Centers for Disease Control and Prevention
Model Performance Evaluation Program

# *Mycobacterium tuberculosis* Complex Drug Susceptibility Testing Program

Report of Results
May 2014
Performance Evaluation Survey

National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention
Division of Tuberculosis Elimination

CS253300

## *Mycobacterium tuberculosis* Complex Drug Susceptibility Testing Report for May 2014 Survey

| | |
|---|---|
| Purpose | The purpose of this report is to present the results of the U.S. Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP) for *Mycobacterium tuberculosis* complex drug susceptibility testing survey sent to participants in May 2014. |
| Report Content | The material in this report was developed and prepared by<br><br>Cortney Stafford, MPH, MT (ASCP), Health Scientist, Laboratory Capacity Team, NCHHSTP, DTBE, LB<br><br>Beverly Metchock, DrPH, D(ABMM), Team Lead, Reference Laboratory, NCHHSTP, DTBE, LB<br><br>Acknowledged contributors: Lois Diem NCHHSTP, DTBE, LB; Mitchell Yakrus NCHHSTP, DTBE, LB; Angela Starks NCHHSTP, DTBE, LB |
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# Table of Contents

***Mycobacterium tuberculosis* Complex Drug Susceptibility Testing MPEP Report for May 2014 Survey**

# Introduction: Overview of MPEP Final Report

The Model Performance Evaluation Program (MPEP) is an educational self-assessment tool in which five isolates of *M. tuberculosis* complex (MTBC) are sent to participating laboratories biannually for staff to monitor their ability to determine drug resistance among the isolates. It is not a formal, graded proficiency testing program. This report includes results for a subset of laboratories performing drug susceptibility tests (DST) for MTBC in the United States. MPEP is a voluntary program, and this report reflects data received from participating laboratory personnel. This aggregate report is prepared in a format that will allow laboratory personnel to compare their DST results with those obtained by other participants using the same methods and drugs, for each isolate. We encourage circulation of this report to personnel who are either involved with DST or reporting and interpreting results 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," M24-A2 [1].

# Expected Susceptibility Testing Results

The tables below provide the anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in May 2014. Although CDC recommends broth-based methods for routine first-line DST of MTBC isolates, Table 1 provides the results obtained by the reference agar proportion method, except for pyrazinamide, where MGIT was the testing method. Table 2 provides molecular results obtained by using DNA sequencing[2].

**Table 1. Expected Conventional Results for May 2014 Survey**

| Growth-based Results | | | | | |
|---|---|---|---|---|---|
| | **First-Line Drugs** | | | | **Second-Line Drugs** |
| | INH | RMP | EMB | PZA | Resistant to: |
| 2014A* | S | R | S | S | OFL |
| 2014B | S | S | S | S | OFL |
| 2014C | S | S | S | S† | OFL |
| 2014D | R | S | S | S | OFL |
| 2014E | S | S | S | S | STR, AMK, KAN, CAP |

Note—S=susceptible, R=resistant

* Most respondents noted resistance to streptomycin for 2014A

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

**Table 2. Expected Molecular Results for May 2014 Survey**

| Mutations Detected | | | | | |
|---|---|---|---|---|---|
| | **First-Line Drugs** | | | **Second-Line Drugs** | |
| MPEP Isolate | ***katG*** | ***rpoB*** | ***embB*** | ***gyrA*** | ***rrs*** |
| 2014A | | His526Asp | | Asp94Gly | |
| 2014B | | Phe514Phe | | Asp94Asn | |
| 2014C | | | | Asp94Phe | |
| 2014D | Thr394Pro | | Glu378Ala | Ala90Val | |
| 2014E | | | | | A1401G |

## Abbreviations and Acronyms

| | |
|---|---|
| AMK | amikacin |
| AP | agar proportion — performed on Middlebrook 7H10 or 7H11 |
| bp | base pair |
| CAP | capreomycin |
| CDC | U.S. Centers for Disease Control and Prevention |
| CIP | ciprofloxacin |
| CLSI | Clinical and Laboratory Standards Institute |
| CYS | cycloserine |
| DNA | deoxyribonucleic acid |
| DST | drug susceptibility testing |
| EMB | ethambutol |
| ETA | ethionamide |
| HMO | Health Maintenance Organization |
| INH | isoniazid |
| KAN | kanamycin |
| LEV | levofloxacin |
| MDR | multidrug resistant |
| MGIT | BACTEC MGIT 960 – Mycobacteria Growth Indicator Tube |
| MIC | minimum inhibitory concentration |
| MOX | moxifloxacin |
| MPEP | Model Performance Evaluation Program |
| MTBC | *Mycobacterium tuberculosis* complex |
| PAS | *p*-aminosalicylic acid |
| PZA | pyrazinamide |
| OFL | ofloxacin |
| R | resistant |
| RBT | rifabutin |
| RMP | rifampin |
| RNA | ribonucleic acid |
| S | susceptible |
| Sensititre | Thermo Scientific Sensititre *Mycobacterium tuberculosis* MIC plate |
| STR | streptomycin |
| TB | tuberculosis |
| VersaTREK | Thermo Scientific VersaTREK Myco susceptibility |
| XDR | extensively drug resistant |

## Technical Notes

The following information pertains to all of the tables and figures for the 2014 MTBC isolates A, B, C, D, and E in this report.

- The source of data in all tables and figures is from the May 2014 MPEP MTBC DST survey, with the exception of Figure 2 that compares data from the May 2014 MPEP survey and the November 2013 MPEP survey.
- The tables indicate the number of reported results (S represents susceptible and R represents resistant) for each drug.
- First-line and second-line drugs have been separated into individual tables for each isolate. Streptomycin is classified as a second-line drug for this report.
- Separate tables for molecular testing are included where data are of note; otherwise, findings are reported in the summary.
- Laboratories that use more than one DST method are encouraged to test isolates with each of those methods at either CLSI-recommended or equivalent critical concentrations. Some laboratories have provided results for multiple DST methods. Consequently, the number of results for some drugs may be greater than 88 (the number of participating laboratories). This report contains all results reported by participating laboratories.
- As a reference, a list of critical concentrations for antituberculosis drugs, by method, can be found at the end of this report.
- The Trek Sensititre system allows determination of a minimum inhibitory concentration (MIC) for each drug in the panel. Laboratories using this method must establish breakpoints to provide a categorical interpretation of S or R.
- Of the 32 laboratories reporting second-line drug results (with the exception of streptomycin), only 6 (19%) tested all three second-line injectable drugs and at least one fluoroquinolone needed to confidently define XDR TB. The second-line injectable drugs are amikacin, kanamycin, and capreomycin. Fluoroquinolones include ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin.

