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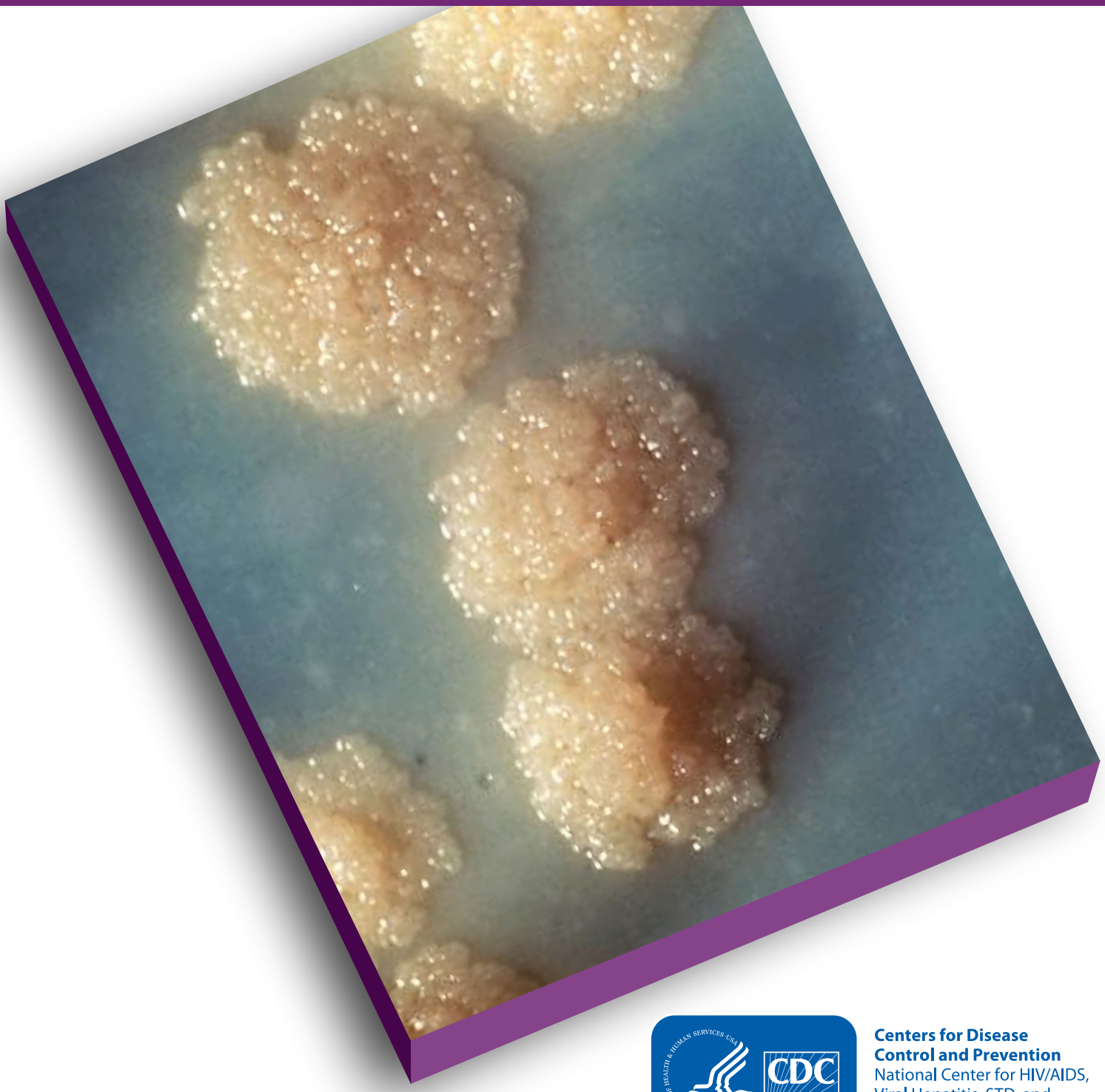
# *Mycobacterium tuberculosis* Complex Drug Susceptibility Testing Program

## Model Performance Evaluation Program
## Report of Results
## September 2020



**Centers for Disease Control and Prevention**
National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention

CS 323576-A

# *Mycobacterium tuberculosis* Complex Drug Susceptibility Testing Report for September 2020 Survey

## Purpose

The purpose of this report is to present results of the U.S. Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP) for *Mycobacterium tuberculosis* complex (MTBC) drug susceptibility testing survey sent to participants in September 2020.

## Report Content

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**Note on Accessibility:**
Find descriptions and explanations of figures in Appendix 1: Accessible Explanation of Figures on page 35.

# Contents

## Abbreviations and Acronyms

| Acronym | Definition |
|---|---|
| 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 |
| FQ | fluoroquinolones |
| 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 |
| Nt | nucleotide |
| PAS | *p*-aminosalicylic acid |
| PZA | pyrazinamide |
| OFL | ofloxacin |
| R | resistant |
| RBT | rifabutin |
| RMP | rifampin |
| RNA | ribonucleic acid |
| S | susceptible |
| Sensititre | Thermo Scientific Sensititre MYCOTB AST or customized plate |
| STR | streptomycin |
| TB | tuberculosis |
| VersaTREK | Thermo Scientific VersaTREK Myco susceptibility |
| XDR | extensively drug resistant |

# 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. The associated 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 standards, participants should refer to consensus documents published by the Clinical and Laboratory Standards Institute (CLSI), "M24: Susceptibility Testing of Mycobacteria, *Nocardiae* spp., and Other Aerobic Actinomycetes" [1]. Recently, World Health Organization (WHO) published two technical reports investigating critical concentrations, by method, for INH, RMP, EMB, PZA and twelve second-line anti-tuberculosis drugs [2, 3]. Based on the systematic review data, recommendations were made for adjustments to critical concentrations for RMP, MOX, LEV, AMK and KAN for some methods.

