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 2010

UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES

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Centers for Disease Control and Prevention, or
U.S. Department of Health and Human Services.

MTB NTM DST Report for November 2010 Samples Survey

Purpose	The purpose of this report is to present the results of the Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program for *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria Drug Susceptibility Testing (MPEP MTB NTM DST) survey sent to participants in November 2010.
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Introduction: Analyses of the November 2010 *M. tuberculosis* and Nontuberculous Mycobacteria Drug Susceptibility Test Results Reported by Participating Laboratories

This report analyzes the laboratory demographic information and drug susceptibility testing results reported to the Centers for Disease Control and Prevention (CDC) by participating laboratories for the panel of five *Mycobacterium tuberculosis* complex (MTBC) isolates shipped in November 2010. Panels were sent to 100 laboratories and all laboratories participated in evaluation of the panels.

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 MTBC isolates.

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

Susceptibility Testing Results for the *M. tuberculosis* Isolates Panel Shipped November 8, 2010

The table below provides the intended results of the panel shipment that was sent to participants in November 2010. Although CDC recommends broth-based methods for routine *M. tuberculosis* complex drug susceptibility testing, this table provides the results obtained by the reference agar proportion method, except in the case of pyrazinamide, where BACTEC™ MGIT™ 960 (MGIT™) was the testing method.

Isolate	Susceptibility Testing Results
A	Resistant to Ethambutol (EMB) @ 5 µg/ml Resistant to Streptomycin (SM) @ 2 µg/ml
B	Resistant to Isoniazid (INH) @0.2 µg/ml Resistant to Ethionamide (ETH) @ 5 µg/ml
C	Resistant to Isoniazid (INH) @ 0.2 µg/ml Resistant to Ethambutol (EMB) @ 5 µg/ml Resistant to Ethionamide (ETH) @ 5 µg/ml
D	Resistant to Isoniazid (INH) @ 0.2 µg/ml Resistant to Ethambutol (EMB) @ 5 µg/ml Resistant to Ethionamide (ETH) @ 5 µg/ml
E	Resistant to Ethambutol (EMB) @ 5 µg/ml Resistant to Streptomycin (SM) @ 2 µg/ml

Descriptive Information About Participant Laboratories

Primary Classification

This report contains the drug susceptibility testing results submitted to CDC by 100 laboratories in 41 states and Puerto Rico.

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

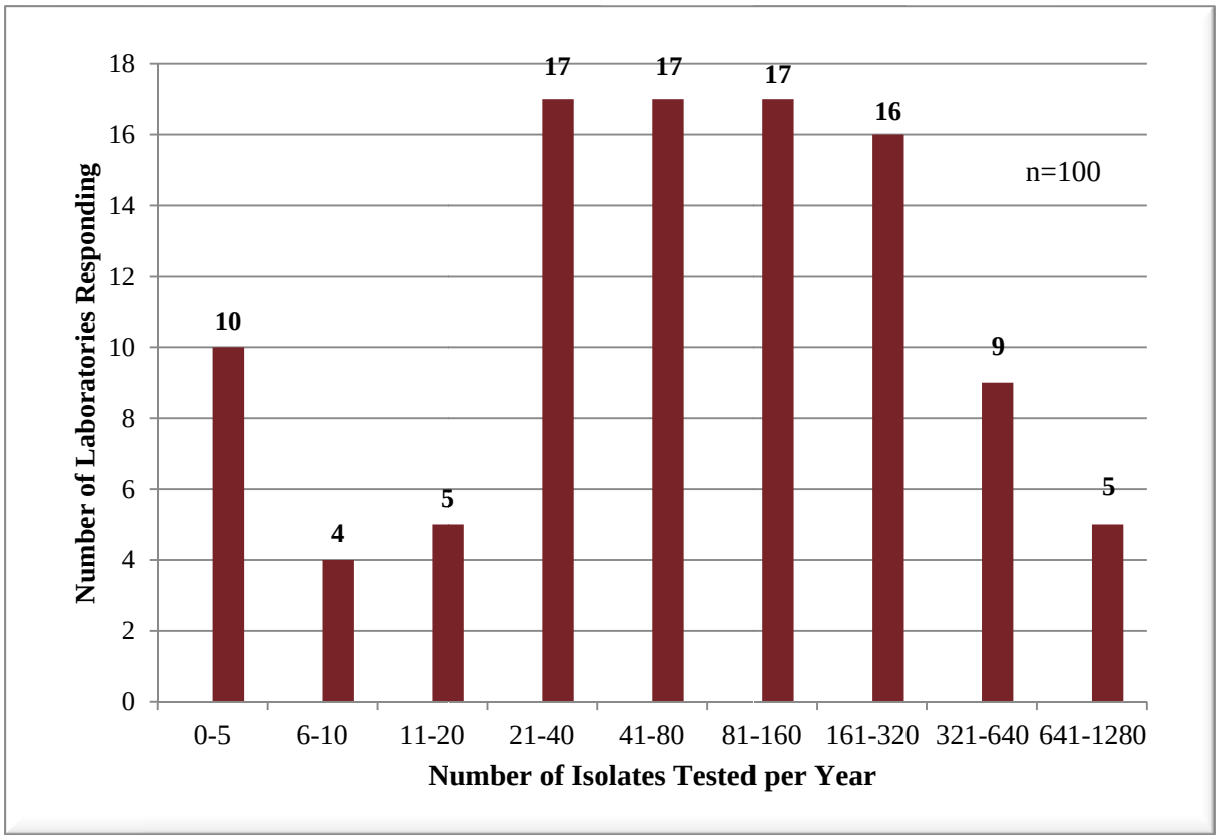
- 63 (63%): Health Department (city, county, state, regional, district, or national reference laboratory);
- 25 (25%): Hospital [city, county, district, community, state, regional, military, Veterans Administration, Federal government (other than military), privately-owned, university, HMO/PPO*-owned and operated, or religious-associated];
- 10 (10%): Independent [e.g., commercial, commercial manufacturer of reagents, HMO satellite clinic, reference laboratory (non-government affiliated)]; and
- 2 (2.0%): Other [Federal government research (nonmilitary)];

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

Annual Number of MTBC Drug Susceptibility Tests Performed by Participants

Figure 1 shows the number of drug susceptibility tests performed on MTBC isolates by the 100 participants in one **calendar year,** January 1–December 31, 2009, excluding quality control isolates. The counts range from zero to 1,055. Nineteen (19) laboratories reported performing less than 21 drug susceptibility tests per year. Two laboratories (one independent commercial laboratory and one health department laboratory not yet functional) reported zero susceptibility tests for the calendar year. To ensure testing proficiency, laboratories with low volumes are encouraged to consider referral of MTBC drug susceptibility testing.