# Descriptive Information about Participant Laboratories

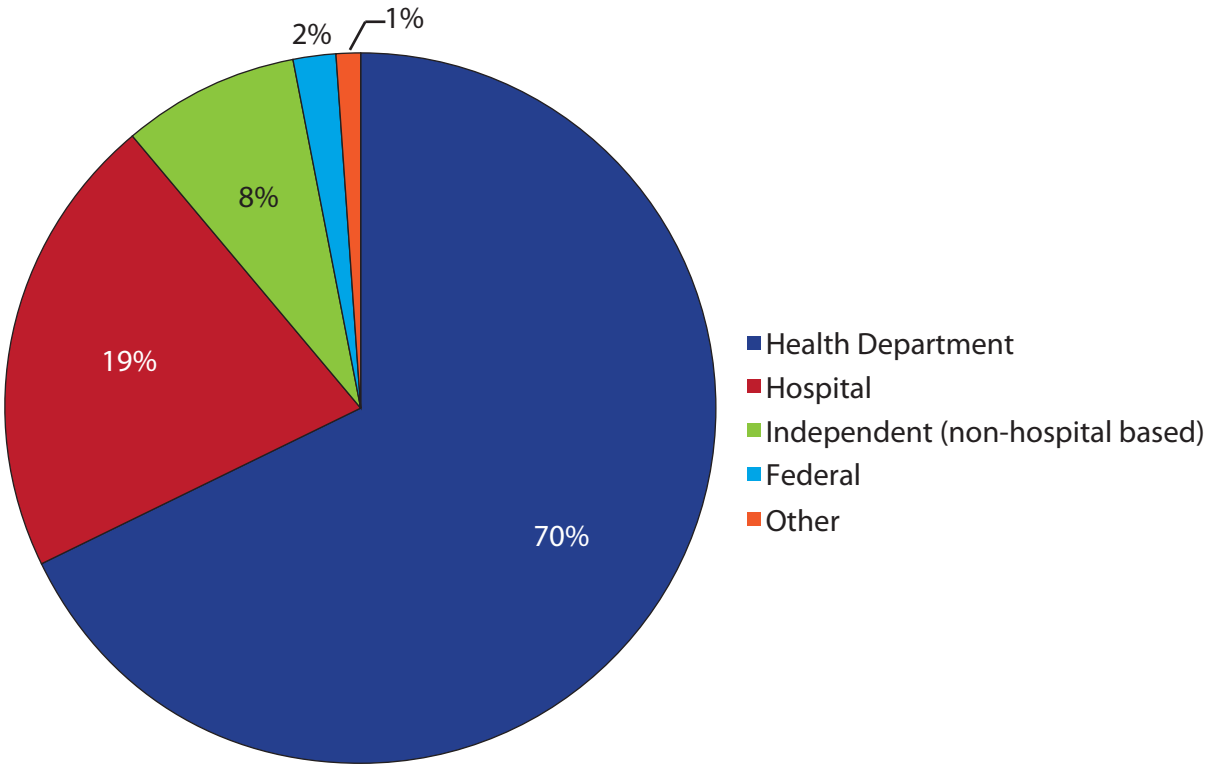
## Primary Classification

This report contains the DST results submitted to CDC by survey participants at 88 laboratories in 42 states.

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

- 61 (70%): Health department (city, country, state, regional, or district laboratory)
- 17 (19%): Hospital laboratory
- 7 (8%): Independent (e.g., commercial, commercial manufacturer of reagents, reference laboratory [non-governmental affiliated])
- 2 (2%): Federal government laboratory
- 1 (1%): Other (quality control manufacturer)

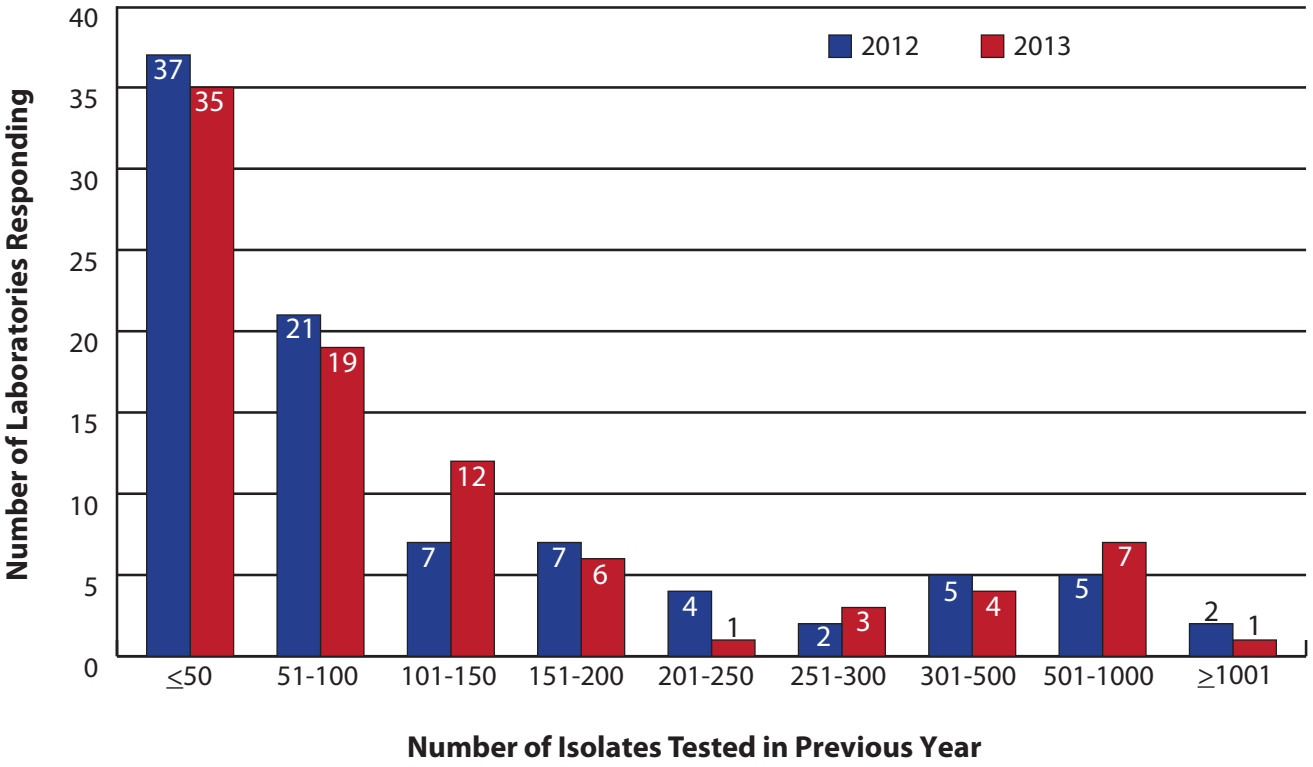
**Figure 1. Primary Classification of Participating Laboratories**



## Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 88 participants in the previous 2 calendar years (excluding isolates used for quality control) is shown in Figure 2. The number of MTBC isolates tested in 2012 is shown for comparison. In 2013, the counts ranged from 0 to 1234 tests and participants at 37 (42%) laboratories reported testing 50 or fewer DST isolates per year. Laboratories with low MTBC DST volumes are encouraged to consider referral of testing because of concerns about maintaining proficiency [3].