# Expected Drug Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in September 2020 are shown in the tables below. Although CDC recommends broth-based methods for routine first-line DST of MTBC isolates, the results obtained by the reference agar proportion method (except for pyrazinamide, in which MGIT was performed) are shown in Table 1. Molecular results obtained by DNA sequencing are listed in Table 2 [4].

### Table 1. Expected Growth-based Results for September 2020 Survey

Note—S=susceptible, R=resistant , V=variable

| Isolate | RMP | INH | EMB | PZA | Second-line Drugs Resistant to: |
|---|---|---|---|---|---|
| 2020F* | S | R† | S | S | ETA |
| 2020G | S | R | S | S | ETA |
| 2020H | S | R | R | S | ETA, STR¥ |
| 2020I* | S | R† | S | S | ETA |
| 2020J | R | S | S | S | |

*Isolates 2020F and 2020I are the same isolate

†Although INH resistance was expected, 80% consensus for a single categorical result of either susceptible or resistant was not achieved for this isolate among participating laboratories. Variable resistance was observed depending on growth-based DST method.

¥Although STR resistance was expected, 80% consensus for a single categorical result of either susceptible or resistant was not achieved for this isolate among participating laboratories. Variable resistance was observed depending on growth-based DST method.

### Table 2. Expected Molecular Results (Mutations Detected in Loci Associated with Resistance) for September 2020 Survey