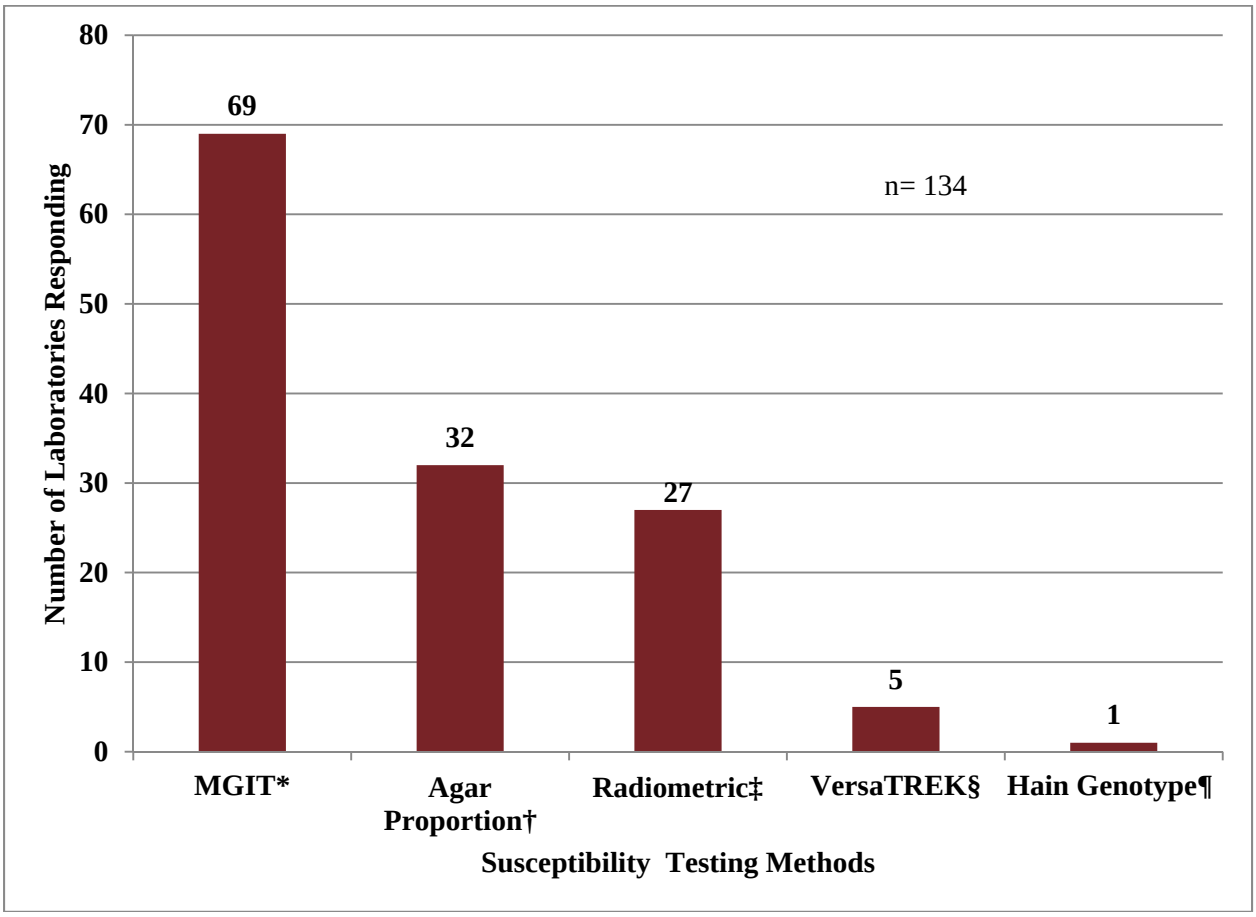
Figure 1: Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in the 2009 Calendar Year



Laboratory Susceptibility Testing Procedures Used by Participants

Participates were asked to report all MTBC susceptibility testing methods that were used to test these isolates. Sixty-eight laboratories used only one method for testing, whereas 30 laboratories used two methods, and two laboratories used three methods. Figure 2 shows the reported susceptibility methods.