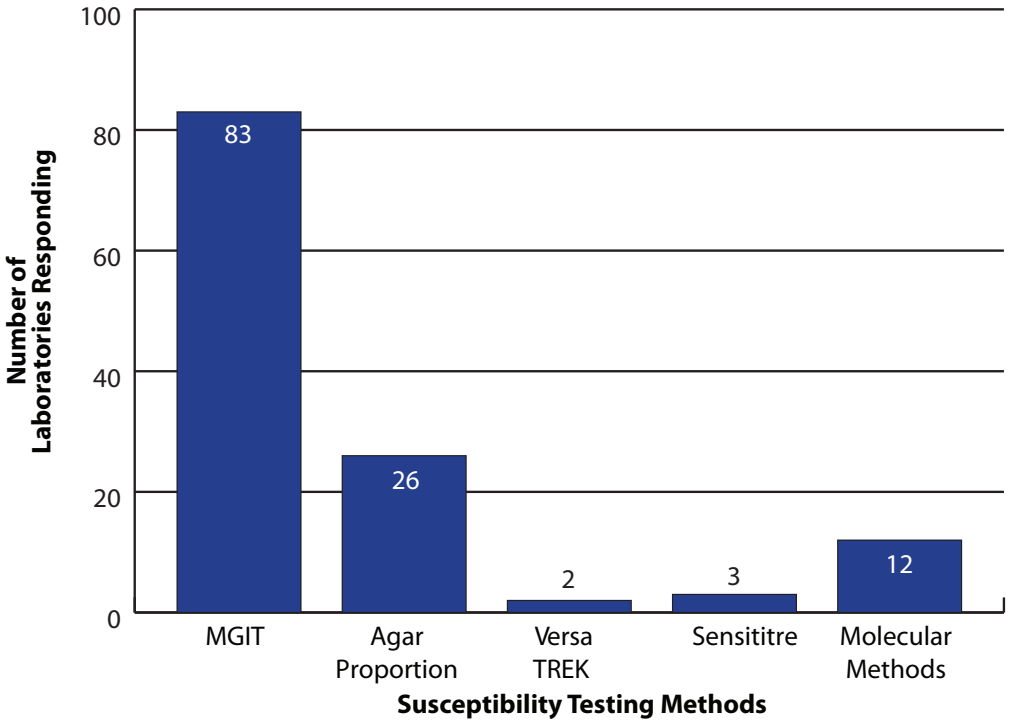
**Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in 2012 and 2013**



## MTBC DST Methods Used by Participants

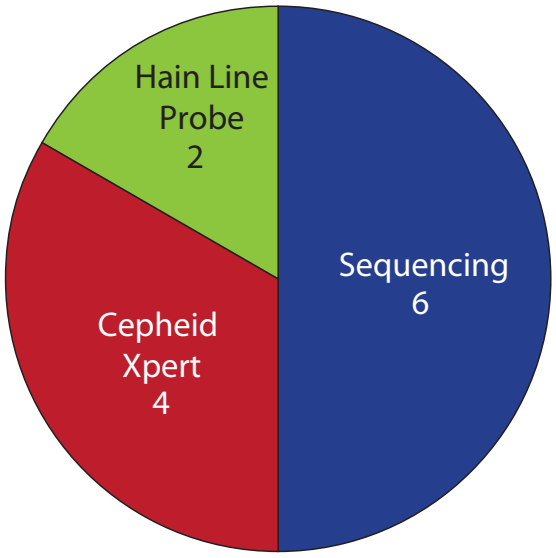
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 56 (64%) laboratories reported only one method, 27 laboratories reported two methods and 4 laboratories noted three susceptibility methods. One laboratory noted four susceptibility methods, including multiple molecular methods.

**Figure 3. MTBC Susceptibility Test Method Used by Participants (n=126)**




The summary of molecular methods reported is shown in Figure 4. The method used by half of the participants was DNA sequencing (50%), including pyrosequencing and Sanger sequencing. Four laboratories reported results for the Cepheid Xpert MTB/RIF assay and two laboratories used the line probe assay, Genotype MTBDRplus by Hain Lifescience.