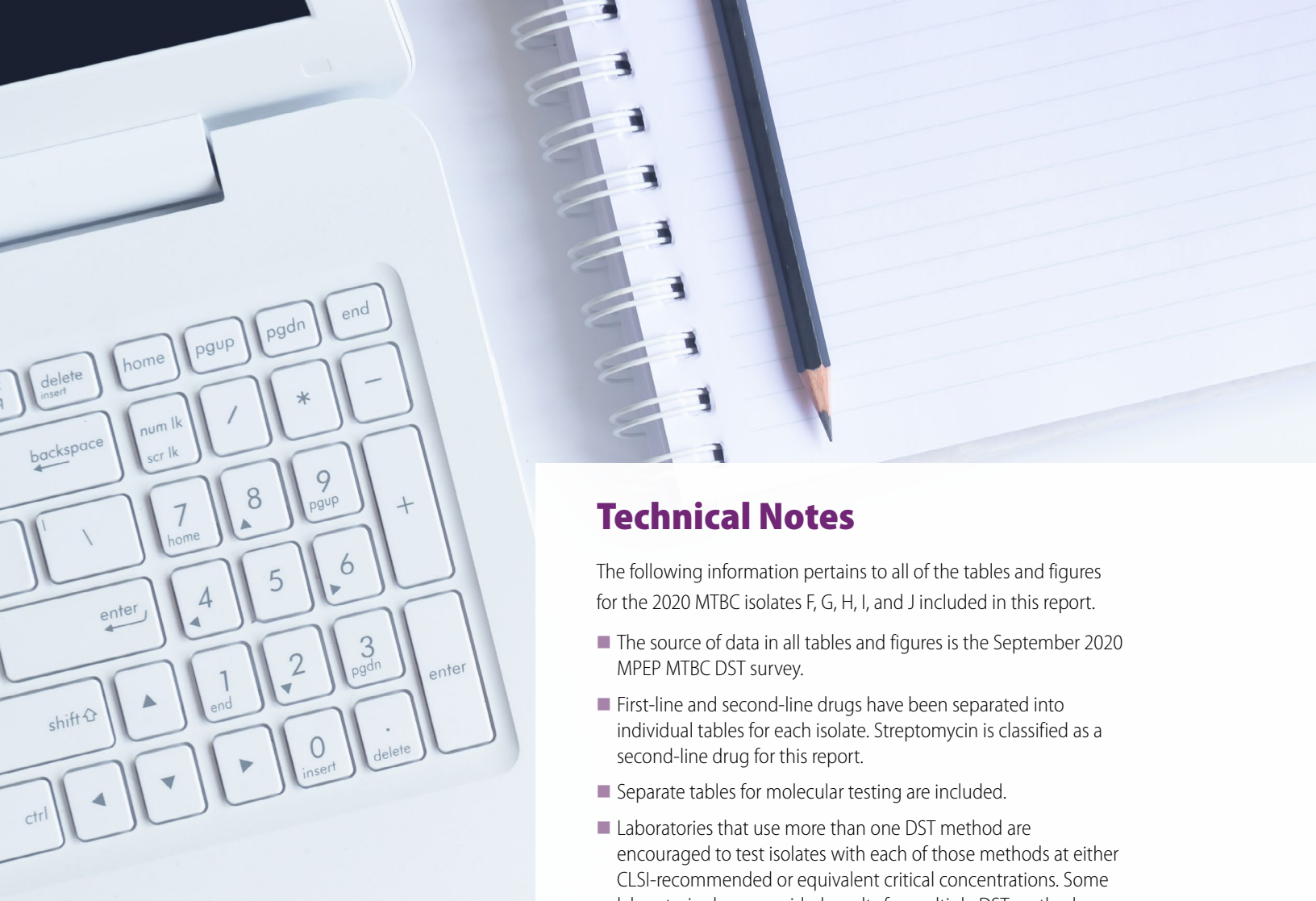
Note—Empty cell=No mutation detected

| Isolate | *rpoB*¥ | *katG* | *inhA* | *fabG1* | *embB* | *ethA* |
|---|---|---|---|---|---|---|
| 2020F | Arg447Arg* (Arg528Arg)† | | | Leu203Leu | | |
| 2020G | | | C-15T | | | |
| 2020H | | Ser315Thr | | | Met306Val | (partial deletion) |
| 2020I | Arg447Arg* (Arg528Arg)† | | | Leu203Leu | | |
| 2020J | Ser450Leu* (Ser531Leu)† | | | | | |

¥Mutation is listed using both the M. tuberculosis and E.coli numbering system [5, 6]

*M. tuberculosis numbering system used

†E. coli numbering system used



## Technical Notes

The following information pertains to all of the tables and figures for the 2020 MTBC isolates F, G, H, I, and J included in this report.

- The source of data in all tables and figures is the September 2020 MPEP MTBC DST survey.
- 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.
- 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 the number of participating laboratories. This report contains all results reported by participating laboratories.
- The Sensititre system allows determination of a minimum inhibitory concentration (MIC) for each drug in the panel. Laboratories using this method may establish breakpoints to provide a categorical interpretation of S or R.
- For participant result tables for first- and second-line DST that have drug-method totals equal to 0, results were not received or the test was not performed.

# Descriptive Information about Participant Laboratories

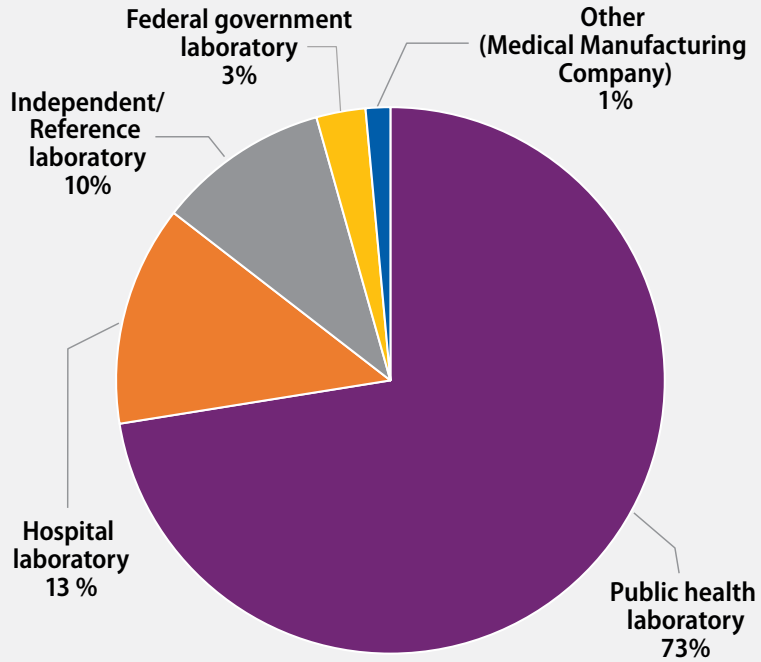
## Primary Classification

This report contains DST results submitted to CDC by survey participants at 69 laboratories in 34 states.

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

- **50 (73%)**: Public health laboratory (e.g., local, county, state)
- **9 (13%)**: Hospital laboratory
- **7 (10%)**: Independent/Reference laboratory (non-hospital based)
- **2 (3%)**: Federal government laboratory
- **1 (1%)**: Other (Medical Manufacturing Company)