Figure 2: Susceptibility Testing Methods Reported by Participant Laboratories

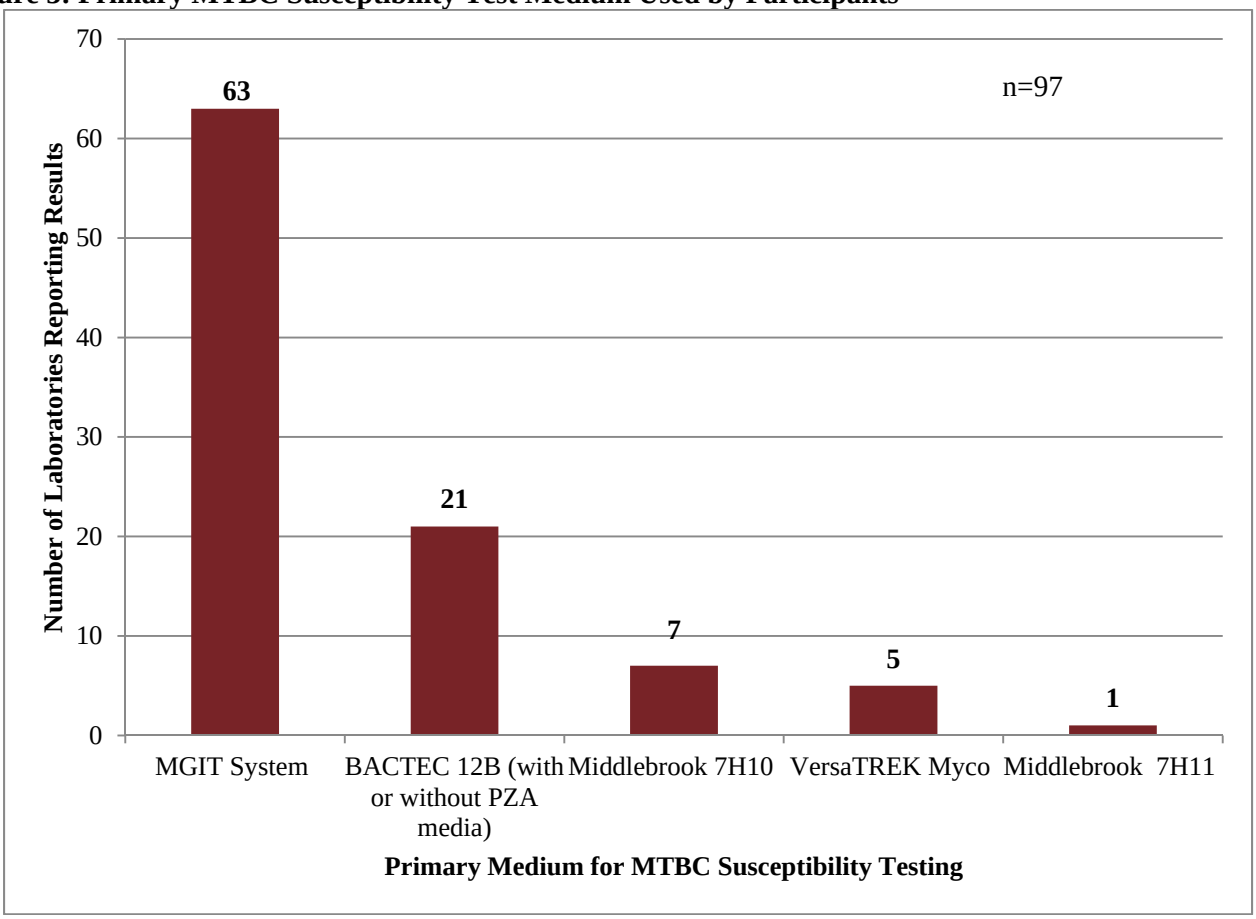


* MGIT™ Mycobacteria Growth Indicator Tube
†Agar proportion using Middlebrook 7H10 or 7H11 medium
‡Radiometric is BACTEC™ 460TB
§ VersaTREK®Myco Susceptibility Kit
¶Hain GenoType® MTBDR*plus* (detects MTBC and its resistance to rifampin and isoniazid) and Hain GenoType® MTBDRsl (a molecular genetics assay which allows the simultaneous detection of the MTBC and its resistance to fluoroquinolones, aminoglycosides, cyclic peptides and/or ethambutol).

The Primary MTBC Susceptibility Testing Media Used by Participants

Participants were asked to indicate the **primary** MTBC susceptibility test medium used by their laboratory for the isolates in the November 2010 shipment. Instructions were to select only one method as their primary method. Three participants selected two methods for this question; therefore, their responses were not included in the analysis regarding primary methods. Figure 3 shows the responses submitted by the remaining 97 participants.

Figure 3: Primary MTBC Susceptibility Test Medium Used by Participants



Of the 69 laboratories that reported using MGIT™ as one of their methods for testing the MTB NTM DST isolates,

- 63 indicated that the MGIT™ method was their primary method for susceptibility testing;
- 5 laboratories indicated Agar proportion (AP) was their primary method using Middlebrook 7H10; and
- 1 laboratory selected more than one primary method.

Of the 27 laboratories who reported using BACTEC™ 460TB as one of their methods for testing the isolates,

- 21 used this as their primary method;
- 4 used MGIT™ as their primary method; and
- 2 used AP as their primary method, (1 using Middlebrook 7H10 and 1 using Middlebrook 7H11).

Of the 32 laboratories who reported using AP as a method for testing the isolates,

- 8 used this as their primary method;
- 7 used BACTEC™ as their primary method;
- 13 used MGIT™ as their primary method;
- 1 used VersaTREK®; and
- 3 labs selected more than one primary method.

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

Nontuberculous Mycobacteria Drug Susceptibility Testing Capacity

When participating laboratories were asked if they performed susceptibility testing of Nontuberculous Mycobacteria (NTM),

- 23 (23%) responded "Yes" and
- 77 (77%) responded "No."

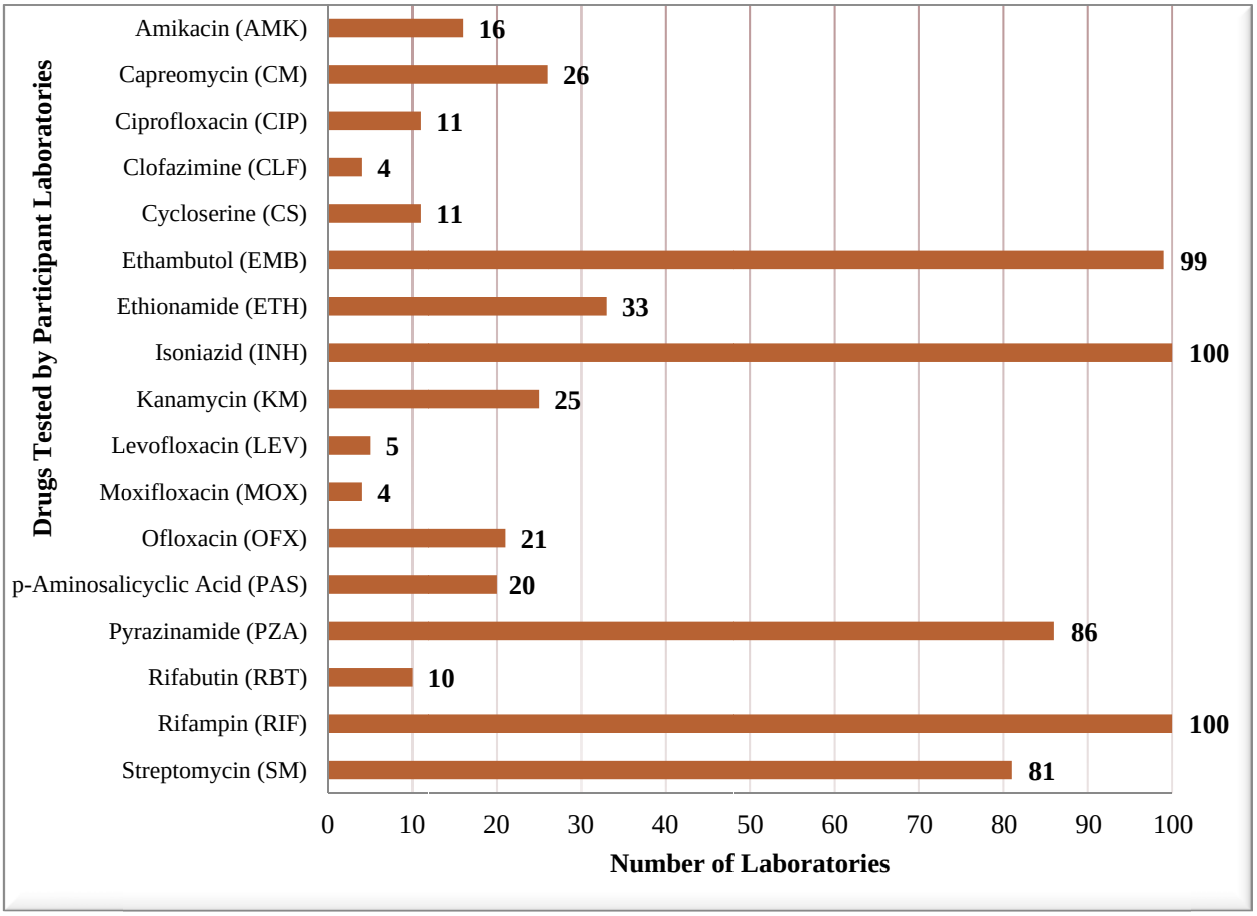
Forty-nine of the 77 laboratories (64%) that do not test NTM isolates in-house responded that they refer those isolates to another laboratory for drug susceptibility testing.