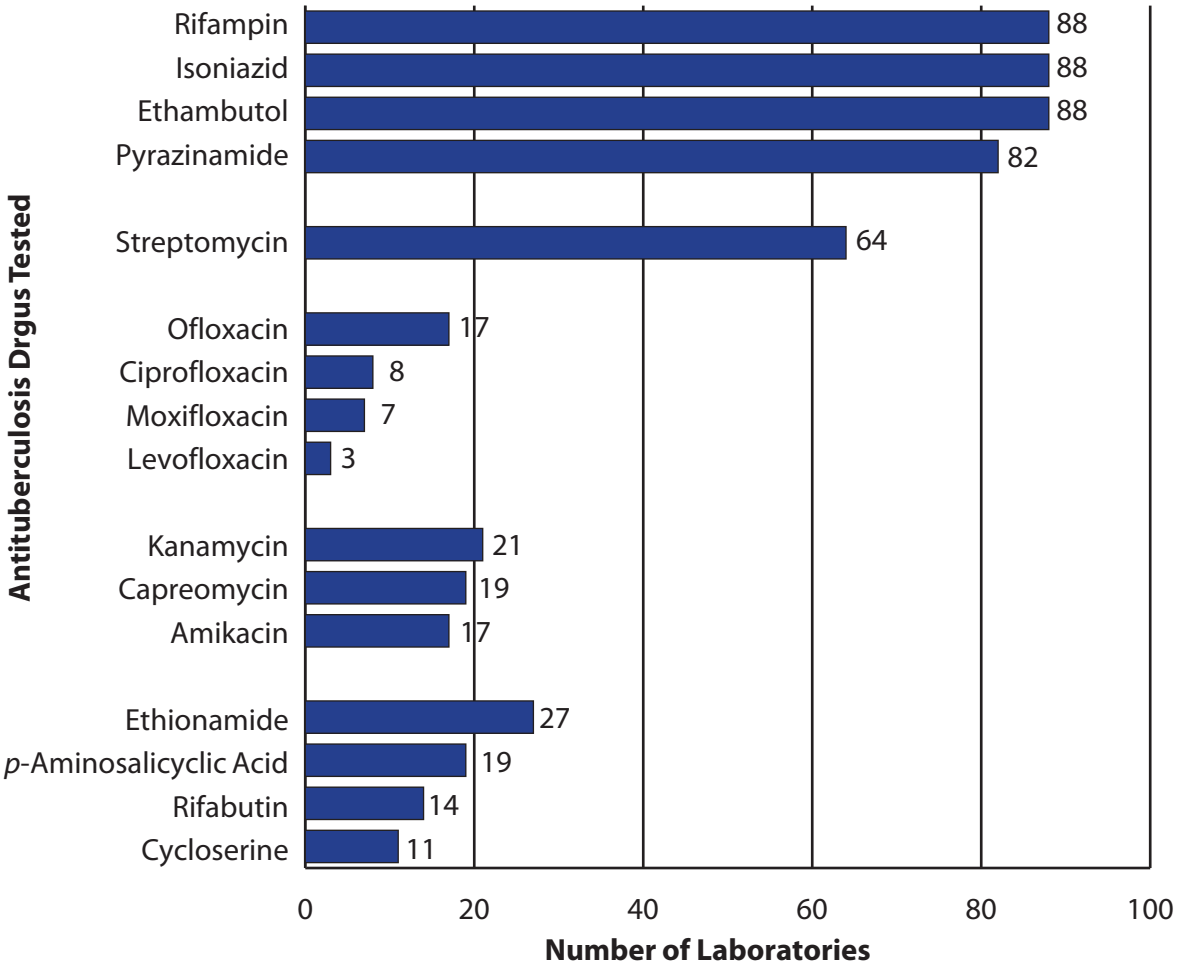
**Figure 4. Molecular Method Used (n=12)**



## Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug is shown in Figure 5. CLSI recommends testing a full panel of first-line drugs (rifampin [RMP], isoniazid [INH], 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 antituberculosis therapy currently recommended for most patients in the United States. All participants reported results for three of the first-line drugs—RMP, INH, and EMB—and 82 (93%) of the participants also reported results for PZA.

**Figure 5. Antituberculosis Drugs Tested by Participants**



# Isolate 2014A

**Expected Result: Resistant to RMP at 1.0 µg/ml and OFL at 2.0 µg/ml by agar proportion**

## Rifampin

Rifampin (RMP) is a bactericidal drug used for treatment of tuberculosis caused by organisms known or presumed to be susceptible to this drug. The critical concentration for AP is 1.0 µg/ml (on Middlebrook 7H10 and 7H11 agars) and the equivalent critical concentration for both MGIT and VersaTREK is 1.0 µg/ml. The mechanism of action of RMP is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase [4]. More than 96% of RMP-resistant isolates contain a mutation in the 81-bp central region of the *rpoB* gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase. The activity of RMP in RMP-resistant isolates depends on both the mutation position and the type of amino acid change.

CDC has recommended that RMP resistance detected by the Xpert MTB/RIF assay should be confirmed by DNA sequencing of genetic loci associated with RMP resistance (i.e., *rpoB*) [5]. The Xpert MTB/RIF assay may generate results that falsely indicate resistance when compared to growth-based methods because of the presence of silent mutations (i.e., nucleotide change but no corresponding change in amino acid) [6]. Sequencing of *rpoB* will allow for clarifying the result and understanding possible discordance between the rapid molecular and the growth-based testing results.

DNA sequence analysis of *rpoB* in Isolate 2014A revealed a C>G point mutation in codon 526 resulting in histidine being replaced by aspartate (His526Asp). Isolates with His526Asp mutations consistently test as resistant to RMP in growth-based assays.

Among four methods, 108 results for RMP were reported for Isolate 2014A. This isolate was reported as **resistant** to RMP by method, as follows

- 100% (25/25) of the results when using AP;
- 100% (78/78) of the results when using MGIT;
- 66% (2/3) of the results when using Sensititre; and
- 100% (2/2) of the results when using VersaTREK.

All eleven (100%) of the molecular results reported for RMP noted that a mutation was detected.

## Ofloxacin

Fluoroquinolones (FQ) are an important class of drugs used to treat tuberculosis resistant to first-line drugs. They are the most commonly prescribed antibiotic class in the United States and have the potential to become part of future first-line regimens [7]. In the United States, resistance to FQ is relatively uncommon in strains of *M. tuberculosis* susceptible to first-line drugs, but treatment with a FQ before diagnosis with tuberculosis is associated with a high risk of FQ resistance and diagnostic delays [7, 8].

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) [9]. DNA sequence of *gyrA* in Isolate 2014A revealed an A>G point mutation in codon 94 of *gyrA* resulting in aspartate being replaced with glycine (Asp94Gly).

Among three methods, 22 results for OFL were reported for Isolate 2014A. This isolate was reported as **resistant** to OFL by method, as follows

- 93% (13/14) of the results when using AP;
- 100% (5/5) of the results when using MGIT; and
- 66% (2/3) of the results when using Sensititre.

Participating laboratories tested a variety of FQ (e.g., OFL, ciprofloxacin, levofloxacin, and moxifloxacin) with most reporting resistance.

The Asp94Gly mutation was detected by the one laboratory that reported molecular testing for FQ.

*Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2014A are listed in Tables, 3, 4, and 5.*

### Table 3. Isolate 2014A—Participant results for first-line DST

| Results by Method for First-Line Drugs | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AP | | | MGIT | | | Sensititre | | | VersaTREK | | |
| **Drug** | **S** | **R** | **Total** | **S** | **R** | **Total** | **S** | **R** | **Total** | **S** | **R** | **Total** |
| **Rifampin** | 0 | 25 | 25 | 0 | 78 | 78 | 1 | 2 | 3 | 0 | 2 | 2 |
| **Isoniazid-Low** | 25 | 0 | 25 | 77 | 1 | 78 | 3 | 0 | 3 | 2 | 0 | 2 |
| **Isoniazid-High** | 22 | 0 | 22 | 30 | 0 | 30 | 3 | 0 | 3 | 2 | 0 | 2 |
| **Ethambutol** | 25 | 0 | 25 | 78 | 0 | 78 | 3 | 0 | 3 | 2 | 0 | 2 |
| **Pyrazinamide** | | | | 81 | 0 | 81 | | | | 1 | 0 | 1 |

Note—S=susceptible, R=resistant

## Table 4. Isolate 2014A—Participant results for second-line DST

| Results by Method for Second-Line Drugs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | AP | | | MGIT | | | Sensititre | | |
| Drug | S | R | Total | S | R | Total | S | R | Total |
| Streptomycin | 0 | 25 | 25 | 3 | 46 | 49 | 0 | 2 | 2* |
| Ofloxacin | 1 | 13 | 14 | 0 | 5 | 5 | 1 | 2 | 3 |
| Ciprofloxacin | 1 | 6 | 7 | 0 | 2 | 2 | | | |
| Levofloxacin | 0 | 2 | 2 | 0 | 1 | 1 | | | |
| Moxifloxacin | 0 | 4 | 4 | 0 | 3 | 3 | 1 | 1 | 2 |
| Amikacin | 12 | 0 | 12 | 4 | 0 | 4 | 2 | 1 | 3 |
| Kanamycin | 19 | 0 | 19 | 3 | 0 | 3 | 1 | 2 | 3 |
| Capreomycin | 17 | 0 | 17 | 4 | 0 | 4 | | | |
| Ethionamide | 22 | 1 | 23 | 6 | 1 | 7 | 3 | 0 | 3 |
| Rifabutin | 1 | 8 | 9 | 0 | 2 | 2 | 1 | 2 | 3 |
| Cycloserine | 8 | 1 | 9 | 1 | 0 | 1 | 2 | 0 | 2 |
| p-Aminosalicylic acid | 17 | 0 | 17 | 3 | 0 | 3 | 3 | 0 | 3 |

Note—S=susceptible, R=resistant

* In addition, one laboratory reported borderline for STR by Sensititre.

## Table 5. Isolate 2014A—Participant results for molecular testing

| Molecular Testing | | | |
|---|---|---|---|
| Drug | Mutation Detected | Mutation Not Detected | Total |
| Rifampin | 11 | 0 | 11 |
| Isoniazid | 0 | 6 | 6 |
| Ethambutol | 0 | 0 | 0 |
| Pyrazinamide | 0 | 2 | 2 |
| Ofloxacin | 1 | 0 | 1 |
| Ciprofloxacin | 1 | 0 | 1 |
| Levofloxacin | 1 | 0 | 1 |
| Moxifloxacin | 1 | 0 | 1 |
| Amikacin | 0 | 1 | 1 |
| Kanamycin | 0 | 1 | 1 |
| Capreomycin | 0 | 1 | 1 |
| Ethionamide | 0 | 1 | 1 |
| Rifabutin | 0 | 1 | 1 |

# Isolate 2014B

**Expected Result: Resistant to OFL at 2.0 µg/ml by agar proportion**

## Ofloxacin

DNA sequencing of *gyrA* in Isolate 2014B revealed a G>A point mutation in codon 94 resulting in aspartate being replaced with asparagine (Asp94Asn).

Among three methods, 16 results for OFL were reported for Isolate 2014B. This isolate was reported as **resistant** to OFL by method, as follows

- 90% (9/10) of the results when using AP;
- 100% (3/3) of the results when using MGIT; and
- 100% (3/3) of the results when using Sensititre.

Participating laboratories tested a variety of FQ (e.g., OFL, ciprofloxacin, levofloxacin, and moxifloxacin) for Isolate 2014B as well, with most reporting resistance.

This mutation was detected by the one laboratory that reported molecular testing for FQ.

## Rifampin

DNA sequence analysis of *rpoB* in Isolate 2014B revealed a C>T point mutation in codon 514 of the *rpoB* locus. However, this mutation does not result in an amino acid change; phenylalanine remains phenylalanine (Phe514Phe). This synonymous (i.e., silent) mutation in *rpoB* is not considered clinically significant and isolates with this mutation reliably test as RMP-susceptible in growth-based systems.

The Xpert MTB/RIF will generate a report of RMP resistance detected for isolates with this mutation. As noted with the previous isolate, sequencing of *rpoB* will allow for clarifying the result and understanding discordance between the Xpert result and results from growth-based testing.

Among four methods, 103 results for RMP were reported for Isolate 2014B. This isolate was reported as **susceptible** to RMP by method, as follows

- 100% (20/20) of the results when using AP;
- 99% (77/78) of the results when using MGIT;
- 100% (3/3) of the results when using Sensititre; and
- 50% (1/2) of the results when using VersaTREK.

Nine of the eleven (82%) molecular results reported for RMP noted that a mutation was detected.

*Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2014B are listed in Tables 6, 7, and 8.*

## Table 6. Isolate 2014B—Participant results for first-line DST

| Results by Method for First-Line Drugs | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AP | | | MGIT | | | Sensititre | | | VersaTREK | | |
| Drug | S | R | Total | S | R | Total | S | R | Total | S | R | Total |
| **Rifampin** | 20 | 0 | 20* | 77 | 1 | 78 | 3 | 0 | 3 | 1 | 1 | 2 |
| **Isoniazid–Low** | 21 | 0 | 21* | 76 | 2 | 78 | 3 | 0 | 3 | 1 | 1 | 2 |
| **Isoniazid–High** | 17 | 0 | 17* | 28 | 0 | 28 | 3 | 0 | 3 | 1 | 1 | 2 |
| **Ethambutol** | 20 | 0 | 20* | 77 | 1 | 78 | 3 | 0 | 3 | 2 | 0 | 2 |
| **Pyrazinamide** | | | | 73 | 8 | 81 | | | | 1 | 0 | 1 |

Note—S=susceptible, R=resistant

*In addition, two laboratories reported no growth for RMP, INH, and EMB by AP.

