2012D, and 2012E sent in the May 2012 shipment. The following information/explanation pertains to all the tables.

Explanation of Tables 1 through 5

- In the following tables, the shaded rows indicate critical concentrations for each test method. For each drug, the critical concentration is defined as the lowest concentration that inhibits 95% of "wild-type" strains of *M. tuberculosis* 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]

- The test results (S represents susceptible and R represents resistant) are listed in the appropriate columns along with a corresponding total number of tests (Sum column) to provide 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.

- Participants should note that the CLSI approved standard "Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes," M24-A2 (ISBN 1-56238-746-4) CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA, 2011 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-A2.[1]

- Concentrations are listed in micrograms per milliliter (µg/ml).

- A concentration of 0.00 is used for results for genetic testing methods (Hain GenoType® MTBDR*plus* Assay [HAIN Lifescience, Germany]; Xpert MTB/RIF[Cepheid] ; and Laboratory Developed Tests].

Isolate 2012A, *M. tuberculosis*–resistant to Rifampin at 1.0µg/ml; Streptomycin at 2.0µg/ml and 10.0µg/ml; and Kanamycin at 5.0µg/ml by Agar Proportion method

Rifampin (RMP) is a first-line drug for treatment of all forms of tuberculosis caused by organisms known or presumed to be susceptible to this drug. It is bactericidal for *M. tuberculosis* at the critical concentration of 1.0µg/ml for AP (on Middlebrook 7H10 and 7H11 agars) and equivalent critical concentrations for BACTEC460™, MGIT960™, and VersaTREK® of 2.0µg/ml, 1.0µg/ml, and 1.0µg/ml, respectively. The mechanism of action of RMP is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase[2, 3]. 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[2, 3]. 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* of Isolate 2012A revealed a point mutation in the *rpoB* locus resulting in serine being replaced by leucin at codon 531 (Ser531Leu). This mutation is associated with resistance to RMP and rifabutin. Ninety-six laboratories reported RMP results for this isolate at the critical concentration. (Some laboratories submitted results from more than one method.) This isolate was reported resistant by:

- 100% (24/24) of the laboratories reporting AP results;
- 100% (11/11) of the laboratories reporting BACTEC™ results;
- 98.6% (72/73) of the laboratories reporting MGIT™ results;
- 100% (4/4) of the laboratories reporting VersaTREK® results; and
- 100% (6/6) of the laboratories reporting molecular method results.

All six laboratories reporting molecular method results reported RMP resistance.

Streptomycin

Streptomycin (SM) belongs to the aminoglycoside class of drugs and its primary mechanism of action is to inhibit the initiation of translations by binding to the 16S rRNA[2,3] In *M. tuberculosis*, the genetic basis of resistance to SM is usually due to mutaions in *rrs* or *rpsL*[3].

Seventy-seven laboratories reported SM results for this isolate at the critical concentration. (Some laboratories submitted results from more than one method.) This isolate was reported resistant by:

- 100% (24/24) of the laboratories reporting AP results;
- 100% (12/12) of the laboratories reporting BACTEC™ results;
- 98.1% (51/52) of the laboratories reporting MGIT™ results;

Kanamycin

Isolate 2012A was also resistant to Kanamycin by the AP method. Mutations in the 16S *r*RNA gene (*rrs*) have been associated with resistance to second-line injectable drugs, kanamycin, amikacin, and capreomycin[4]. In addition, low-level kanamycin resistance, but not amikacin resistance, is associated with mutations in the promoter region of the *eis* gene which results in the overexpression of the encoded aminoglycoside acetyltransferase[5, 6]. DNA sequence analysis of the *rrs* and *eis* of 2012A revealed no mutations in rrs and a G-10A mutation in *eis*.

The recommended testing concentration for KM by 7H10 Agar medium is 5µg/ml and by 7H11 Agar medium is 6µg/ml.

While fifteen laboratories reported 7H10 agar medium results for KM for this isolate, only nine reported results at the critical concentration. (Six laboratories reported results at 6µg/ml.)

While four laboratories reported 7H11 agar medium results for this isolate, only three reported results at the critical concentration. (One laboratory reported results at 5µg/ml.)

This isolate was reported resistant by:

- 77.8% (7/9) of the laboratories reporting 7H10 AP results at recommended concentration;
- 33.3% (1/3) of the laboratories reporting 7H11 AP results at recommended concentration;
- 100% (3/3) of the laboratories reporting BACTEC™ results.

See Table 1 for the complete results submitted by all participants for Isolate 2012A.