Antituberculous Drugs Used by Participants

CLSI recommends a full panel of first-line (primary) drugs (isoniazid [INH], rifampin [RIF], ethambutol [EMB], and pyrazinamide [PZA])1, since it represents a combination of tests that provides the clinician with comprehensive information related to the four-drug therapy currently recommended for treatment of most patients in the United States with tuberculosis. Of the 100 participants, 99 tested three of the first-line drugs—INH, RIF, and EMB; 86 (86%) of these also tested PZA.

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

Figure 4: Antituberculous Drugs Used by 100 Participants



Note: Providing test results for all drugs that are reported to CDC by participants should not be construed as a recommendation or endorsement for testing particular drugs or drug concentrations with MTBC isolated from patients. It is assumed that some of the drugs are being tested for research purposes or potential use in the few referral institutions that may treat patients with MTBC isolates resistant to almost all standard drugs. 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 monoresistance 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."1 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 physicians expert in the treatment and care of tuberculosis.

Explanation of Tables 1 through 5

1. In the following tables, the shaded rows indicate critical concentrations for each test method. For each drug, the critical concentration is 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]

2. The test results (S represents susceptible or 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.

3. 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]

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

5. A concentration of 0.00 is entered for results associated with genetic testing [Hain GenoType® MTBDR*plus* Assay or Hain GenoType® MTBDRsl Kit (HAIN Lifescience, Germany)] for which no drug concentration is required.

Isolate A, *M. tuberculosis*–resistant to Ethambutol at 5 µg/ml and to Streptomycin at 2 µg/ml by Agar Proportion

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] EMB targets the arabinosyl transferases (*emb*CAB operon), thereby inhibiting the biosynthesis of the cell wall components arabinogalactan and lipoarabinomannan.[2,3]

Conventional culture based methods of EMB susceptibility testing are problematic.[4] 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.[3,5]
DNA sequence analysis of *emb*B of Isolate A revealed a Gly406Asp mutation. This mutation is highly associated with EMB resistance.[6]

Ninety-seven laboratories reported EMB results for this isolate at the critical concentration (5.0 µg/ml for AP using Middlebrook 7H10; 7.5 µg/ml for AP using Middlebrook 7H11; 2.5 µg/ml for BACTEC™, 5.0 µg/ml for MGIT™, and 5.0 µg/ml for VersaTREK®), (Some laboratories submitted results for more than one method.) This isolate was reported resistant to EMB by method as follows:

- 41% (9/22) of the results when using AP (Middlebrook 7H10) (Of these 2 were reported as borderline);
- 0% (0/1) of the results when using AP (Middlebrook 7H11);
- 60% (12/20) of the results when using BACTEC (Of these 2 were reported as borderline);
- 2% (1/64) of the results when using MGIT; and
- 0% (0/5) of the results when using VersaTREK®.

The laboratory using Hain GenoType® MTBDR*sl* reported Isolate A as susceptible to EMB.

(Note—See section on Summary and Discussion of Results for information on method-dependent discrepancies.)

Streptomycin

Streptomycin (SM) is an aminoglycoside antibiotic that is active against a variety of bacteria including MTBC. SM kills actively growing bacilli but is inactive against non-growing or intracellular bacilli.2 The site of action of SM is the 30S subunit of the ribosome at the ribosomal protein S12 and the 16S rRNA. Resistance to SM is caused by mutations in the S12 protein encoded by *rps*L gene and 16S rRNA encoded by *rrs* gene.2

Eight-one laboratories reported SM results for this isolate at the critical concentration (2.0 µg/ml for AP; 2.0

µg/ml for BACTEC™, 1.0 µg/ml for MGIT™).

(Note—Some laboratories submitted results for more than one method.)

This isolate was reported resistant to SM by method as follows:

- 92 % (24/26) of the results when using AP;
- 100 % (20/20) of the results when using BACTEC™; and
- 98 % (46/47) of the results when using MGIT™.

SM is no longer considered a first-line drug1 although it is included in commercially available BACTEC and MGIT panels. Of 61 laboratories reporting only first-line drug results, 49 (79%) reported SM results. Laboratories may wish to consider the cost-effectiveness of routine SM testing as part of their first-line panel.

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

Table 1: Participant results for *M. tuberculosis*, Isolate A–resistant to EMB at 5 µg/ml and SM at 2 µg/ml by AP

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Tests* Results		
DRUG	Conc	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00										1		1
Isoniazid	0.10				21		21	68		68	5		5
Isoniazid	0.20	23		23									
Isoniazid	0.40				8		8	24		24	5		5
Isoniazid	1.00	24		24	1		1						
Isoniazid	5.00	4		4									
Rifampin	0.00										1		1
Rifampin	1.00	25		25	2		2	66		66	5		5
Rifampin	2.00				21		21	2		2			
Rifampin	5.00	3		3									
Pyrazinamide	20.00				1		1						
Pyrazinamide	100.00				17		17	63	1	64			
Pyrazinamide	300.00				1		1				5		5
Pyrazinamide	900.00				1		1						
Ethambutol	0.00										1		1
Ethambutol	2.50				8	12†	20	2		2			
Ethambutol	5.00	13	9†	22	3		3	63	1	64	5		5
Ethambutol	7.50	1		1	2		2	1		1			
Ethambutol	8.00										5		5
Ethambutol	10.00	9	1	10	1		1						
Streptomycin	1.00							1	46	47			
Streptomycin	2.00	2	24	26		20	20		3	3			
Streptomycin	4.00		1	1		1	1	10	3	13			
Streptomycin	6.00				1	1	2						
Streptomycin	10.00	19	3	22	1		1						
Ethionamide	1.25					1	1						
Ethionamide	5.00	21		21	2		2	2		2			
Ethionamide	10.00	4		4									
Kanamycin	0.00										1		1
Kanamycin	2.50				1		1						
Kanamycin	5.00	11		11	1		1						
Kanamycin	6.00	10		10									
Capreomycin	0.00										1		1
Capreomycin	2.50				1		1						
Capreomycin	3.00							2		2			
Capreomycin	5.00				2		2						
Capreomycin	10.00	18		18									
Cycloserine	25.00	1		1									
Cycloserine	30.00	8		8									
Cycloserine	50.00	1		1									
Cycloserine	60.00	2		2									
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	4		4									

* VersaTREK®, Hain GenoType®, or both

† Includes borderline results

Table 1 continued: *M. tuberculosis*, Isolate A–resistant to EMB at 5 µg/ml and SM at 2 µg/ml

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Tests* Results		
DRUG	Conc	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Amikacin	0.00										1		1
Amikacin	1.50							2		2			
Amikacin	2.00	1		1	1		1						
Amikacin	4.00	2		2									
Amikacin	5.00	1		1	1		1						
Amikacin	6.00	7		7									
Amikacin	12.00	2		2									
Ofloxacin	0.00											1	1
Ofloxacin	1.25				1		1						
Ofloxacin	2.00	14		14	4		4						
Ofloxacin	4.00				1		1						
Clofazimine	0.06					1	1						
Clofazimine	0.12					1	1						
Clofazimine	0.25				1		1						
Clofazimine	1.00	1		1									
Rifabutin	0.50	5		5	1		1						
Rifabutin	1.00	2		2									
Rifabutin	2.00	4		4									
Ciprofloxacin	1.00	1		1									
Ciprofloxacin	2.00	7		7	1		1						
Levofloxacin	1.00	1		1									
Levofloxacin	1.50							2		2			
Levofloxacin	2.00				1		1						
Moxifloxacin	0.25								1	1			
Moxifloxacin	0.50	1		1									
Moxifloxacin	1.00	2		2									

* VersaTREK®, Hain GenoType®, or both
† Includes borderline results

Isolate B, *M. tuberculosis*–resistant to Isoniazid at 0.2 µg/ml and to Ethionamide at 5 µg/ml by Agar Proportion

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 KatG encoded by the *kat*G gene.[2,5] 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.[2] 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 B revealed a cytosine to thymine transition at the nucleotide positioned 15 bases upstream of the start codon (C(-15)T)in *inh*A; *kat*G was wild-type (i.e., no mutations were detected).