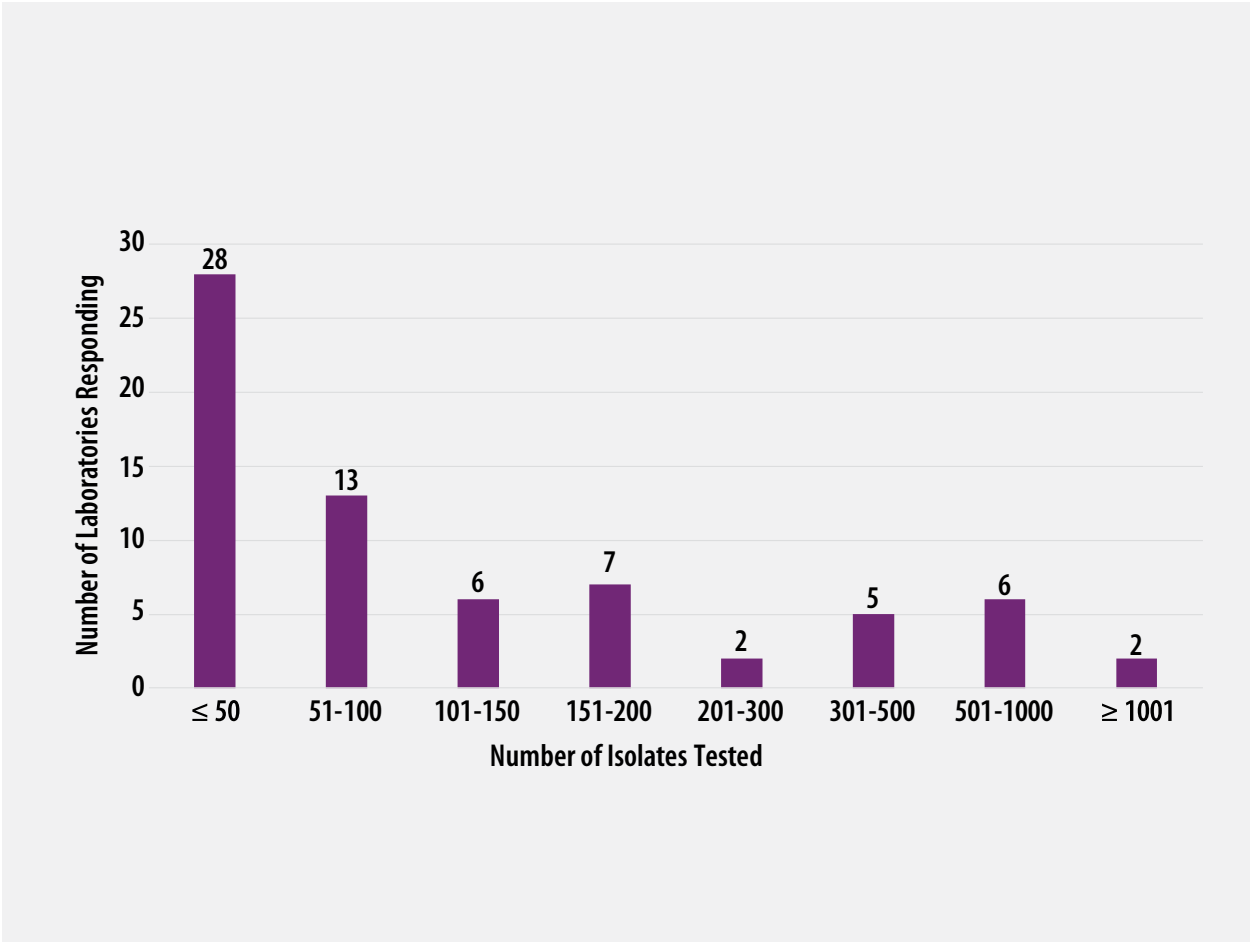
### Figure 1. Primary Classification of Participating Laboratories, September 2020



## Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 69 participants in 2019 (excluding isolates used for quality control) is shown in Figure 2. In 2019, the counts ranged from 0 to 1,039 tests. Participants at 28 (41%) 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 [7].

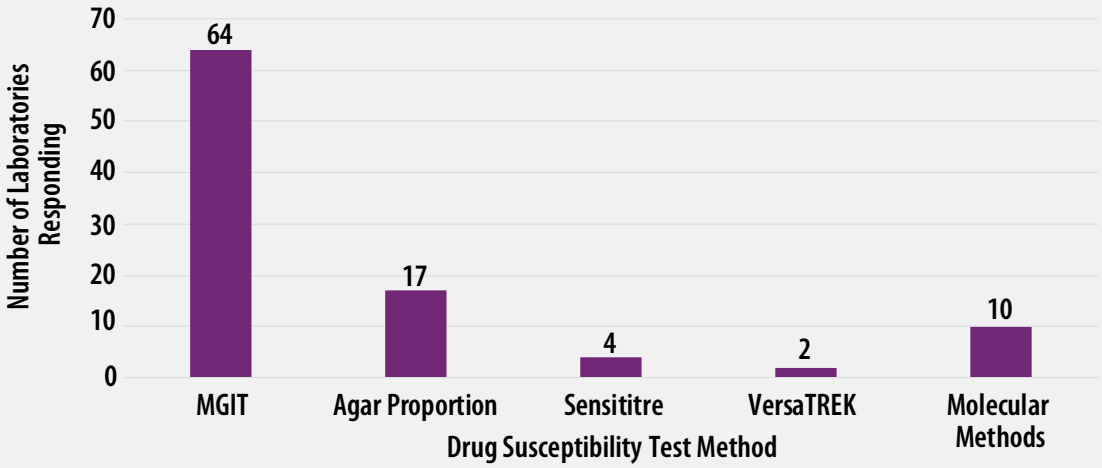
**Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=69)**



## MTBC DST Methods Used by Participants

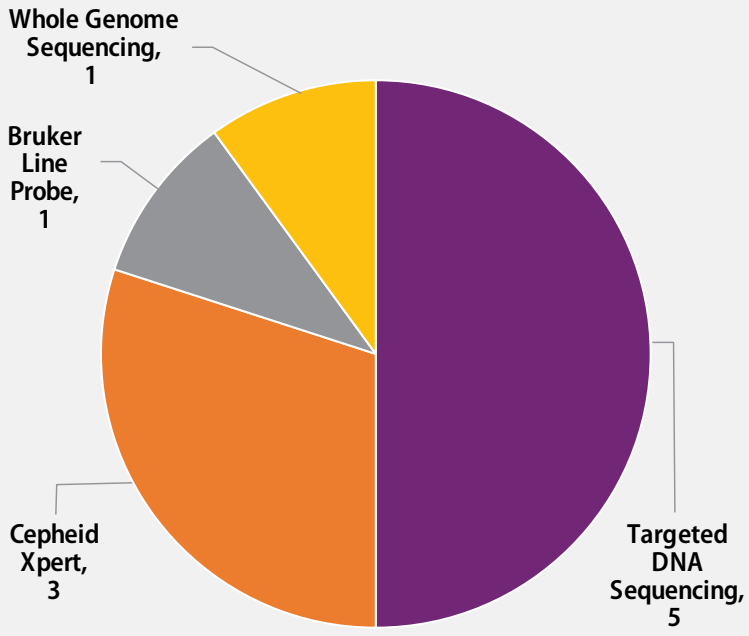
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Of participating laboratories, 46 (66%) reported results for only one method, 20 (29%) reported two methods, and 4 (5%) noted three susceptibility methods.