Table 1: Participant results for Isolate 2012A, *M. tuberculosis*–resistant to Rifampin at 1.0µg/ml; Streptomycin at 2.0µg/ml and 10.0µg/ml; and Kanamycin at 5.0µg/ml by Agar Proportion method

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00										3		3
Isoniazid	0.01							1		1			
Isoniazid	0.10				11		11	70		70	4		4
Isoniazid	0.20	22		22				2		2			
Isoniazid	0.40				3		3	21	1	22	4		4
Isoniazid	1.00	23		23	1		1						
Isoniazid	5.00	5		5									
Rifampin	0.00											6	6
Rifampin	1.00		24	24		3	3	1	72	73		4	4
Rifampin	2.00					11	11						
Rifampin	5.00		4	4		1	1						
Rifampin	10.00					1	1						
Pyrazinamide	0.00										1		1
Pyrazinamide	100.00				8	1	9	68	3	71			
Pyrazinamide	300.00				1		1					2	2
Ethambutol	0.00										1		1
Ethambutol	2.50				11		11						
Ethambutol	5.00	18	1	19	2		2	72		72	4		4
Ethambutol	7.50	2		2	1		1	1		1			
Ethambutol	8.00										4		4
Ethambutol	10.00	9		9									
Ethambutol	25.00	1		1									
Streptomycin	1.00					1	1	1	51	52			
Streptomycin	2.00		24	24		12	12		1	1			
Streptomycin	4.00		2	2		1	1		11	11			
Streptomycin	6.00					1	1						
Streptomycin	10.00	2	20	22		1	1						
Streptomycin	50.00		1	1									
Ethionamide	1.25					2	2						
Ethionamide	2.50					1	1						
Ethionamide	5.00	6	12	18					4	4			
Ethionamide	10.00	1	3	4					2	2			
Kanamycin	0.00										1		1
Kanamycin	2.50					1	1						
Kanamycin	5.00	2	8	10		3	3						
Kanamycin	6.00	6	3	9									
Kanamycin	10.00					1	1						
Capreomycin	0.00										1		1
Capreomycin	1.25				1		1						
Capreomycin	2.50				1		1	1		1			
Capreomycin	3.00							3		3			
Capreomycin	10.00	18		18									
Cycloserine	30.00	10		10									
Cycloserine	60.00	1		1									

* VersaTREK®, Hain GenoType®, or other Molecular Methods

Table 1 continued: Participant results for Isolate 2012A, *M. tuberculosis*–resistant to Rifampin at 1.0µg/ml; Streptomycin at 2.0µg/ml and 10.0µg/ml; and Kanamycin at 5.0µg/ml by Agar Proportion method

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
p-Aminosalicylic acid	2.00	16		16									
p-Aminosalicylic acid	4.00							1		1			
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	3		3									
Amikacin	0.00										1		1
Amikacin	1.00	1		1				1		1			
Amikacin	1.50							2		2			
Amikacin	2.00	1		1									
Amikacin	2.50	1		1									
Amikacin	4.00	4		4									
Amikacin	5.00	1		1									
Amikacin	6.00	5		5									
Amikacin	12.00	2		2									
Ofloxacin	0.00										1		1
Ofloxacin	0.60	1		1									
Ofloxacin	1.00	3		3									
Ofloxacin	1.25				1		1						
Ofloxacin	1.50							1		1			
Ofloxacin	2.00	14		14	2		2	1		1			
Ciprofloxacin	0.00										1		1
Ciprofloxacin	1.00	2		2				1		1			
Ciprofloxacin	2.00	6		6									
Azithromycin	3.00		1	1									
Clarithromycin	3.00		1	1									
Clofazimine	0.50				1		1						
Clofazimine	1.00	1		1									
Levofloxacin	1.50							1		1			
Levofloxacin	2.00							1		1			
Moxifloxacin	0.13							1		1			
Moxifloxacin	0.25							2		2			
Moxifloxacin	0.50	1		1									
Moxifloxacin	1.00	1		1									
Rifabutin	0.05					1	1						
Rifabutin	0.25					1	1						
Rifabutin	0.50		5	5		2	2						
Rifabutin	1.00		2	2					1	1			
Rifabutin	2.00	1	5	6									
Rifabutin	2.50					1	1						
Rifabutin	5.00		1	1									

* VersaTREK®, Hain GenoType®, XPERT MTB/RIF or other Molecular Methods

Isolate 2012B, *M. tuberculosis*– resistant to Isoniazid at 0.2µg/ml and 1.0µg/ml; Ethambutol at 5.0µg/ml; Amikacin at 4.0µg/ml; Capreomycin at 10.0µg/ml; and Kanamycin at 5.0µg/ml by Agar Proportion method

Isoniazid

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. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *kat*G gene[2, 3]. The target of activated INH is enoyl-acyl-carrier protein reductase (*Inh*A) which is required for mycolic acid biosynthesis. There are two described mechanisms that account for the majority of INH resistance[2, 3]. The most common method, mutations in *kat*G, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the *inh*A gene which are generally associated with low-level resistance to INH and are less frequent than *kat*G mutations. DNA sequence analysis of *inh*A and *kat*G of Isolate 2012B revealed a G>C point mutation in the *kat*G locus resulting in serine being replaced by threonine at codon 315 (Ser315Thr); *inhA* was wild-type (i.e., no mutations were detected).