The recommended critical concentration and additional higher concentrations for testing INH using the AP method are, respectively, 0.2 µg/ml and 1.0 µg/ml. The equivalent concentrations for BACTEC™, MGIT™, and VersaTREK® are 0.1 µg/ml and 0.4 µg/ml. All participants tested this isolate with at least one concentration, but 3 laboratories (2 reporting BACTEC™ results and 1 reporting AP results) did not report results at the critical concentration.

Ninety-seven 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 at the critical concentration of INH by method as follows:

- 78% (21/27) of the results when using AP;
- 100% (21/21) of the results when using BACTEC™;
- 96 % (64/67) of the results when using MGIT™;
- 80% (4/5) of the results when using VersaTREK®.

The three laboratories that did not report results at the critical concentration reported this isolate as susceptible. The laboratory using Hain GenoType® MTBDR*plus* reported INH resistance.

All laboratories reporting results at the higher concentrations of INH reported this isolate to be susceptible.

Ethionamide

Ethionamide (ETH) is a second-line drug used to treat drug-resistant TB. It is a derivative of nicotinic acid and is bactericidal against MTBC. Like INH, ETH is a prodrug that is activated by a monooxygenase and it inhibits the same target as INH (InhA).[2] Mutations in *inh*A confer resistance to ETH in addition to resistance to INH. ETH susceptibility testing is problematic.[7]

Twenty-two laboratories reported ETH results for this isolate at the critical concentration for AP (5.0 µg/ml); 11 (50%) reported resistance.

Pyrazinamide

Isolate B is susceptible to PZA.

Susceptiblity testing using a radiometric method (i.e., BACTEC) is presently considered the reference method for PZA susceptibility testing.1 Some broth-based methods for PZA testing have been reported to be problematic.[8]

Eighty-one laboratories reported PZA results for this isolate at the critical concentration (100 µg/ml for BACTEC™, 100 µg/ml for MGIT™, and 300 µg/ml for VersaTREK®) (Some laboratories submitted results from more than one method.) This isolate was reported resistant to PZA at the critical concentration by method as follows:

- 0% (0/17) of the results when using BACTEC™;
- 11 % (7/62) of the results when using MGIT™; and
- 0% (0/3) of the results when using VersaTREK®.

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

Table 2: Participant results for *M. tuberculosis*, Isolate B–resistant to INH at 0.2 µg /ml and ETH at 5 µg /ml by AP

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Tests* Results		
DRUG	Conc	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00											1	1
Isoniazid	0.10					21	21	3	64	67	1	4	5
Isoniazid	0.20	6	21	27	1		1						
Isoniazid	0.40				8		8	36		36	5		5
Isoniazid	1.00	29		29	2		2						
Isoniazid	5.00	4		4	1		1						
Rifampin	0.00										1		1
Rifampin	1.00	27		27	2		2	66		66	5		5
Rifampin	2.00				21		21	2		2			
Rifampin	5.00	4		4									
Pyrazinamide	0.00										1		1
Pyrazinamide	20.00				1		1						
Pyrazinamide	100.00				17		17	55	7	62			
Pyrazinamide	300.00				1		1				3		3
Pyrazinamide	900.00				1		1						
Ethambutol	0.00										1		1
Ethambutol	2.50				19	1	20	2		2			
Ethambutol	5.00	24		24	1	1	2	65		65	5		5
Ethambutol	7.50	1		1	2		2	1		1			
Ethambutol	8.00										5		5
Ethambutol	10.00	11		11									
Streptomycin	1.00							48		48			
Streptomycin	2.00	26	1	27	20		20	3		3			
Streptomycin	4.00	1		1				8		8			
Streptomycin	6.00				1		1						
Streptomycin	10.00	21		21									
Ethionamide	1.25					1	1						
Ethionamide	2.50					1	1						
Ethionamide	5.00	11	11	22	1	1	2		2	2			
Ethionamide	10.00	2	2	4									
Kanamycin	0.00										1		1
Kanamycin	5.00	11		11	1		1						
Kanamycin	6.00	12		12									
Capreomycin	0.00										1		1
Capreomycin	3.00							2		2			
Capreomycin	5.00				3		3						
Capreomycin	10.00	20		20									
Cycloserine	25.00	1		1									
Cycloserine	30.00	8		8									
Cycloserine	50.00	1		1									
Cycloserine	60.00	1		1									
p-Aminosalicylic acid	2.00	17		17									
p-Aminosalicylic acid	4.00				1		1						
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	3		3									

* VersaTREK®, Hain GenoType®, or both

Table 2 continued: *M. tuberculosis*, Isolate B–resistant to INH at 0.2 µg /ml and ETH at 5 µg /ml

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Tests* Results		
DRUG	Conc	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Amikacin	0.00										1		1
Amikacin	1.50							2		2			
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	12.00	2		2									
Ofloxacin	0.00										1		1
Ofloxacin	1.00	1		1	1		1						
Ofloxacin	2.00	13	1	14	3	1	4	1		1			
Ofloxacin	4.00				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	1		1									
Rifabutin	0.50	5		5	1		1						
Rifabutin	1.00	2		2									
Rifabutin	2.00	4		4									
Ciprofloxacin	1.00	2		2	1		1						
Ciprofloxacin	2.00	8		8	1		1						
Levofloxacin	1.00	1		1									
Levofloxacin	1.50							2		2			
Levofloxacin	2.00				2		2						
Moxifloxacin	0.25							1		1			
Moxifloxacin	0.50	1		1									
Moxifloxacin	1.00	2		2									

* VersaTREK®, Hain GenoType®, or both

Isolate C, *M. tuberculosis*–resistant to Isoniazid at 0.2 µg/ml, Ethambutol at 5 µg/ml, and Ethionamide at 5 µg/ml by Agar Proportion

Isoniazid

As noted in the section for Isolate B, there are two described mechanisms that account for the majority of INH resistance. Mutations in *kat*G are generally associated with high-level resistance to INH. Mutations in the promoter region of the *inh*A gene 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 C revealed a cytosine to thymine transition at the nucleotide positioned 15 bases upstream of the start codon (C(-15)T) in *inh*A; *kat*G was wild-type (i.e., no mutations were detected).