## Table 7. Isolate 2014B—Participant results for second-line DST

| Results by Method for Second-Line Drugs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | AP | | | MGIT | | | Sensititre | | |
| Drug | S | R | Total | S | R | Total | S | R | Total |
| **Streptomycin** | 16 | 2 | 18*# | 50 | 0 | 50 | 3 | 0 | 3 |
| **Ofloxacin** | 1 | 9 | 10# | 0 | 3 | 3 | 0 | 3 | 3 |
| **Ciprofloxacin** | 1 | 4 | 5* | 0 | 1 | 1 | | | |
| **Levofloxacin** | 0 | 1 | 1# | 0 | 1 | 1 | | | |
| **Moxifloxacin** | 0 | 2 | 2# | 0 | 2 | 2 | 1 | 1 | 2 |
| **Amikacin** | 9 | 0 | 9# | 4 | 0 | 4 | 3 | 0 | 3 |
| **Kanamycin** | 14 | 0 | 14*# | 2 | 0 | 2 | 3 | 0 | 3 |
| **Capreomycin** | 13 | 0 | 13*# | 4 | 0 | 4 | | | |
| **Ethionamide** | 17 | 0 | 17*# | 6 | 0 | 6 | 3 | 0 | 3 |
| **Rifabutin** | 8 | 0 | 8# | 2 | 0 | 2 | 3 | 0 | 3 |
| **Cycloserine** | 5 | 0 | 5*# | | | † | 3 | 0 | 3 |
| **p-Aminosalicylic acid** | 12 | 0 | 12# | 2 | 0 | 2† | 2 | 1 | 3 |

Note—S=susceptible, R=resistant

* In addition, one laboratory reported no growth for STR, CIP, KAN, CAP, ETA, and CYS by AP.

# In addition, one laboratory reported no growth for STR, OFL, LEV, MOX, KAN, AMK, CAP, ETA, RBT, CYS, and PAS by AP.

† In addition, one laboratory reported borderline for CYS and PAS by MGIT.

## Table 8. Isolate 2014B—Participant results for molecular testing

| Molecular Testing | | | |
|---|---|---|---|
| **Drug** | **Mutation Detected** | **Mutation Not Detected** | **Total** |
| Rifampin | 9* | 2 | 11 |
| Isoniazid | 0 | 6 | 6 |
| Ethambutol | 0 | 0 | 0 |
| Pyrazinamide | 0 | 2 | 2 |
| Ofloxacin | 1 | 0 | 1 |
| Ciprofloxacin | 1 | 0 | 1 |
| Levofloxacin | 1 | 0 | 1 |
| Moxifloxacin | 1 | 0 | 1 |
| Amikacin | 0 | 1 | 1 |
| Kanamycin | 0 | 1 | 1 |
| Capreomycin | 0 | 1 | 1 |
| Ethionamide | 0 | 1 | 1 |
| Rifabutin | 1 | 0 | 1 |

*Three laboratories noted the mutation detected was a silent mutation

# Isolate 2014C

**Expected Result: Resistant to OFL at 2.0 µg/ml by agar proportion**

## Ofloxacin

DNA sequence of *gyrA* in Isolate 2014C revealed a GA>TT point mutation in codon 94 of *gyrA* resulting in aspartate being replaced with phenylalanine (Asp94Phe).

Among three methods, 17 results for OFL were reported for Isolate 2014C. This isolate was reported as **resistant** to OFL by method, as follows

- 92% (11/12) of the results when using AP;
- 100% (4/4) of the results when using MGIT; and
- 100% (3/3) of the results when using Sensititre.

Participating laboratories tested a variety of FQ (e.g., OFL, ciprofloxacin, levofloxacin, and moxifloxacin) for Isolate 2014C as well, with most reporting resistance.

This mutation was detected by the one laboratory that reported molecular testing for FQ.

## Pyrazinamide

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

- 78% (63/81) of the results when using MGIT; and
- 0% (0/1) of the results when using VersaTREK.

Three laboratories reported molecular results; none reported detection of a mutation for PZA.

Issues with false resistance to PZA have been reported [10], and as indicated by these results, remains a potential concern.

*Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2014C are listed in Tables 9, 10, and 11.*

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### Table 9. Isolate 2014C—Participant results for first-line DST

| Results by Method for First-Line Drugs | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AP | | | MGIT | | | Sensititre | | | VersaTREK | | |
| **Drug** | **S** | **R** | **Total** | **S** | **R** | **Total** | **S** | **R** | **Total** | **S** | **R** | **Total** |
| **Rifampin** | 25 | 0 | 25 | 77 | 0 | 77* | 2 | 1 | 3 | 2 | 0 | 2 |
| **Isoniazid–Low** | 25 | 0 | 25 | 77 | 0 | 77* | 3 | 0 | 3 | 2 | 0 | 2 |
| **Isoniazid–High** | 21 | 0 | 21 | 28 | 1 | 28* | 3 | 0 | 3 | 2 | 0 | 2 |
| **Ethambutol** | 25 | 0 | 25 | 75 | 2 | 77* | 3 | 0 | 3 | 2 | 0 | 2 |
| **Pyrazinamide** | | | | 18 | 63 | 81 | | | | 1 | 0 | 1 |

Note—S=susceptible, R=resistant

*In addition, one laboratory reported no growth for RMP, INH, and EMB by MGIT.

## Table 10. Isolate 2014C—Participant results for second-line DST

| Results by Method for Second-Line Drugs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | AP | | | MGIT | | | Sensititre | | |
| Drug | S | R | Total | S | R | Total | S | R | Total |
| Streptomycin | 23 | 0 | 23 | 50 | 0 | 50 | 2 | 1 | 3 |
| Ofloxacin | 1 | 11 | 12 | 0 | 4 | 4 | 0 | 3 | 3 |
| Ciprofloxacin | 1 | 6 | 7 | 0 | 1 | 1 | | | |
| Levofloxacin | 0 | 2 | 2 | 0 | 1 | 1 | | | |
| Moxifloxacin | 0 | 3 | 3 | 0 | 3 | 3 | 0 | 2 | 2 |
| Amikacin | 11 | 0 | 11 | 4 | 0 | 4 | 3 | 0 | 3 |
| Kanamycin | 18 | 0 | 18 | 2 | 0 | 2 | 3 | 0 | 3 |
| Capreomycin | 16 | 0 | 16 | 4 | 0 | 4 | | | |
| Ethionamide | 20 | 0 | 20 | 4 | 2 | 6 | 3 | 0 | 3 |
| Rifabutin | 9 | 0 | 9 | 2 | 0 | 2 | 2 | 1 | 3 |
| Cycloserine | 8 | 1 | 9 | 1 | 0 | 1 | 2 | 0 | 2 |
| p-Aminosalicylic acid | 16 | 0 | 16 | 3 | 0 | 3 | 2 | 1 | 3 |

Note—S=susceptible, R=resistant

## Table 11. Isolate 2014C—Participant results for molecular testing

| Molecular Testing | | | |
|---|---|---|---|
| Drug | Mutation Detected | Mutation Not Detected | Total |
| Rifampin | 0 | 10 | 10 |
| Isoniazid | 0 | 6 | 6 |
| Ethambutol | 0 | 0 | 0 |
| Pyrazinamide | 0 | 3 | 3 |
| Ofloxacin | 1 | 0 | 1 |
| Ciprofloxacin | 1 | 0 | 1 |
| Levofloxacin | 1 | 0 | 1 |
| Moxifloxacin | 1 | 0 | 1 |
| Amikacin | 0 | 1 | 1 |
| Kanamycin | 0 | 1 | 1 |
| Capreomycin | 0 | 1 | 1 |
| Ethionamide | 0 | 1 | 1 |
| Rifabutin | 0 | 1 | 1 |