The recommended critical concentration and additional higher concentrations for testing INH using the AP method are 0.2 µg/ml and 1.0 µg/ml respectively. The equivalent concentrations for BACTEC™, MGIT™, and VersaTREK® are 0.1 µg/ml and 0.4 µg/ml. It is recommended that all laboratories perform testing at the critical concentration; if resistant, then testing at the higher recommended concentration should be performed.

Ninety-four laboratories reported INH results for this isolate at the critical concentration. (Some laboratories submitted results for more than one method.) This isolate was reported resistant by:

- 100% (24/24) of the laboratories reporting AP results;
- 100% (11/11) of the laboratories reporting BACTEC™ results;
- 97.2% (69/71) of the laboratories reporting MGIT™ results;
- 100% (4/4) of the laboratories reporting VersaTREK® results.

Two laboratories did not report results at the critical concentration.

Most Laboratories also reported resistance at recommended higher concentration.

The laboratories using Hain GenoType® MTBDR*plus* and laboratory developed tests reported INH 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[2, 3]. EMB targets the arabinosyl transferases (*emb*CAB operon), thereby inhibiting the biosynthesis of the cell wall components arabinogalactan and lipoarabinomannan[7].

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, 7]. Conventional culture based methods of EMB susceptibility testing are problematic [8] and false susceptibility has been reported [2]. DNA sequence analysis of *emb*B of Isolate 2012B revealed a mutation resulting methionine replaced by isoleucine at codon 306 (Met306Ile). This mutation is highly associated with EMB resistance[7].

Ninety-six laboratories reported EMB results for this isolate at the critical concentration. (Some laboratories submitted results for more than one method.) This isolate was reported resistant by:

- 70% (14/20) of the laboratories reporting 7H10 AP results;
- 0% (0.20) of the laboratories reporting 7H11 AP results;
- 90.1% (10/11) of the laboratories reporting BACTEC™ results;
- 71.2% (52/73) of the laboratories reporting MGIT™ results;
- 50% (2/4) of the laboratories reporting VersaTREK® results.

Second-line injectable drugs

Kanamycin and Amikacin are aminoglycoside antibiotics while Capreomycin is a cyclic peptide antibiotic. All three exert their activity at the level of protein translation. The most common mechanism of cross resistance to all three drugs is an A1401G mutation in the *rrs* gene coding for 16S rRNA [4].

Isolate 2012B was resistant to Amikacin, Capreomycin, and Kanamycin by the AP method.. DNA sequence analysis of the *rrs* gene of Isolate 2012B revealed the A1401G mutation.

Amikacin

Fourteen laboratories reported Amikacin results for this isolate. (Some laboratories submitted results from more than one method.) This isolate was reported resistant by:

- 100% (4/4) of the laboratories reporting AP results at the recommended critical concentration.

Capreomycin

Twenty-three laboratories reported Capreomycin results for this isolate. (Some laboratories submitted results from more than one method.) This isolate was reported resistant by:

- 88.9% (16/18) of the laboratories reporting AP results at the recommended critical concentration.

Kanamycin

The recommended testing concentration for KM by 7H10 Agar medium is 5µg/ml and by 7H11 Agar medium is 6µg/ml.

While fifteen laboratories reported 7H10 agar medium results for KM for this isolate, only nine reported results at the critical concentration. (Six laboratories reported results at 6µg/ml.)

While four laboratories reported 7H11 agar medium results for this isolate, only three reported results at the critical concentration. (One laboratory reported results at 5µg/ml.)

This isolate was reported resistant by:

- 100% (9/9) of the laboratories reporting AP 7H10 results at the recommended critical concentration.
- 100% (3/3) of the laboratories reporting AP 7H11 results at the recommended critical concentration.
- 100% (3/3) of the laboratories reporting BACTEC results at the recommended critical concentration.

The laboratory using Hain GenoType® MTBDR*sl* also reported resistance to Amikacin, Capreopmycin, and Kanamycin. The target for detection of resistance to second-line injectable drugs in the MTBDR*sl* test is *rrs*.

See Table 2 for the complete results submitted by all participants for Isolate 2012B.