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

- 93 % (25/27) of the results when using AP;
- 100% (21/21) of the results when using BACTEC™;
- 100 % (65/65) of the results when using MGIT™; and
- 100% (5/5) of the results when using VersaTREK®.

The three laboratories that did not report results at the critical concentration reported this isolate as susceptible. The laboratory using Hain GenoType® MTBDR*plus* reported INH resistance.

Three percent (2/64) of laboratories reporting results at the higher concentrations of INH reported this isolate to be resistant.

Ethambutol

As noted in the section for Isolate A, mutations in *emb*B codons 306, 406, and 497 are among the most frequent mutations in EMB-resistant isolates, and serve as predictors of EMB resistance. DNA sequence analysis of *emb*B of Isolate C revealed a Met306Val mutation. This mutation is highly associated with EMB resistance.[3,5]

Ninety-eight laboratories reported EMB results for this isolate at the critical concentration (5.0 µg/ml for AP using Middlebrook 7H10; 7.5 µg/ml for AP using Middlebrook 7H11; 2.5 µg/ml for BACTEC™, 5.0 µg/ml for MGIT™, and 5.0 µg/ml for VersaTREK®), (Some laboratories submitted results from more than one method.) This isolate was reported resistant to EMB by method as follows:

- 89 % (23/26) of the results when using AP;
- 95% (19/20) of the results when using BACTEC™;
- 21% (13/61) of the results when using MGIT™;
- 80% (4/5) of the results when using VersaTREK®;

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

(Note—See section on Summary and Discussion of Results for information on method-dependent discrepancies.)

Ethionamide

Twenty-three laboratories reported ETH results for this isolate at the critical concentration for AP (5.0 µg/ml); 18 (78%) reported resistance.

Pyrazinamide

Isolate C is susceptible to PZA.

Eight-three laboratories reported PZA results for this isolate at the critical concentration (100 µg/ml for BACTEC™, 100 µg/ml for MGIT™, and 300 µg/ml for VersaTREK®) (Some laboratories submitted results for more than one method.) This isolate was reported resistant at the critical concentration by method as follows:

- 0% (0/17) of the results when using BACTEC™;
- 32% (20/62) of the results when using MGIT™; and
- 100% (3/3) of the results when using VersaTREK®.

(Note—See section on Summary and Discussion of Results for information on method-dependent discrepancies.)

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

Table 3: Participant results for *M. tuberculosis*, Isolate C–resistant to INH at 0.2 µg/ml, EMB at 5 µg/ml, and ETH at 5 µg/ml by AP

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Tests* Results		
DRUG	Conc	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00											1	1
Isoniazid	0.10					21	21		65	65		5	5
Isoniazid	0.20	2	25	27	1		1						
Isoniazid	0.40				8		8	33	2	35	5		5
Isoniazid	1.00	29		29	2		2						
Isoniazid	5.00	4		4	1		1						
Rifampin	0.00										1		1
Rifampin	1.00	27		27	2		2	64		64	5		5
Rifampin	2.00				21		21	2		2			
Rifampin	5.00	4		4									
Pyrazinamide	0.00										1		1
Pyrazinamide	20.00				1		1						
Pyrazinamide	100.00				17		17	42	20	62			
Pyrazinamide	300.00				1		1					3	3
Pyrazinamide	900.00				1		1						
Ethambutol	0.00											1	1
Ethambutol	2.50				1	19	20	2		2			
Ethambutol	5.00	3	23	26		3	3	48	13	61	1	4	5
Ethambutol	7.50	1		1	3		3	1		1			
Ethambutol	8.00										5		5
Ethambutol	10.00	10	2	12	1		1						
Streptomycin	1.00							48		48			
Streptomycin	2.00	26	1	27	19	1	20	2	1	3			
Streptomycin	4.00	1		1				9		9			
Streptomycin	6.00				1		1						
Streptomycin	10.00	21		21									
Ethionamide	1.25					2	2						
Ethionamide	2.50					1	1						
Ethionamide	5.00	5	18	23		2	2		2	2			
Ethionamide	10.00	1	3	4									
Kanamycin	0.00										1		1
Kanamycin	2.50				1		1						
Kanamycin	5.00	11		11	2		2						
Kanamycin	6.00	12		12									
Capreomycin	0.00										1		1
Capreomycin	1.25				1		1						
Capreomycin	2.50				1		1						
Capreomycin	3.00							2		2			
Capreomycin	5.00				3		3						
Capreomycin	10.00	20		20									
Cycloserine	25.00	1		1									
Cycloserine	30.00	9		9									
Cycloserine	50.00	1		1									
Cycloserine	60.00	2		2									

* VersaTREK®, Hain GenoType®, or both

Table 3 continued: *M. tuberculosis,* Isolate C–resistant to INH at 0.2 µg/ml, EMB at 5 µg/ml, and ETH at 5 µg/ml

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Tests* Results		
DRUG	Conc	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
p-Aminosalicylic acid	2.00	18		18									
p-Aminosalicylic acid	4.00				1		1						
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	4		4									
Amikacin	0.00										1		1
Amikacin	1.50							2		2			
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	12.00	2		2									
Ofloxacin	0.00										1		1
Ofloxacin	1.00	1		1	1		1						
Ofloxacin	1.25					1	1						
Ofloxacin	2.00	13	1	14	4	1	5	1		1			
Ofloxacin	2.50				1		1						
Ofloxacin	4.00				1		1						
Ofloxacin	5.00				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	1		1									
Rifabutin	0.50	5		5	2		2						
Rifabutin	1.00	2		2									
Rifabutin	2.00	4		4									
Ciprofloxacin	1.00	2		2	1		1						
Ciprofloxacin	2.00	8		8	1		1						
Levofloxacin	1.00	1		1									
Levofloxacin	1.50							2		2			
Levofloxacin	2.00				2		2						
Moxifloxacin	0.25							1		1			
Moxifloxacin	0.50	1		1									
Moxifloxacin	1.00	2		2									

* VersaTREK®, Hain GenoType®, or both

Isolate D, *M. tuberculosis*–resistant to Isoniazid at 0.2 µg/ml, Ethambutol at 5 µg/ml, and Ethionamide at 5 µg/ml by Agar Proportion

Isolate D is a duplicate of Isolate C.

Isoniazid

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

- 85% (23/27) of the results when using AP;
- 100% (21/21) of the results when using BACTEC™;
- 97% (63/65) of the results when using MGIT™;
- 100% (5/5) of the results when using VersaTREK®;

The 3 laboratories that did not report results at the critical concentration reported this isolate as susceptible. The laboratory using Hain GenoType® MTBDR*plus* reported INH resistance.