### Figure 3. MTBC Drug Susceptibility Test Method Used by Participants (n=97)




Ten molecular methods reported by participants are shown in Figure 4. The method used most frequently by laboratories (5) was targeted DNA sequencing (50%), including pyrosequencing and Sanger sequencing. Three (30%) laboratories reported use of the Cepheid Xpert MTB/RIF assay, one (10%) reported results for line probe assays, Genotype MTBDR*plus* and MTBDRsl by Bruker, and one (10%) reported results from whole genome sequencing.

### Figure 4. Molecular Method Reported (n=10)

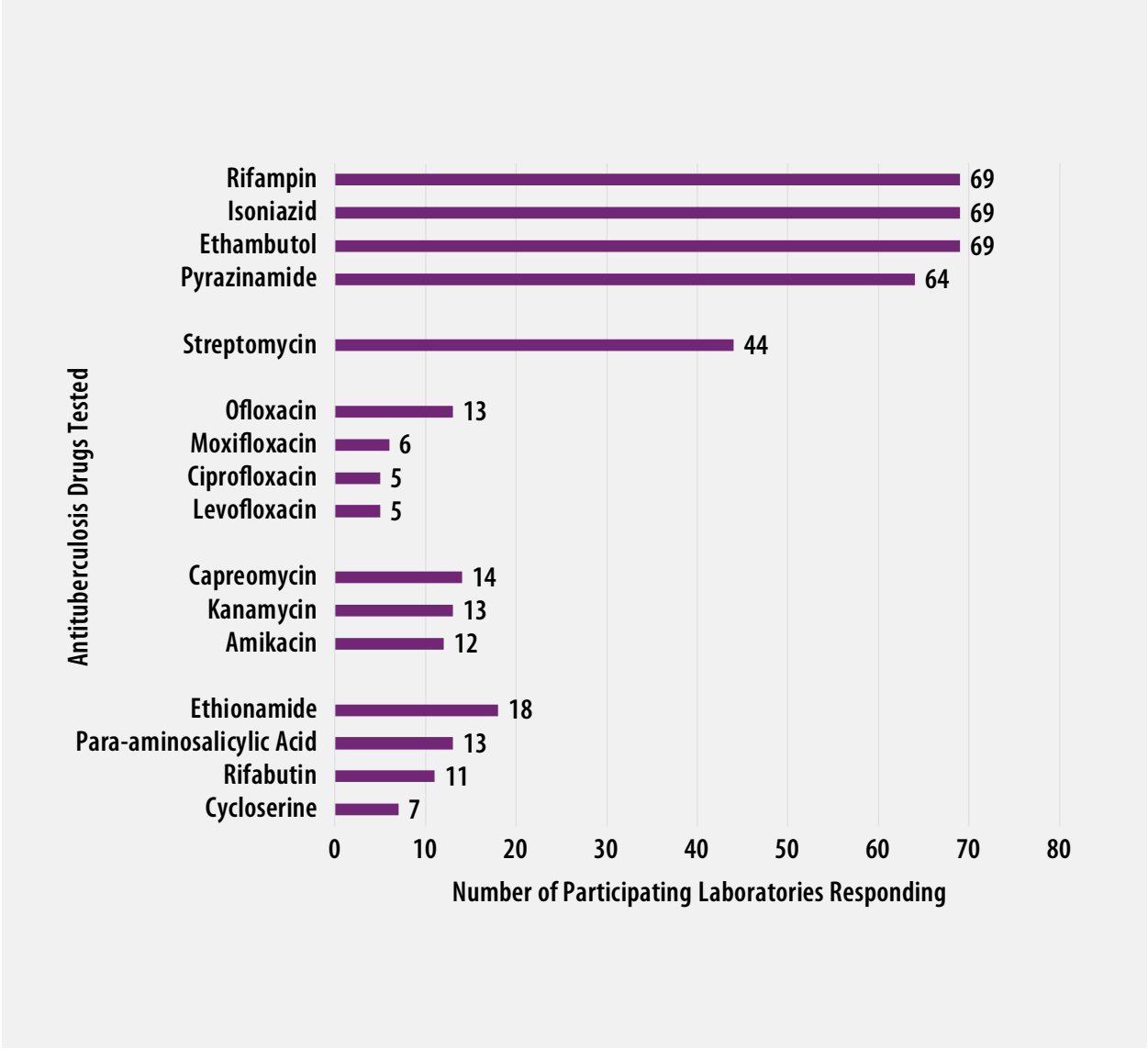


## Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the September 2020 survey is presented 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. All participants reported results for three of the first-line drugs (RMP, INH and EMB) and 64 (93%) also reported results for PZA by growth-based DST methods. One laboratory performs molecular testing for PZA via sequencing of *pncA*, in place of growth-based DST.