Table 2: Participant results for Isolate 2012B, *M. tuberculosis*– resistant to Isoniazid at 0.2µg/ml and 1.0µg/ml; Ethambutol at 5.0µg/ml; Amikacin at 4.0µg/ml; Capreomycin at 10.0µg/ml; and Kanamycin at 5.0µg/ml by Agar Proportion method

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00											3	3
Isoniazid	0.01								1	1			
Isoniazid	0.10					11	11	2	69	71		4	4
Isoniazid	0.20		24	24		1	1		2	2			
Isoniazid	0.40					3	3	1	34	35		4	4
Isoniazid	1.00		26	26		2	2		1	1			
Isoniazid	2.00					1	1						
Isoniazid	5.00		5	5	1		1						
Rifampin	0.00										6		6
Rifampin	1.00	23		23	3		3	74		74	4		4
Rifampin	2.00				10		10						
Rifampin	5.00	4		4									
Pyrazinamide	100.00				8		8	70	2	72			
Pyrazinamide	300.00				1		1				3		3
Ethambutol	0.00											1	1
Ethambutol	2.50				1	10	11						
Ethambutol	5.00	6	14	20		3	3	21	52	73	2	2	4
Ethambutol	7.50	2		2	1	1	2	1		1			
Ethambutol	8.00										4		4
Ethambutol	10.00	10	1	11	1		1						
Ethambutol	25.00		1	1									
Streptomycin	1.00				1		1	52		52			
Streptomycin	2.00	23		23	12		12	1		1			
Streptomycin	4.00	1		1				7		7			
Streptomycin	10.00	20		20									
Streptomycin	50.00	1		1									
Ethionamide	1.25				1	1	2						
Ethionamide	2.50				1		1						
Ethionamide	5.00	12	6	18				1	3	4			
Ethionamide	10.00	2	2	4					1	1			
Kanamycin	0.00											1	1
Kanamycin	2.50					1	1						
Kanamycin	5.00		10	10		3	3						
Kanamycin	6.00		9	9									
Kanamycin	10.00					1	1						
Capreomycin	0.00											1	1
Capreomycin	1.25					1	1						
Capreomycin	2.50					1	1		1	1			
Capreomycin	3.00								3	3			
Capreomycin	5.00					1	1						
Capreomycin	10.00	2	16	18	1		1						
Cycloserine	30.00	10		10									
Cycloserine	60.00	1		1									

* VersaTREK®, Hain GenoType®, XPERT MTB/RIF or other Molecular Methods

Table 2 Continued: Participant results for Isolate 2012B, *M. tuberculosis*– resistant to Isoniazid at 0.2µg/ml and 1.0µg/ml; Ethambutol at 5.0µg/ml; Amikacin at 4.0µg/ml; Capreomycin at 10.0µg/ml; and Kanamycin at 5.0µg/ml by Agar Proportion method

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
p-Aminosalicylic acid	2.00	16		16									
p-Aminosalicylic acid	4.00							1		1			
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	3		3									
Amikacin	0.00											1	1
Amikacin	1.00		1	1					1	1			
Amikacin	1.50								2	2			
Amikacin	2.00		1	1									
Amikacin	2.50		1	1									
Amikacin	4.00		4	4									
Amikacin	5.00		1	1									
Amikacin	6.00	1	4	5									
Amikacin	12.00		2	2									
Ofloxacin	0.00										1		1
Ofloxacin	0.60	1		1									
Ofloxacin	1.00	3		3									
Ofloxacin	1.25				1		1						
Ofloxacin	1.50							1		1			
Ofloxacin	2.00	14		14	2		2	2		2			
Ciprofloxacin	0.00										1		1
Ciprofloxacin	1.00	2		2				1		1			
Ciprofloxacin	2.00	6		6									
Clofazimine	0.50				1		1						
Clofazimine	1.00	1		1									
Levofloxacin	1.50							1		1			
Levofloxacin	2.00							1		1			
Moxifloxacin	0.13							1		1			
Moxifloxacin	0.25							2		2			
Moxifloxacin	0.50	1		1									
Moxifloxacin	1.00	1		1									
Rifabutin	0.50	5		5	1		1						
Rifabutin	1.00	2		2				1		1			
Rifabutin	2.00	6		6									
Rifabutin	2.50				1		1						
Rifabutin	5.00	1		1									

* VersaTREK®, Hain GenoType®, XPERT MTB/RIF or other Molecular Methods

Isolate 2012C, *M. tuberculosis*–resistant to Amikacin at 4.0µg/ml; Capreomycin at 10.0µg/ml; and Kanamycin at 5.0µg/ml by Agar Proportion method

Second- line drugs

Isolate 2012C was also resistant to Amikacin, Capreomycin, and Kanamycin by the AP method. DNA sequence analysis of the *rrs* gene of Isolate 2012C revealed an A1401G mutation which is highly associated with resistance to second line injectable drugs[4].

Amikacin

Fourteen laboratories reported Amikacin results for this isolate. (Some laboratories submitted results from more than one method.) This isolate was reported resistant by:

- 100% (4/4) of the laboratories reporting AP results at the recommended critical concentration.