Two percent (1/64) laboratories reporting results at the higher concentrations of INH reported this isolate to be resistant.

Ethambutol

Ninety-eight laboratories reported EMB results for this isolate at the critical concentration (5.0 µg/ml for AP using Middlebrook 7H10; 7.5 µg/ml for AP using Middlebrook 7H11; 2.5 µg/ml for BACTEC™, 5.0 µg/ml for MGIT™, and 5.0 µg/ml for VersaTREK®). (Some laboratories submitted results from more than one method.) This isolate was reported resistant to EMB by method as follows:

- 92 % (24/26) of the results when using AP (Of these one result was reported as borderline);
- 95% (19/20) of the results when using BACTEC™;
- 25% (15/61) of the results when using MGIT™ (Of these one result was reported as borderline);
- 80% (4/5) of the results when using VersaTREK®;

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

(Note—See section on Summary and Discussion of Results for information on method-dependent discrepancies.)

Ethionamide

Twenty-three laboratories reported ETH results for this isolate at the critical concentration for AP (5.0 µg/ml); 18 (78%) reported resistance.

Pyrazinamide

Eighty-three laboratories reported PZA results for this isolate at the critical concentration (100 µg/ml for BACTEC™, 100 µg/ml for MGIT™, and 300 µg/ml for VersaTREK®) (Some laboratories submitted results from more than one method.) This isolate was reported resistant at the critical concentration by method as follows:

- 0% (0/17) of the results when using BACTEC™;
- 33% (21/63) of the results when using MGIT™; and
- 100% (3/3) of the results when using VersaTREK®.

(Note—See section on Summary and Discussion of Results for information on method-dependent discrepancies.)

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

Table 4: Participant results for *M. tuberculosis*, Isolate D–resistant to INH at 0.2 µg/ml, EMB at 5 µg/ml, and ETH at 5 µg/ml by AP

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Tests* Results		
DRUG	Conc	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00											1	1
Isoniazid	0.10					21	21	2	63	65		5	5
Isoniazid	0.20	4	23	27	1		1						
Isoniazid	0.40				8		8	34	1	35	5		5
Isoniazid	1.00	29		29	2		2						
Isoniazid	5.00	4		4	1		1						
Rifampin	0.00										1		1
Rifampin	1.00	27		27	2		2	65		65	5		5
Rifampin	2.00				20	1	21	2		2			
Rifampin	5.00	4		4									
Pyrazinamide	0.00										1		1
Pyrazinamide	20.00				1		1						
Pyrazinamide	100.00				17		17	41	21	62			
Pyrazinamide	300.00				1		1					3	3
Pyrazinamide	900.00				1		1						
Ethambutol	0.00											1	1
Ethambutol	2.50				1	19	20	2		2			
Ethambutol	5.00	2	24†	26		3	3	46	16†	62	1	4	5
Ethambutol	7.50	1		1	2	1	3	1		1			
Ethambutol	8.00										5		5
Ethambutol	10.00	10	3	13	1		1						
Streptomycin	1.00							48		48			
Streptomycin	2.00	26	1	27	20		20	3		3			
Streptomycin	4.00	1		1				9		9			
Streptomycin	6.00				1		1						
Streptomycin	10.00	21		21									
Ethionamide	1.25					2	2						
Ethionamide	2.50					1	1						
Ethionamide	5.00	5	18	23		2	2		2	2			
Ethionamide	10.00	1	3	4									
Kanamycin	0.00										1		1
Kanamycin	2.50				1		1						
Kanamycin	5.00	11		11	2		2						
Kanamycin	6.00	12		12									
Capreomycin	0.00										1		1
Capreomycin	1.25				1		1						
Capreomycin	2.50				1		1						
Capreomycin	3.00							2		2			
Capreomycin	5.00				3		3						
Capreomycin	10.00	20		20									
Cycloserine	25.00	1		1									
Cycloserine	30.00	9		9									
Cycloserine	50.00	1		1									
Cycloserine	60.00	2		2									

* VersaTREK®, Hain GenoType®, or both
† Includes borderline results

Table 4 continued: *M. tuberculosis,* Isolate D–resistant to INH at 0.2 µg/ml, EMB at 5 µg/ml, and ETH at 5 µg/ml

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Tests* Results		
DRUG	Conc	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
p-Aminosalicylic acid	2.00	17	1	18									
p-Aminosalicylic acid	4.00				1		1						
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	3	1	4									
Amikacin	0.00										1		1
Amikacin	1.50							2		2			
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	12.00	2		2									
Ofloxacin	0.00										1		1
Ofloxacin	1.00	1		1	1		1						
Ofloxacin	1.25					1	1						
Ofloxacin	2.00	14		14	4	1	5	1		1			
Ofloxacin	2.50				1		1						
Ofloxacin	4.00				1		1						
Ofloxacin	5.00				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	1		1									
Rifabutin	0.50	5		5	2		2						
Rifabutin	1.00	2		2									
Rifabutin	2.00	4		4									
Ciprofloxacin	1.00	2		2	1		1						
Ciprofloxacin	2.00	8		8	1		1						
Levofloxacin	1.00	1		1									
Levofloxacin	1.50							2		2			
Levofloxacin	2.00				2		2						
Moxifloxacin	0.25							1		1			
Moxifloxacin	0.50	1		1									
Moxifloxacin	1.00	2		2									

* VersaTREK®, Hain GenoType®, or both
† Includes borderline results

Isolate E, *M. tuberculosis*–resistant to Ethambutol at 5 µg/ml and to Streptomycin at 2 µg/ml by Agar Proportion

Isolate E is a duplicate of Isolate A.

Ethambutol

Ninety-eight laboratories reported EMB results for this isolate at the critical concentration (5.0 µg/ml for AP using Middlebrook 7H10; 7.5 µg/ml for AP using Middlebrook 7H11; 2.5 µg/ml for BACTEC™, 5.0 µg/ml for MGIT™, and 5.0 µg/ml for VersaTREK®), (Some laboratories submitted results from more than one method.) This isolate was reported resistant to EMB by method as follows:

- 38% (9/24) of the results when using AP (7H10) (Of these one result was reported as borderline);
- 0% (0/1) of the results when using AP (7H11);
- 65% (13/20) of the results when using BACTEC™ (Of these 2 were reported as borderline);
- 2% (1/64) of the results when using MGIT™; and
- 0% (0/5) of the results when using VersaTREK®.

The laboratory using Hain GenoType® MTBDR*sl* reported Isolate A as susceptible.

(Note—See section on Summary and Discussion of Results for information on method-dependent discrepancies.)

Streptomycin

Eighty-one laboratories reported SM results for this isolate at the critical concentration (2.0 µg/ml for AP; 2.0 µg/ml for BACTEC™, 1.0 µg/ml for MGIT™). (Some laboratories submitted results from more than one method.) This isolate was reported resistant to SM by method as follows:

- 85 % (23/27) of the results when using AP;
- 100 % (20/20) of the results when using BACTEC™; and
- 100% (47/47) of the results when using MGIT™.