# Isolate 2014D

**Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml and OFL at 0.2 µg/ml by agar proportion**

## Isoniazid

Isoniazid (INH) is the most widely used first-line antituberculosis drug. It is a cornerstone of regimens used to treat TB disease and latent infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *kat*G gene [2, 4]. The target of activated INH is enoyl-acyl-carrier protein reductase (*inh*A) which is required for mycolic acid biosynthesis. Two mechanisms account for the majority of INH resistance [2, 4, 9]. The most common mechanism, mutations in *kat*G, is generally associated with high-level resistance to INH. Resistance to INH can also occur by mutations in the promoter region of the *inh*A gene which are generally associated with low-level resistance to INH and are less frequent than *katG* mutations. DNA sequence analysis of *inh*A and *kat*G of Isolate 2014D revealed A>C point mutation at codon 394 in the *katG* locus resulting in threonine being replaced by proline (Thr394Pro); *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, respectively, 0.2 µg/ml and 1.0 µg/ml. The equivalent concentrations for MGIT and VersaTREK are 0.1 µg/ml and 0.4 µg/ml.

For Isolate 2014D, 109 INH results were reported. This isolate was reported **resistant** to INH at the critical concentration by method, as follows

- 100% (26/26) of the results when using AP;
- 100% (78/78) of the results when using MGIT;
- 66% (2/3) of the results when using Sensititre; and
- 100% (2/2) of the results when using VersaTREK.

Seventy (97%) results were reported as **resistant** at the higher concentrations of INH as well.

Seven laboratories reported molecular results; only one (14%) laboratory reported the detection of a mutation for INH.

## Ofloxacin

DNA sequence of *gyrA* in Isolate 2014D revealed a C>T point mutation in codon 90 of *gyrA* resulting in alanine being replaced with valine (Ala90Val).

Among three methods, 22 results for OFL were reported for Isolate 2014D. This isolate was reported as **resistant** to OFL by method, as follows

- 93% (13/14) of the results when using AP;
- 100% (5/5) of the results when using MGIT; and
- 100% (3/3) of the results when using Sensititre.

Participating laboratories tested a variety of FQ (e.g., OFL, ciprofloxacin, levofloxacin, and moxifloxacin) for Isolate 2014D as well, with most reporting resistance. However, as noted on Table 13, discrepant results for ciprofloxacin and moxifloxacin by AP were reported by a few laboratories.

This mutation was detected by the one laboratory that performed molecular testing for FQ.

*Complete first-line DST, second-line DST, molecular results submitted by all participants for Isolate 2014D are listed in Tables 12, 13, and 14.*

## Table 12. Isolate 2014D—Participant results for first-line DST

| Results by Method for First-Line Drugs | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AP | | | MGIT | | | Sensititre | | | VersaTREK | | |
| Drug | S | R | Total | S | R | Total | S | R | Total | S | R | Total |
| **Rifampin** | 25 | 0 | 25 | 78 | 0 | 78 | 3 | 0 | 3 | 2 | 0 | 2 |
| **Isoniazid–Low** | 0 | 26 | 26 | 0 | 78 | 78 | 1 | 2 | 3 | 0 | 2 | 2 |
| **Isoniazid–High** | 1 | 23 | 24 | 0 | 43 | 43 | 1 | 2 | 3 | 0 | 2 | 2 |
| **Ethambutol** | 24 | 1 | 25 | 78 | 0 | 78 | 3 | 0 | 3 | 2 | 0 | 2 |
| **Pyrazinamide** | | | | 71 | 9 | 80 | | | | 1 | 0 | 1 |

Note—S=susceptible, R=resistant

## Table 13. Isolate 2014D—Participant results for second-line DST

| Results by Method for Second-Line Drugs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | AP | | | MGIT | | | Sensititre | | |
| Drug | S | R | Total | S | R | Total | S | R | Total |
| **Streptomycin** | 24 | 0 | 24 | 50 | 0 | 50 | 3 | 0 | 3 |
| **Ofloxacin** | 1 | 13 | 14 | 0 | 5 | 5 | 0 | 3 | 3 |
| **Ciprofloxacin** | 3 | 4 | 7 | 0 | 2 | 2 | | | |
| **Levofloxacin** | 0 | 2 | 2 | 0 | 1 | 1 | | | |
| **Moxifloxacin** | 3 | 1 | 4 | 0 | 3 | 3 | 1 | 1 | 2 |
| **Amikacin** | 12 | 0 | 12 | 4 | 0 | 4 | 3 | 0 | 3 |
| **Kanamycin** | 19 | 0 | 19 | 3 | 0 | 3 | 3 | 0 | 3 |
| **Capreomycin** | 17 | 0 | 17 | 4 | 0 | 4 | | | |
| **Ethionamide** | 21 | 1 | 22 | 7 | 0 | 7 | 3 | 0 | 3 |
| **Rifabutin** | 9 | 0 | 9 | 2 | 0 | 2 | 3 | 0 | 3 |
| **Cycloserine** | 9 | 0 | 9 | 1 | 0 | 1 | 2 | 1 | 3 |
| **p-Aminosalicylic acid** | 16 | 0 | 16 | 4 | 0 | 4 | 2 | 1 | 3 |

Note—S=susceptible, R=resistant

**Table 14. Isolate 2014D—Participant results for molecular testing**

| Molecular Testing | | | |
|---|---|---|---|
| **Drug** | **Mutation Detected** | **Mutation Not Detected** | **Total** |
| **Rifampin** | 0 | 10 | 10 |
| **Isoniazid** | 1 | 6 | 7 |
| **Ethambutol** | 0 | 0 | 0 |
| **Pyrazinamide** | 0 | 2 | 2 |
| **Ofloxacin** | 1 | 0 | 1 |
| **Ciprofloxacin** | 1 | 0 | 1 |
| **Levofloxacin** | 1 | 0 | 1 |
| **Moxifloxacin** | 1 | 0 | 1 |
| **Amikacin** | 0 | 1 | 1 |
| **Kanamycin** | 0 | 1 | 1 |
| **Capreomycin** | 0 | 1 | 1 |
| **Ethionamide** | 0 | 1 | 1 |
| **Rifabutin** | 0 | 1 | 1 |

# Isolate 2014E

**Expected Result: Resistant to STR at 2.0 µg/ml, AMK at 4.0 µg/ml, CAP at 10.0 µg/ml, and KAN at 5.0 µg/ml by agar proportion**

## Streptomycin

Streptomycin (STR) 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[4, 9]. In *M. tuberculosis* complex, the genetic basis of resistance to STR is usually due to mutations in *rrs* or *rpsL*[9].