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Capreomycin

Twenty-one laboratories reported Capreomycin results for this isolate. (Some laboratories submitted results from more than one method.) This isolate was reported resistant by:

- 94.4% (17/18) of the laboratories reporting AP results at the recommended critical concentration.

Kanamycin

The recommended testing concentration for KM by 7H10 Agar medium is 5µg/ml and by 7H11 Agar medium is 6µg/ml.

While fifteen laboratories reported 7H10 agar medium results for KM for this isolate, only nine reported results at the critical concentration. (Six laboratories reported results at 6µg/ml.)

While three laboratories reported 7H11 agar medium results for this isolate, only two reported results at the critical concentration. (One laboratory reported results at 5µg/ml.)

This isolate was reported resistant by:

- 100% (9/9) of the laboratories reporting AP 7H10 results at the recommended critical concentration.
- 100% (2/2) of the laboratories reporting AP 7H11 results at the recommended critical concentration.
- 100% (1/1) of the laboratories reporting BACTEC results at the recommended critical concentration.

The laboratory using Hain GenoType® MTBDR*sl* also reported resistance to AMikacin, Capreopmycin, and Kanamycin. The target in the MTBDR*sl* test is the *rrs* gene.

See Table 3 for the complete results submitted by all participants for Isolate 2012C.

Table 3: Participant results for Isolate 2012C, *M. tuberculosis*–resistant to Amikacin at 4.0µg/ml; Capreomycin at 10.0µg/ml; and Kanamycin at 5.0µg/ml by Agar Proportion method

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00										3		3
Isoniazid	0.01							1		1			
Isoniazid	0.10				11		11	71		71	4		4
Isoniazid	0.20	21		21				2		2			
Isoniazid	0.40				3		3	23	1	24	4		4
Isoniazid	1.00	22		22	1		1						
Isoniazid	5.00	5		5									
Rifampin	0.00										6		6
Rifampin	1.00	22		22	3		3	74		74	4		4
Rifampin	2.00				10		10						
Rifampin	5.00	3		3									
Pyrazinamide	0.00										1		1
Pyrazinamide	100.00				7	1	8	72		72			
Pyrazinamide	300.00				1		1					2	2
Ethambutol	0.00										1		1
Ethambutol	2.50				11		11						
Ethambutol	5.00	18		18	2		2	73		73	4		4
Ethambutol	7.50	2		2	1		1	1		1			
Ethambutol	8.00										4		4
Ethambutol	10.00	8		8									
Ethambutol	25.00		1	1									
Streptomycin	1.00				1		1	51	1†	52			
Streptomycin	2.00	22		22	12		12	1		1			
Streptomycin	4.00	1		1				8		8			
Streptomycin	10.00	19		19									
Streptomycin	50.00	1		1									
Ethionamide	1.25				1		1						
Ethionamide	2.50				1		1						
Ethionamide	5.00	17		17				4		4			
Ethionamide	10.00	4		4									
Kanamycin	0.00											1	1
Kanamycin	5.00		10	10		1	1						
Kanamycin	6.00		8	8									
Capreomycin	0.00											1	1
Capreomycin	2.50								1	1			
Capreomycin	3.00								3	3			
Capreomycin	10.00	1	17	18									
Cycloserine	30.00	9		9									
Cycloserine	60.00	1		1									

* VersaTREK®, Hain GenoType®, XPERT MTB/RIF or Molecular Methods

† Borderline result

Table 3 Continued: Participant results for Isolate 2012C, *M. tuberculosis*–resistant to Amikacin at 4.0µg/ml; Capreomycin at 10.0µg/ml; and Kanamycin at 5.0µg/ml by Agar Proportion method

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	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	3		3									
Amikacin	0.00											1	1
Amikacin	1.00		1	1					1	1			
Amikacin	1.50								2	2			
Amikacin	2.00		1	1									
Amikacin	2.50		1	1									
Amikacin	4.00		4	4									
Amikacin	5.00		1	1									
Amikacin	6.00		5	5									
Amikacin	12.00		2	2									
Ofloxacin	0.00										1		1
Ofloxacin	0.60	1		1									
Ofloxacin	1.00	2		2									
Ofloxacin	1.50							1		1			
Ofloxacin	2.00	14		14	1		1	1		1			
Ciprofloxacin	0.00										1		1
Ciprofloxacin	1.00	1		1				1		1			
Ciprofloxacin	2.00	6		6									
Clofazimine	1.00	1		1									
Levofloxacin	1.50							1		1			
Levofloxacin	2.00							1		1			
Moxifloxacin	0.13							1		1			
Moxifloxacin	0.25							2		2			
Moxifloxacin	0.50	1		1									
Moxifloxacin	1.00	1		1									
Rifabutin	0.50	5		5									
Rifabutin	1.00	2		2				1		1			
Rifabutin	2.00	6		6									
Rifabutin	2.50				1		1						
Rifabutin	5.00	1		1									

