Centers for Disease Control and Prevention
Model Performance Evaluation Program

*Mycobacterium tuberculosis*
Drug Susceptibility Testing Program

Report of Results
November 2012
Performance Evaluation Survey

United States Department of Health and Human Services

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MTB DST Report for November 2012 Samples Survey

Purpose
The purpose of this report is to present the results of the Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program for *Mycobacterium tuberculosis* drug susceptibility testing survey sent to participants in November 2012.

Report Content
The material in this report was developed and prepared by

Cortney Stafford, MPH, MT (ASCP), Health Scientist, Laboratory Capacity Team, NCHHSTP, DTBE, LB

Beverly Metchock, DrPH, D(ABMM), Team Lead, Reference Laboratory, NCHHSTP, DTBE, LB

Acknowledged contributors: Lois Diem CDC/Atlanta, Mitch Yakrus CDC/Atlanta, Angela Starks CDC/Atlanta

Contact Information
Comments and inquiries regarding this report should be directed to the Model Performance Evaluation Program (MPEP)

TBMPEP@cdc.gov

404-639-4013
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Introduction: Overview of MPEP Final Report

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

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

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

Expected Susceptibility Testing Results

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

<table>
<thead>
<tr>
<th></th>
<th>First-line Drugs</th>
<th>Second-line Drugs</th>
<th>Resistant to:</th>
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</thead>
<tbody>
<tr>
<td>INH</td>
<td>RMP</td>
<td>EMB</td>
<td>PZA</td>
</tr>
<tr>
<td>2012F</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2012G</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2012H</td>
<td>S</td>
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<td>S</td>
</tr>
<tr>
<td>2012I</td>
<td>S</td>
<td>R</td>
<td>R*</td>
</tr>
<tr>
<td>2012J</td>
<td>S</td>
<td>S</td>
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</tr>
</tbody>
</table>

*Less than 80% of reported results agreed with the expected result.*
Descriptive Information about Participant Laboratories

Primary Classification

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

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

- 62 (70%): Health Department (city, country, state, regional, district, or national reference laboratory)
- 17 (19%): Hospital [city, county, district, community, state, regional, military, Veterans Administration, Federal government (other than military), privately-owned, university, HMO/PPO*-owned and operated, or religious-associated]
- 7 (8%): Independent [e.g., commercial, commercial manufacturer of reagents, HMO* satellite clinic, reference laboratory (non-governmental affiliated)]
- 3 (3%): Other [Federal government research (nonmilitary)].

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

Figure 1. Primary Classification of Participating Laboratories

Health Department (e.g. city, county, state, regional, district, national reference laboratory)

Hospital

Independent (non-hospital based)

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

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

![Figure 2. Annual Volume of *M. tuberculosis* Isolates Tested for Drug Susceptibility in 2011 (N=89)](chart.png)
**Laboratory Susceptibility Testing Procedures Used**

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

![Figure 3. Susceptibility Testing Methods Reported (N=133)](image)

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

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

![Figure 4. Initial M. tuberculosis Susceptibility Test Method Used (N=89)](image)
Antituberculosis Drugs Tested

CLSI recommends a full panel of first-line drugs (isoniazid [INH], rifampin [RMP], ethambutol [EMB], and pyrazinamide [PZA])[1], because it represents a combination of tests that provides the clinician with comprehensive information related to the four-drug therapy currently recommended for treatment of most patients in the United States with tuberculosis. All participants reported results for three of the first-line drugs—INH, RMP, and EMB; 79 (89%) of the participants also reported results for PZA.

Figure 5. Antituberculosis Drugs Tested

![Antituberculosis Drugs Tested Chart]

- Rifampin: 89
- Isoniazid: 89
- Ethambutol: 89
- Pyrazinamide: 79
- Streptomycin: 65
- Ciprofloxacin: 9
- Ofloxacin: 20
- Levofloxacin: 3
- Moxifloxacin: 7
- Amikacin: 14
- Kanamycin: 24
- Capreomycin: 23
- Ethionamide: 30
- Rifabutin: 43
- Cycloserine: 10
- p-Aminosalicylic Acid: 20
- Number of Laboratories
Basic Information for Isolates F-J

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

- First-line and second-line drugs have been separated into individual tables for each isolate. Streptomycin is now included as part of the second-line table.

- Laboratories that use more than one DST method are encouraged to test isolates with each of those methods at the CLSI-recommended, or equivalent, critical concentrations. Also, some laboratories provided results for additional drug concentrations. Consequently, the number of results for some drugs may be greater than 89 (the number of participating laboratories). This report contains all results reported by participating laboratories, including drug concentrations with only one result.

- The tables indicate the number of reported results (S represents Susceptible and R represents Resistant) for each drug at the noted concentration.

- Separate tables for molecular testing are included where data is of note; otherwise findings are reported in the summary. If results are not provided for molecular tests, laboratories did not detect a mutation.

- A list of critical concentrations for antituberculosis drugs, by method, can be found at the end of this report.

- Of the 31 laboratories reporting second-line drug results (with the exception of streptomycin), only 9 (29%) tested the three second-line injectables and one fluoroquinolone needed to define XDR TB.
Isolate F

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

Isoniazid
Isoniazid is the most widely used first-line antituberculosis drug. It is a cornerstone of all effective regimens for the treatment of TB disease and latent infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme KatG encoded by the katG gene [3, 4]. The target of activated INH is enoyl-acyl-carrier protein reductase (InhA) which is required for mycolic acid biosynthesis. There are two described mechanisms that account for the majority of INH resistance [3, 4]. The most common mechanism, mutations in katG, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the inhA gene which are generally associated with low-level resistance. DNA sequence analysis of inhA and katG of Isolate F revealed no mutations; this is known to occur in approximately 10-15% of isolates found to be INH resistant.