For 21 laboratories reporting second-line drug results (with the exception of streptomycin), four (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

### Figure 5. Antituberculosis Drugs Tested by Participants



# Isolate 2020F

Expected Result: Resistant to INH at 0.2 µg/ml and ETA at 5.0 µg/ml by agar proportion

## Isoniazid

Isoniazid (INH) is the most widely used first-line antituberculosis drug and is a cornerstone of regimens used to treat TB disease and latent TB infection. INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *katG* gene [4, 8]. The target of activated INH is enoyl-acyl-carrier protein reductase (encoded by the *inhA* gene); this binding inhibits cell wall mycolic acid biosynthesis. There are two mechanisms that account for the majority of INH resistance [4, 8, 9]. 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 to INH and are less frequent than *katG* mutations. Approximately 10–15% of isolates found to be INH-resistant have no mutations detected in either of these loci. Numerous loci have been investigated to identify additional genes correlated with INH resistance. The *fabG1* (also known as *mabA*) gene, like *inhA*, is involved in mycolic acid biosynthesis and at least one mutation in this region has been associated with low-level INH resistance [10, 11]. In MTBC, *ahpC* codes for an alkyl hydroperoxide reductase that is associated with resistance to reactive oxygen and reactive nitrogen intermediates; consequently, it is believed that mutations in the promoter region could be surrogate markers for INH resistance [8].

DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* for Isolate 2020F revealed a G>A point mutation in *fabG1* at codon 203 resulting in the synonymous/silent mutation Leu203Leu; *inhA*, *katG*, and *ahpC* were wild-type (i.e., no mutations were detected).

Within *fabG1*, the silent/synonymous mutation (i.e., nucleotide change but no corresponding change in amino acid) Leu203Leu has been found to confer INH resistance through the formation of an alternative promoter, thereby increasing the transcriptional levels of *inhA* [11]. Although silent mutations were previously believed to not play a role in drug resistance, the Leu203Leu mutation demonstrates that silent mutations could be associated with resistance depending on the specific gene and the location of the mutation.

The recommended critical concentration and additional higher concentrations for testing INH using the AP method are 0.2 µg/ml and 1.0 µg/ml, respectively. The equivalent concentrations for MGIT and VersaTREK are 0.1 µg/ml and 0.4 µg/ml [1].

For Isolate 2020F, 78 INH results were reported for the critical concentration. This isolate was reported **resistant** to INH by method, as follows:

- **94% (15/16)** of the results when using AP
- **49% (28/57)** of the results when using MGIT
- **0% (0/3)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

No results were reported as resistant at the higher concentrations of INH. Only 28 (48%) laboratories performing MGIT DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a different method.

Of the 6 molecular results reported for INH, 3 (50%) laboratories reported detection of a mutation in *fabG1*, all specifically noting the Leu203Leu mutation.

Three of the laboratories performing Sensititre reported INH MIC values as 0.12 µg/ml (n=2) and 0.25 µg/ml (n=1).

## Ethionamide

Resistance to INH and ethionamide (ETA) can occur by mutations in the *fabG1–inhA* regulatory region, which are generally associated with low-level resistance to INH. Mutations in *ethA* also confer resistance to ETA, without concomitant resistance to INH [12].

Sequencing analysis of *ethA* was not performed and as previously noted, sequencing of the *inhA* gene revealed wild-type (i.e., no mutations were detected). The synonymous/silent mutation Leu203Leu was detected in the *fabG1* locus for Isolate 2020F.

For Isolate 2020F, 17 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **84% (11/13)** of the results when using AP
- **67% (2/3)** of the results when using MGIT
- **0% (0/1)** of the results when using Sensititre

One laboratory performing Sensititre reported an ETA MIC value as 2.5 µg/ml (n=1) with a categorical result of susceptible. One additional laboratory reported an ETA MIC value as 10 µg/ml (n=1) but as no interpretation was indicated by this laboratory, the result was excluded from Table 9.

## Rifampin

Rifampin (RMP) is a bactericidal drug used as part of a standard first-line regimen for the treatment of TB. RMP's mechanism of action is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase [8]. The primary mechanism of resistance is a mutation within the 81-bp central region of the *rpoB* gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase [9]. Mutations in codons 450, 445 and 435 (E. coli numbering system corresponding to 531, 526 and 516) are among the most frequent mutations in RMP-resistant isolates and serve as predictors of RMP resistance [8, 9]. The activity of RMP on isolates with *rpoB* mutations 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 be confirmed by DNA sequencing of *rpoB* [13]. The Xpert MTB/RIF assay could generate results that falsely indicate resistance when compared to growth-based methods because of the presence of silent/synonymous mutations [14]. Sequencing of *rpoB* will allow for clarification of the result and understanding of possible discordance between rapid molecular and growth-based testing results.

DNA sequence analysis of *rpoB* in Isolate 2020F revealed a C>T point mutation in codon 447 (E. coli numbering 528) of the *rpoB* locus. However, this mutation does not result in an amino acid change; arginine remains arginine (Arg447Arg). Unlike the *fabG1* silent mutation in this isolate that was associated with INH resistance, the Arg447Arg 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. However, as noted above, the Xpert MTB/RIF assay could indicate RMP resistance for this isolate and sequencing of *rpoB* should be performed.

For Isolate 2020F, 80 results for RMP were reported. This isolate was reported as **susceptible** to RMP by method, as follows:

- **100% (16/16)** of the results when using AP
- **100% (59/59)** of the results when using MGIT
- **100% (3/3)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Of the 8 molecular results reported for RMP, 5 (63%) laboratories reported mutation detected with 4 laboratories specifically noting the Arg447Arg silent mutation. Three laboratories reported mutation not detected, however this may be due to the detection of a silent mutation not associated with resistance.