* VersaTREK®, Hain GenoType®, XPERT MTB/RIF or Molecular Methods

Isolate 2012D, *M. tuberculosis*– resistant to Ofloxacin at 2.0µg/ml by Agar Proportion method

This isolate is susceptible to all first line drugs at recommended testing concentrations. This isolate was reported resistant by:

- 4.8% (1/21) of the laboratories reporting for INH by AP method;
- 5.6% (1/18) of the laboratories reporting for EMB by AP method;
- 1.4% (1/74) of the laboratories reporting for EMB by MGIT™ method;
- 1.4% (1/71) of the laboratories reporting PZA by MGIT™ method.

Ofloxacin

Fluoroquinolones (FQ) are important class of drugs to treat tuberculosis resistant to first-line drugs. They are the most commonly prescribed antibiotic class in the United States and they have the potential to become part of future first-line antituberculosis regimens[9]. Resistance to FQ is relatively low in strains of *M. tuberculosis* susceptible to first-line drugs but receipt of FQ before tuberculosis (TB) diagnosis is associated with a high risk of FQ-resistant TB and delays in diagnosis[9, 10].

Resistance to FQ has been mainly attributed to mutations in a 21-bp region of the *M. tuberculosis gyrA* gene, often called the quinolone resistance determining region (QRDR)[2, 3].

DNA sequence analysis of the *gyrA* gene of Isolate 2012D revealed a Ser91Pro mutation which is highly associated with resistance to fluoroquinolones[2, 3].

Eighteen laboratories reported Ofloxacin results for this isolate at the critical concentration. (Some laboratories submitted results from more than one method.) This isolate was reported resistant by:

- 100% (14/14) of the laboratories reporting AP results.

The laboratory using Hain GenoType® MTBDR*sl* also reported Ofloxacin resistance.

See Table 4 for the complete results submitted by all participants for Isolate 2012D.

Table 4: Participant results for Isolate 2012D, *M. tuberculosis*– resistant to Ofloxacin at 2.0µg/ml by Agar Proportion method

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00										3		3
Isoniazid	0.01							1		1			
Isoniazid	0.10				10		10	72		72	4		4
Isoniazid	0.20	20	1	21				2		2			
Isoniazid	0.40				3		3	24		24	4		4
Isoniazid	1.00	21	1	22	1		1						
Isoniazid	5.00	4	1	5									
Rifampin	0.00										6		6
Rifampin	1.00	22		22	3		3	75		75	4		4
Rifampin	2.00				9		9						
Rifampin	5.00	3		3									
Pyrazinamide	100.00				8		8	70	1	71			
Pyrazinamide	300.00				1		1				2		2
Ethambutol	0.00										1		1
Ethambutol	2.50				10		10						
Ethambutol	5.00	17	1	18	2		2	73	1	74	4		4
Ethambutol	7.50	2		2	1		1	1		1			
Ethambutol	8.00										4		4
Ethambutol	10.00	8		8									
Ethambutol	25.00		1	1									
Streptomycin	1.00				1		1	52		52			
Streptomycin	2.00	22		22	11		11	1		1			
Streptomycin	4.00	1		1				7		7			
Streptomycin	10.00	19		19									
Streptomycin	50.00	1		1									
Ethionamide	1.25				1		1						
Ethionamide	2.50				1		1						
Ethionamide	5.00	17		17				4		4			
Ethionamide	10.00	4		4									
Kanamycin	0.00										1		1
Kanamycin	5.00	10		10	1		1						
Kanamycin	6.00	8		8									
Capreomycin	0.00										1		1
Capreomycin	2.50							1		1			
Capreomycin	3.00							3		3			
Capreomycin	10.00	18		18									
Cycloserine	30.00	9		9									
Cycloserine	60.00	1		1									

* VersaTREK®, Hain GenoType®, XPERT MTB/RIF or Molecular Methods

Table 4 Continued: Participant results for Isolate 2012D, *M. tuberculosis*– resistant to Ofloxacin at 2.0µg/ml by Agar Proportion method