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

Table 5: Participant results for *M. tuberculosis*, Isolate E–resistant to EMB at 5 µg/ml and SM at 2 µg/ml by AP

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Tests* Results		
DRUG	Conc	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
Isoniazid	0.00										1		1
Isoniazid	0.10				21		21	68		68	5		5
Isoniazid	0.20	23	1	24									
Isoniazid	0.40				8		8	22		22	5		5
Isoniazid	1.00	25		25	1		1						
Isoniazid	5.00	4		4									
Rifampin	0.00										1		1
Rifampin	1.00	26		26	2		2	66		66	5		5
Rifampin	2.00				21		21	2		2			
Rifampin	5.00	3		3									
Pyrazinamide	20.00				1		1						
Pyrazinamide	100.00				17		17	64		64			
Pyrazinamide	300.00				1		1				4		4
Pyrazinamide	900.00				1		1						
Ethambutol	0.00										1		1
Ethambutol	2.50				7	13†	20	2		2			
Ethambutol	5.00	15	9†	24	3		3	63	1	64	5		5
Ethambutol	7.50	1		1	2		2						
Ethambutol	8.00										5		5
Ethambutol	10.00	11		11	1		1						
Streptomycin	1.00								47	47			
Streptomycin	2.00	4	23	27		20	20		3	3			
Streptomycin	4.00		1	1		1	1	11	2	13			
Streptomycin	6.00				1	1	2						
Streptomycin	10.00	20	2	22	1		1						
Ethionamide	1.25					1	1						
Ethionamide	5.00	22		22	2		2	2		2			
Ethionamide	10.00	4		4									
Kanamycin	0.00										1		1
Kanamycin	2.50				1		1						
Kanamycin	5.00	11		11	1		1						
Kanamycin	6.00	11		11									
Capreomycin	0.00										1		1
Capreomycin	2.50				1		1						
Capreomycin	3.00							2		2			
Capreomycin	5.00				2		2						
Capreomycin	10.00	19		19									
Cycloserine	25.00	1		1									
Cycloserine	30.00	9		9									
Cycloserine	50.00	1		1									
Cycloserine	60.00	2		2									

* VersaTREK®, Hain GenoType®, or both

† Includes borderline results

Table 5 continued: *M. tuberculosis,* Isolate E–resistant to EMB at 5 µg/ml and SM at 2 µg/ml

		Test Method											
		AP Results			BACTEC Results			MGIT Results			Other Tests* Results		
DRUG	Conc	S	R	Sum	S	R	Sum	S	R	Sum	S	R	Sum
p-Aminosalicylic acid	2.00	17		17									
p-Aminosalicylic acid	4.00				1		1						
p-Aminosalicylic acid	8.00	2		2									
p-Aminosalicylic acid	10.00	4		4									
Amikacin	0.00										1		1
Amikacin	1.50							2		2			
Amikacin	2.00	1		1	1		1						
Amikacin	4.00	2		2									
Amikacin	5.00	1		1	1		1						
Amikacin	6.00	7		7									
Amikacin	12.00	2		2									
Ofloxacin	0.00										1		1
Ofloxacin	1.25				1		1						
Ofloxacin	2.00	14		14	4		4						
Ofloxacin	4.00				1		1						
Clofazimine	0.06					1	1						
Clofazimine	0.12					1	1						
Clofazimine	0.25				1		1						
Clofazimine	1.00	1		1									
Rifabutin	0.50	5		5	1		1						
Rifabutin	1.00	2		2									
Rifabutin	2.00	4		4									
Ciprofloxacin	1.00	1		1									
Ciprofloxacin	2.00	8		8	1		1						
Levofloxacin	1.00	1		1									
Levofloxacin	1.50							2		2			
Levofloxacin	2.00				1		1						
Moxifloxacin	0.25							1		1			
Moxifloxacin	0.50	1		1									
Moxifloxacin	1.00	2		2									

* VersaTREK®, Hain GenoType®, or both
† Includes borderline results

Summary and Discussion of Results

Three unique isolates of MTBC were included in the November 2010 MPEP panel; two of these three isolates were sent in duplicate (A=E and C=D). The results of the challenge were surprising for both Ethambutol (EMB) and Pyrazinamide (PZA).

Ethambutol

Isolates A, C, D, and E were intended to be resistant to EMB and the isolates were shown to have mutations in the *embB* locus that are highly associated with EMB resistance. However, most laboratories did not detect EMB resistance in isolates A and E, and many did not detect EMB resistance for isolates C and D. Not detecting EMB resistance appears to be method and isolate-dependent.

Based on the results for the recent MPEP panel, one could postulate that the BACTEC 460 method is a more sensitive method than AP or other broth-based methods for detecting EMB resistance. It may be necessary to interpret conventional growth-based testing results in conjunction with molecular results to determine optimal treatment options until the weaknesses in the current testing methods are understood and addressed.

Pyrazinamide

All isolates were intended to be susceptible to PZA. The CLSI-recommended method for PZA testing is the BACTEC 460 radiometric system. All laboratories reporting BACTEC 460 results reported all of the challenge isolates and susceptible to PZA. However, for laboratories reporting MGIT and VersaTREK results, about a third reporting MGIT and all reporting VersaTREK reported isolates C and D resistant to PZA, a few laboratories reporting MGIT reported isolate B as PZA resistant, and no laboratories reported isolates A and E as resistant. Detection of false resistance to PZA appears to be method and isolate-dependent.

The problem of PZA false resistance when using MGIT has been described in the literature.[8] Due to the potential for false resistant results, laboratories may consider retesting PZA-resistant isolates with the BACTEC 460 method, if available. Unfortunately the BACTEC 460 will not be commercially available in the near future. Thus, laboratories must work to find a successful testing algorithm for providing accurate PZA susceptibility results.

Abbreviations Used in This Report

AMK	amikacin
AP	agar proportion
BACTEC™	BACTEC™ 460TB
bp	base pair
BSL	Biosafety Level
CDC	Centers for Disease Control and Prevention (CDC)
CIP	ciprofloxacin
CLF	clofazimine
CLSI	Clinical Laboratory and Standards Institute
CM	capreomycin
CS	cycloserine
DNA	deoxyribonucleic acid
DST	Drug Susceptibility Testing
EMB	ethambutol
HMO	Health Maintenance Organization
INH	isoniazid
KM	kanamycin
LEV	levofloxacin
MGIT™	BACTEC™ MGIT™ 960 (Mycobacteria Growth Indicator Tube)
MOX	moxifloxacin
MPEP MTB NTM DST	Model Performance Evaluation Program for *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria Drug Susceptibility Testing
MTBC	*Mycobacterium tuberculosis* complex
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
RIF	rifampin
RNA	ribonucleic acid
SM	streptomycin
ETH	ethionamide
VersaTREK®	VersaTREK®Myco Susceptibility Kit

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