Three of the laboratories performing Sensititre reported RMP MIC values as ≤0.12 µg/ml (n=1), 0.12 µg/ml (n=1) and 0.25 µg/ml (n=1).

*Complete first-line DST, second-line DST and molecular results submitted by all participants for Isolate 2020F are listed in Tables 3–10.*

*Three laboratories noted no growth for at least one antituberculosis drug tested for Isolate 2020F*

### Table 3. Isolate 2020F—Participant Results for First-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 16 | 0 | 16 |
| Isoniazid—Low | 1 | 15 | 16 |
| Isoniazid—High | 16 | 0 | 16 |
| Ethambutol | 16 | 0 | 16 |

### Table 4. Isolate 2020F—Participant Results for First-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 59 | 0 | 59 |
| Isoniazid—Low | 29 | 28 | 57* |
| Isoniazid—High | 28 | 0 | 28 |
| Ethambutol | 58 | 1 | 59 |
| Pyrazinamide | 61 | 1 | 62 |

* One additional laboratory reported 'Borderline' for INH by MGIT.

### Table 5. Isolate 2020F—Participant Results for First-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 3 | 0 | 3 |
| Isoniazid—Low | 3 | 0 | 3 |
| Isoniazid—High | 1 | 0 | 1 |
| Ethambutol | 3 | 0 | 3 |

### Table 6. Isolate 2020F—Participant Results for First-Line DST by VersaTREK

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 0 | 2 | 2 |
| Isoniazid—High | 2 | 0 | 2 |
| Ethambutol | 2 | 0 | 2 |
| Pyrazinamide | 1 | 0 | 1 |

### Table 7. Isolate 2020F—Participant Results for Second-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 14 | 0 | 14 |
| Ofloxacin | 10 | 0 | 10 |
| Ciprofloxacin | 4 | 0 | 4 |
| Levofloxacin | 2 | 0 | 2 |
| Moxifloxacin | 2 | 0 | 2 |
| Amikacin | 7 | 0 | 7 |
| Kanamycin | 10 | 0 | 10 |
| Capreomycin | 9 | 0 | 9 |
| Ethionamide | 2 | 11 | 13 |
| Rifabutin | 7 | 0 | 7 |
| Cycloserine | 4 | 0 | 4 |
| p-Aminosalicylic acid | 9 | 0 | 9 |

## Table 8. Isolate 2020F—Participant Results for Second-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 30 | 0 | 30 |
| Ofloxacin | 3 | 0 | 3 |
| Ciprofloxacin | 0 | 0 | 0 |
| Levofloxacin | 2 | 0 | 2 |
| Moxifloxacin | 2 | 0 | 2 |
| Amikacin | 3 | 0 | 3 |
| Kanamycin | 1 | 0 | 1 |
| Capreomycin | 3 | 0 | 3 |
| Ethionamide | 1 | 2 | 3 |
| Rifabutin | 2 | 0 | 2 |
| Cycloserine | 0 | 0 | 0 |
| p-Aminosalicylic acid | 1 | 0 | 1 |

## Table 9. Isolate 2020F—Participant Results for Second-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 2 | 0 | 2* |
| Ofloxacin | 1 | 0 | 1* |
| Ciprofloxacin | 0 | 0 | 0 |
| Levofloxacin | 1 | 0 | 1 |
| Moxifloxacin | 1 | 0 | 1* |
| Amikacin | 2 | 0 | 2* |
| Kanamycin | 1 | 0 | 1* |
| Capreomycin | 1 | 0 | 1 |
| Ethionamide | 1 | 0 | 1* |
| Rifabutin | 2 | 0 | 2* |
| Cycloserine | 1 | 0 | 1* |
| p-Aminosalicylic acid | 2 | 0 | 2* |

* One additional laboratory reported 'No Interpretation' for STR, OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre.

## Table 10. Isolate 2020F—Participant Results for Molecular Testing

| Drug | Mutation Detected | Mutation Not Detected | Total |
|---|---|---|---|
| Rifampin | 5 | 3 | 8 |
| Isoniazid | 3 | 3 | 6 |
| Ethambutol | 0 | 4 | 4 |
| Pyrazinamide | 0 | 2 | 2 |
| Ofloxacin | 0 | 3 | 3 |
| Ciprofloxacin | 0 | 3 | 3 |
| Levofloxacin | 0 | 4 | 4 |
| Moxifloxacin | 0 | 4 | 4 |
| Amikacin | 0 | 3 | 3 |
| Kanamycin | 0 | 3 | 3 |
| Capreomycin | 0 | 3 | 3 |
| Ethionamide | 2 | 0 | 2 |
| Rifabutin | 1 | 3 | 4 |

# Isolate 2020G

Expected Result: Resistant to INH at 0.2 µg/ml and ETA at 5.0 µg/ml by agar proportion

## Isoniazid

DNA sequence analysis of *inhA*, *katG*, *fabG1*, and *ahpC* of Isolate 2020G revealed a C>T point mutation at nucleotide position -15 of the promoter region of the *inhA* gene (C-15T); *katG*, *fabG1* and *ahpC* were wild-type (i.e., no mutations were detected). Mutations in the promoter region of the *inhA* gene are generally associated with low-level resistance to INH.