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
p-Aminosalicylic acid	2.00	13	1	14									
p-Aminosalicylic acid	4.00							1		1			
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	3		3									
Amikacin	0.00										1		1
Amikacin	1.00	1		1				1		1			
Amikacin	1.50							2		2			
Amikacin	2.00	1		1									
Amikacin	2.50	1		1									
Amikacin	4.00	4		4									
Amikacin	5.00	1		1									
Amikacin	6.00	5		5									
Amikacin	12.00	2		2									
Ofloxacin	0.00											1	1
Ofloxacin	0.60		1	1									
Ofloxacin	1.00		2	2									
Ofloxacin	1.50								1	1			
Ofloxacin	2.00		14	14		1	1		1	1			
Ciprofloxacin	0.00											1	1
Ciprofloxacin	1.00		1	1					1	1			
Ciprofloxacin	2.00		6	6									
Clofazimine	1.00	1		1									
Levofloxacin	1.50								1	1			
Levofloxacin	2.00								1	1			
Moxifloxacin	0.13								1	1			
Moxifloxacin	0.25								2	2			
Moxifloxacin	0.50		1	1									
Moxifloxacin	1.00	1		1									
Rifabutin	0.50	5		5									
Rifabutin	1.00	2		2				1		1			
Rifabutin	2.00	6		6									
Rifabutin	2.50				1		1						
Rifabutin	5.00	1		1									

* VersaTREK®, Hain GenoType®, XPERT MTB/RIF or Molecular Methods

Isolate 2012E, *M. tuberculosis*–resistant to Streptomycin at 2.0µg/ml and 10.0µg/ml by Agar Proportion method

Streptomycin

As previously stated, streptomycin (SM) belongs to the aminoglycoside class of drugs and its primary mechanism of action is to inhibit the initiation of translations by binding to the 16S rRNA. In *M. tuberculosis*, the genetic basis of resistance to SM is usually due to mutations in *rrs* or *rpsL*[3].
76 laboratories reported SM results for this isolate at the critical concentration. (Some laboratories submitted results from more than one method. This isolate was reported resistant by:

- 100% (24/24) of the laboratories reporting AP results;
- 90.9% (10/11) of the laboratories reporting BACTEC™ results;
- 98.1% (52/53) of the laboratories reporting MGIT™ results.

Pyrazinamide

The expected PZA result for isolate 2012E was susceptible. However, many laboratories reported it as resistant.

Pyrazinamide (PZA) is an important first-line drug used with INH and RMP for treatment of tuberculosis. The role of PZA is to shorten TB treatment to 6 months because it kills a population of persistent and semi-dormant bacilli in the acidic pH environment in the lesions that are not killed by other drugs. Pyrazinamide is a prodrug that requires conversion to its active form, pyrazinoic acid, by the pyrazinamidase (PZase) encoded by the *pnc*A gene of *M. tuberculosis*. Resistance to PZA is usually caused by diverse nucleotide changes scattered throughout the *pncA* gene, and PZA-resistant *M. tuberculosis* strains lose PZase activity[2, 3] .

Standard culture-based PZA susceptibility tests are difficult to perform as a result of poor buffering of test media, the use of acidic medium pH that inhibits growth, and excessively large inoculum that reduce the activity of PZA[11]. Among culture based DST methods, the BACTEC™ radiometric method is probably the most reliable and is currently the reference method for choice for PZA DST [1]. MGIT had widely replaced the BACTEC™ radiometric method. However, MGIT™ may over report PZA resistance [11, 12]. Tests for PZase activity and for the detection of mutations in *pnc*A may be used as alternative methods for the detection of PZA resistance in M. tuberculosis [11, 12].

Eighty laboratories reported PZA results at the critical concentration for this isolate. (Some laboratories submitted results from more than one method). This isolate was reported resistant by:

50.0% (4/8) of the laboratories reporting BACTEC™ results;

73.9% (51/69) of the laboratories reporting MGIT™ results, one laboratory reported contaminated; and

50.0% (1/2) of the laboratories reporting VersaTREK® results.

One laboratory reported susceptible using Laboratory Developed Test.

Isolate 2012E did not have a mutation detected in *pnc*A. Further study is needed to determine whether it is truly susceptible to PZA.

See Table 5 for the complete results submitted by all participants for Isolate 2012E.

Table 5: Participant results for Isolate 2012E, *M. tuberculosis*–resistant to Streptomycin at 2.0µg/ml and 10.0µg/ml by Agar Proportion method