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

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

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

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

Streptomycin

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

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

See Tables 1 and 2 for the complete results submitted by all participants for Isolate 2012F.
Table 1. Isolate F—Participant results for first-line drug susceptibility testing

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<thead>
<tr>
<th>Drug</th>
<th>Conc. (µg/ml)</th>
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<th>BACTEC 460</th>
<th>MGIT</th>
<th>VersaTREK</th>
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<tbody>
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*In addition, one laboratory reported borderline for ethambutol by MGIT
** In addition, one laboratory reported borderline for pyrazinamide by MGIT
Table 2. Isolate F—Participant results for second-line drug susceptibility testing

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

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

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

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

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

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. (µg/ml)</th>
<th>AP</th>
<th>BACTEC 460</th>
<th>MGIT</th>
<th>VersaTREK</th>
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Table 4. Isolate G—Participant results for second-line drug susceptibility testing

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Isolate H

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

Streptomycin

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

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

Pyrazinamide

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

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

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

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

See Tables 5 and 6 for the complete results submitted by all participants for Isolate 2012H.
Table 5. Isolate H—Participant results for first-line drug susceptibility testing

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* In addition, one laboratory reported borderline for rifampin by agar proportion.
** In addition, one laboratory reported borderline for pyrazinamide by MGIT.
Table 6. Isolate H—Participant results for second-line drug susceptibility testing

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

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

**Rifampin**

Rifampin is a first-line drug for treatment of all forms of tuberculosis caused by organisms known or presumed to be susceptible to this drug. It is bactericidal for *M. tuberculosis* at the critical concentration of 1.0 μg/ml for AP (on Middlebrook 7H10 and 7H11 agars) and equivalent critical concentrations for BACTEC™ 460, MGIT™, and VersaTREK® of 2.0 μg/ml, 1.0 μg/ml, and 1.0 μg/ml, respectively. The mechanism of action of RMP is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase. More than 96% of RMP-resistant isolates contain a mutation in the 81-base pair (bp) central region of the *rpoB* gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase [3, 4]. The activity of RMP in RMP-resistant isolates depends on both the mutation position and the type of amino acid change in the *rpoB* gene. Mutations in codons 531, 526, and 516 are among the most frequent mutations in RMP-resistant isolates and serve as predictors of RMP resistance. DNA sequence analysis of *rpoB* in Isolate 2012I revealed a C>T point mutation in the *rpoB* locus resulting in histidine being replaced by tyrosine at codon 526 (His526Tyr).

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

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

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

**Rifabutin**

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

**Ethambutol**

Ethambutol (EMB) is an important first-line drug for the treatment of tuberculosis and is used in combination with INH, RMP, and PZA to prevent emergence of drug resistance. EMB is a
bacteriostatic agent that is active against growing bacilli and has no effect on non-replicating bacilli [3, 4]. EMB targets the arabinosyl transferases (embCAB operon), thereby inhibiting the biosynthesis of the cell wall components arabinogalactan and lipoarabinomannan [6].

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

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

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

**Pyrazinamide**

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

**Isoniazid**

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

*See Tables 7, 8, and 9 for the complete results submitted by all participants for Isolate 2012I.*
### Table 7. Isolate I—Participant results for first-line drug susceptibility testing

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. (µg/ml)</th>
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<th>BACTEC 460</th>
<th>MGIT</th>
<th>VersaTREK</th>
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*In addition, one laboratory reported borderline for rifampin by agar proportion.
**In addition, one laboratory reported borderline for isoniazid at 0.1 and 0.4 by MGIT
#In addition, one laboratory reported borderline for pyrazinamide by MGIT
### Table 8. Isolate I—Participant results for second-line drug susceptibility testing

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<th>MGIT</th>
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*In addition, one laboratory reported borderline for streptomycin by agar proportion and MGIT.
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<td>Capreomycin</td>
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Isolate J

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

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

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

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

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. (µg/ml)</th>
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<th>BACTEC 460</th>
<th>MGIT</th>
<th>VersaTREK</th>
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<td></td>
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*In addition, one laboratory reported borderline for ethambutol by MGIT
## Table 11. Isolate J—Participant results for second-line drug susceptibility testing

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Glossary

AP     agar proportion – performed on Middlebrook 7H10 or 7H11
BACTEC™ 460  BACTEC™ 460TB – a radiometric broth based DST method
bp     base pair
CDC    U.S. Centers for Disease Control and Prevention
CLSI   Clinical Laboratory and Standards Institute
DNA    deoxyribonucleic acid
DST    drug susceptibility testing
HMO    Health Maintenance Organization
MDR    multidrug-resistant
MGIT™  BACTEC™ MGIT™ 960 – Mycobacteria Growth Indicator Tube
MPEP   Model Performance Evaluation Program
MTBC   Mycobacterium tuberculosis complex
PPO    Preferred Provider Organization
RNA    ribonucleic acid
TB     Tuberculosis
VersaTREK®  VersaTREK® Myco susceptibility kit
XDR    extensively drug-resistant

RMP    rifampin
INH    isoniazid
EMB    ethambutol
PZA    pyrazinamide
RBT    rifabutin
SM     streptomycin
# Equivalent Critical Concentrations

(Concentrations listed as µg/ml)

## Agar Proportion

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NOTE: Critical concentrations as indicated in CLSI M24-A2 document [1]

*The higher concentration of INH and EMB should be tested as second-line drugs after resistance at the critical concentration is detected.

†Breakpoints for establishing susceptibility have not be determined

## Broth Based Media

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NOTE: Critical concentrations as indicated in applicable manufacturer package inserts

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