For Isolate 2014E, 76 STR results were reported. The isolate was reported **resistant** to STR by method, as follows

- 100% (24/24) of the results when using AP;
- 96% (48/50) of the results when using MGIT; and
- 50% (1/2) of the results when using Sensititre.

## Second-line injectable drugs

Kanamycin (KAN) and amikacin (AMK) are aminoglycoside antibiotics while capreomycin (CAP) 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[11]. Isolate 2014E was resistant to the second-line injectable drugs (AMK, KAN, and CAP) by the AP method. DNA sequence analysis of the *rrs* gene revealed the A1401G mutation.

For Isolate 2014E, 76 results were reported for KAN, AMK, and CAP. The isolate was reported **resistant** to the second-line injectables by method, as follows

- 95% (43/45) of the results when using AP;
- 100% (11/11) of the results when using MGIT; and
- 66% (4/6) of the results when using Sensititre.

*Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2014E are listed in Tables 15, 16, and 17.*

### Table 15. Isolate 2014E—Participant results for first-line DST

| Results by Method for First-Line Drugs | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AP | | | MGIT | | | Sensititre | | | VersaTREK | | |
| **Drug** | **S** | **R** | **Total** | **S** | **R** | **Total** | **S** | **R** | **Total** | **S** | **R** | **Total** |
| **Rifampin** | 24 | 0 | 24 | 78 | 0 | 78 | 3 | 0 | 3 | 2 | 0 | 2 |
| **Isoniazid–Low** | 24 | 0 | 24 | 78 | 0 | 78 | 2 | 1 | 3 | 2 | 0 | 2 |
| **Isoniazid–High** | 20 | 0 | 20 | 29 | 1 | 30 | 2 | 1 | 3 | 2 | 0 | 2 |
| **Ethambutol** | 23 | 1 | 24 | 77 | 1 | 78 | 3 | 0 | 3 | 2 | 0 | 2 |
| **Pyrazinamide** | | | | 80 | 1 | 81 | | | | 1 | 0 | 1 |

Note—S=susceptible, R=resistant

## Table 16. Isolate 2014E—Participant results for second-line DST

| Results by Method for Second-Line Drugs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | AP | | | MGIT | | | Sensititre | | |
| Drug | S | R | Total | S | R | Total | S | R | Total |
| Streptomycin | 0 | 24 | 24 | 2 | 48 | 50 | 1 | 1 | 2 |
| Ofloxacin | 13 | 0 | 13 | 5 | 0 | 5 | 1 | 2 | 3 |
| Ciprofloxacin | 6 | 0 | 6 | 2 | 0 | 2 | | | |
| Levofloxacin | 2 | 0 | 2 | 1 | 0 | 1 | | | |
| Moxifloxacin | 4 | 0 | 4 | 3 | 0 | 3 | 2 | 0 | 2 |
| Amikacin | 0 | 11 | 11 | 0 | 4 | 4 | 1 | 2 | 3 |
| Kanamycin | 2 | 16 | 18 | 0 | 3 | 3 | 1 | 2 | 3 |
| Capreomycin | 0 | 16 | 16 | 0 | 4 | 4 | | | |
| Ethionamide | 21 | 0 | 21 | 7 | 0 | 7 | 3 | 0 | 3 |
| Rifabutin | 9 | 0 | 9 | 2 | 0 | 2 | 3 | 0 | 3 |
| Cycloserine | 8 | 0 | 8 | 1 | 0 | 1 | 2 | 1 | 3 |
| p-Aminosalicylic acid | 15 | 0 | 15 | 4 | 0 | 4 | 2 | 1 | 3 |

Note—S=susceptible, R=resistant

## Table 17. Isolate 2014E—Participant results for molecular testing

| Molecular Testing | | | |
|---|---|---|---|
| Drug | Mutation Detected | Mutation Not Detected | Total |
| Rifampin | 0 | 10 | 10 |
| Isoniazid | 0 | 6 | 6 |
| Ethambutol | 0 | 0 | 0 |
| Pyrazinamide | 0 | 2 | 2 |
| Ofloxacin | 0 | 1 | 1 |
| Ciprofloxacin | 0 | 1 | 1 |
| Levofloxacin | 0 | 1 | 1 |
| Moxifloxacin | 0 | 1 | 1 |
| Amikacin | 1 | 0 | 1 |
| Kanamycin | 1 | 0 | 1 |
| Capreomycin | 1 | 0 | 1 |
| Ethionamide | 0 | 1 | 1 |
| Rifabutin | 0 | 1 | 1 |

# Equivalent Critical Concentrations

(Concentrations listed as µg/ml)

## Agar Proportion

| | 7H10 agar | 7H11 agar |
|---|---|---|
| **First-line Drugs** | | |
| Isoniazid | 0.2 and 1.0* | 0.2 and 1.0* |
| Rifampin | 1.0 | 1.0 |
| Ethambutol | 5.0 and 10.0* | 7.5 |
| Pyrazinamide | Not recommended | Not recommended |
| **Second-line Drugs** | | |
| Streptomycin | 2.0 and 10.0 | 2.0 and 10.0 |
| Amikacin | 4.0 | -† |
| Capreomycin | 10.0 | 10.0 |
| Kanamycin | 5.0 | 6.0 |
| Levofloxacin | 1.0 | -† |
| Moxifloxacin | 0.5 | 0.5 |
| Ofloxacin | 2.0 | 2.0 |
| Ethionamide | 5.0 | 10.0 |
| Rifabutin | 0.5 | 0.5 |
| *p*-Aminosalicylic acid | 2.0 | 8.0 |

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

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

† Breakpoints for establishing susceptibility have not be determined

## Broth Based Media

| | MGIT | VersaTREK |
|---|---|---|
| **First-line Drugs** | | |
| Isoniazid | 0.1 (and 0.4*) | 0.1 (and 0.4*) |
| Rifampin | 1.0 | 1.0 |
| Ethambutol | 5.0 | 5.0 (and 8.0*) |
| Pyrazinamide | 100.0 | 300.0 |
| **Second-line Drug** | | |
| Streptomycin | 1.0 (and 4.0*) | |

NOTE: Critical concentrations as indicated in applicable manufacturer package inserts

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

# References


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