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Results*		
Drug	Conc.	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00										3		3
Isoniazid	0.01							1		1			
Isoniazid	0.10				10		10	70	1	71	4		4
Isoniazid	0.20	22		22				2		2			
Isoniazid	0.40				3		3	24		24	4		4
Isoniazid	1.00	23		23	1		1						
Isoniazid	5.00	5		5									
Rifampin	0.00										6		6
Rifampin	1.00	23		23	3		3	75		75	4		4
Rifampin	2.00				9		9						
Rifampin	5.00	4		4									
Pyrazinamide	0.00										1		1
Pyrazinamide	100.00				4	4†	8	18	51	69			
Pyrazinamide	300.00					1	1				1	1	2
Ethambutol	0.00										1		1
Ethambutol	2.50				10		10						
Ethambutol	5.00	19		19	2		2	72	2	74	4		4
Ethambutol	7.50	2		2	1		1	1		1			
Ethambutol	8.00										4		4
Ethambutol	10.00	9		9									
Ethambutol	25.00	1		1									
Streptomycin	1.00					1	1	1	52	53			
Streptomycin	2.00		24	24	1	10	11		1	1			
Streptomycin	4.00		2	2		1	1		11	11			
Streptomycin	6.00					1	1						
Streptomycin	10.00		22	22		1	1						
Streptomycin	50.00		1	1									
Ethionamide	1.25					1	1						
Ethionamide	2.50				1		1						
Ethionamide	5.00	16	2	18				4		4			
Ethionamide	10.00	3	1	4									
Kanamycin	0.00										1		1
Kanamycin	5.00	10		10	1		1						
Kanamycin	6.00	9		9									
Capreomycin	0.00										1		1
Capreomycin	2.50							1		1			
Capreomycin	3.00							3		3			
Capreomycin	10.00	18		18									
Cycloserine	30.00	9		9									
Cycloserine	60.00	1		1									

* VersaTREK®, Hain GenoType®, XPERT MTB/RIF or other Molecular Methods

† Includes one borderline result

Table 5 Continued: Isolate 2012E, *M. tuberculosis*–resistant to Streptomycin at 2.0µg/ml and 10.0µg/ml by Agar Proportion method

		Test Method											
		AP			BACTEC			MGIT			Other		
Drug	Conc.	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	3		3									
Amikacin	0.00										1		1
Amikacin	1.00	1		1				1		1			
Amikacin	1.50							2		2			
Amikacin	2.00	1		1									
Amikacin	2.50	1		1									
Amikacin	4.00	4		4									
Amikacin	5.00	1		1									
Amikacin	6.00	5		5									
Amikacin	12.00	2		2									
Ofloxacin	0.00										1		1
Ofloxacin	0.60	1		1									
Ofloxacin	1.00	3		3									
Ofloxacin	1.50							1		1			
Ofloxacin	2.00	14		14	1		1	1		1			
Ciprofloxacin	0.00										1		1
Ciprofloxacin	1.00	2		2				1		1			
Ciprofloxacin	2.00	6		6									
Clofazimine	1.00	1		1									
Levofloxacin	1.50							1		1			
Levofloxacin	2.00							1		1			
Moxifloxacin	0.13							1		1			
Moxifloxacin	0.25							2		2			
Moxifloxacin	0.50	1		1									
Moxifloxacin	1.00	1		1									
Rifabutin	0.50	4		4									
Rifabutin	1.00	1		1				1		1			
Rifabutin	2.00	5		5									
Rifabutin	2.50				1		1						
Rifabutin	5.00	1		1									

* VersaTREK®, Hain GenoType®, XPERT MTB/RIF or Molecular Methods

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
ETH	ethionamide
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 *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria Drug Susceptibility Testing
NIH	National Institutes of Health
NTM	Nontuberculous Mycobacteria
OFX	ofloxacin
PAS	p-aminosalicyclic acid
PPO	Preferred Provider Organization
PZA	pyrazinamide
QRDR	quinolone-resistance-determining region
RBT	rifabutin
RMP	rifampin
RNA	ribonucleic acid
SM	streptomycin
VersaTREK®	VersaTREK®Myco Susceptibility Kit

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. 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.
3. Zhang, Y. and W.W. Yew, *Mechanisms of drug resistance in Mycobacterium tuberculosis.* Int J Tuberc Lung Dis, 2009. **13**(11): p. 1320-30.
4. Maus, C.E., B.B. Plikaytis, and T.M. Shinnick, *Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis.* Antimicrob Agents Chemother, 2005. **49**(8): p. 3192-7.
5. Zaunbrecher, M.A., et al., *Overexpression of the chromosomally encoded aminoglycoside acetyltransferase eis confers kanamycin resistance in Mycobacterium tuberculosis.* Proc Natl Acad Sci U S A, 2009. **106**(47): p. 20004-9.
6. 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.
7. 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.
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. Devasia, R.A., et al., *Fluoroquinolone resistance in Mycobacterium tuberculosis: the effect of duration and timing of fluoroquinolone exposure.* Am J Respir Crit Care Med, 2009. **180**(4): p. 365-70.
10. Chen, T.C., et al., *Fluoroquinolones are associated with delayed treatment and resistance in tuberculosis: a systematic review and meta-analysis.* Int J Infect Dis, 2011. **15**(3): p. e211-6.
11. Chang, K.C., W.W. Yew, and Y. Zhang, *Pyrazinamide susceptibility testing in Mycobacterium tuberculosis: a systematic review with meta-analyses.* Antimicrob Agents Chemother, 2011. **55**(10): p. 4499-505.
12. 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.