For Isolate 2020G, 82 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (17/17)** of the results when using AP
- **100% (61/61)** of the results when using MGIT
- **50% (1/2)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Five (9%) results were reported as resistant at the higher concentrations of INH. Only 34 (56%) laboratories performing MGIT DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a second DST method.

Of the 6 molecular results reported for INH, all (100%) laboratories reported detection of a mutation with 5 laboratories specifically noting the C-15T mutation.

Two of the laboratories performing Sensititre reported INH MIC values as 0.25 µg/ml (n=1) and 0.5 µg/ml (n=1). A third laboratory reported an INH MIC value as 0.25 µg/ml (n=1) and indicated a result of borderline.

*Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2020G are listed in Tables 11–18*

### Table 11. Isolate 2020G—Participant Results for First-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 17 | 0 | 17 |
| Isoniazid—Low | 0 | 17 | 17 |
| Isoniazid—High | 17 | 0 | 17 |
| Ethambutol | 17 | 0 | 17 |

### Table 12. Isolate 2020G—Participant Results for First-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 62 | 0 | 62 |
| Isoniazid—Low | 0 | 61 | 61 |
| Isoniazid—High | 29 | 5 | 34 |
| Ethambutol | 62 | 0 | 62 |
| Pyrazinamide | 60 | 3 | 63 |

### Table 13. Isolate 2020G—Participant Results for First-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 3 | 0 | 3 |
| Isoniazid—Low | 1 | 1 | 2* |
| Isoniazid—High | 0 | 0 | 0* |
| Ethambutol | 3 | 0 | 3 |

* One additional laboratory reported borderline for INH by Sensititre.

### Table 14. Isolate 2020G—Participant Results for First-Line DST by VersaTREK

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 0 | 2 | 2 |
| Isoniazid—High | 2 | 0 | 2 |
| Ethambutol | 2 | 0 | 2 |
| Pyrazinamide | 1 | 0 | 1 |

### Table 15. Isolate 2020G—Participant Results for Second-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 15 | 0 | 15 |
| Ofloxacin | 10 | 0 | 10 |
| Ciprofloxacin | 5 | 0 | 5 |
| Levofloxacin | 2 | 0 | 2 |
| Moxifloxacin | 2 | 0 | 2 |
| Amikacin | 7 | 0 | 7 |
| Kanamycin | 11 | 0 | 11 |
| Capreomycin | 10 | 0 | 10 |
| Ethionamide | 1 | 13 | 14 |
| Rifabutin | 7 | 0 | 7 |
| Cycloserine | 5 | 0 | 5 |
| p-Aminosalicylic acid | 10 | 0 | 10 |

### Table 16. Isolate 2020G—Participant Results for Second-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 32 | 0 | 32 |
| Ofloxacin | 3 | 0 | 3 |
| Ciprofloxacin | 0 | 0 | 0 |
| Levofloxacin | 2 | 0 | 2 |
| Moxifloxacin | 2 | 0 | 2 |
| Amikacin | 3 | 0 | 3 |
| Kanamycin | 1 | 0 | 1 |
| Capreomycin | 3 | 0 | 3 |
| Ethionamide | 0 | 3 | 3 |
| Rifabutin | 2 | 0 | 2 |
| Cycloserine | 0 | 0 | 0 |
| p-Aminosalicylic acid | 1 | 0 | 1 |

## Table 17. Isolate 2020G—Participant Results for Second-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 2 | 0 | 2* |
| Ofloxacin | 1 | 0 | 1* |
| Ciprofloxacin | 0 | 0 | 0 |
| Levofloxacin | 1 | 0 | 1 |
| Moxifloxacin | 1 | 0 | 1* |
| Amikacin | 2 | 0 | 2* |
| Kanamycin | 1 | 0 | 1* |
| Capreomycin | 1 | 0 | 1 |
| Ethionamide | 1 | 0 | 1* |
| Rifabutin | 2 | 0 | 2* |
| Cycloserine | 0 | 0 | 0* |
| p-Aminosalicylic acid | 2 | 0 | 2* |

* One additional laboratory reported 'No Interpretation' for STR, OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre.

## Table 18. Isolate 2020G—Participant Results for Molecular Testing

| Drug | Mutation Detected | Mutation Not Detected | Total |
|---|---|---|---|
| Rifampin | 0 | 8 | 8 |
| Isoniazid | 6 | 0 | 6 |
| Ethambutol | 0 | 4 | 4 |
| Pyrazinamide | 1* | 1 | 2 |
| Ofloxacin | 0 | 3 | 3 |
| Ciprofloxacin | 0 | 3 | 3 |
| Levofloxacin | 0 | 4 | 4 |
| Moxifloxacin | 0 | 4 | 4 |
| Amikacin | 0 | 3 | 3 |
| Kanamycin | 0 | 3 | 3 |
| Capreomycin | 0 | 2 | 2 |
| Ethionamide | 2 | 0 | 2 |
| Rifabutin | 0 | 4 | 4 |

* One laboratory noted the detection of a mutation not associated with PZA resistance..

# Isolate 2020H

Expected Result: Resistant to INH at 0.2 µg/ml and 1.0 µg/ml, EMB at 5.0 µg/ml, ETA at 5.0 µg/ml and STR at 2.0 µg/ml by agar proportion

## Isoniazid

As previously noted, resistance to INH most commonly occurs due to mutations in the *katG* gene or the promoter region of the *inhA* gene, however, mutations in *fabG1* and *ahpC* can also cause resistance. DNA sequence analysis of Isolate 2020H revealed a G>C point mutation at codon 315 in the *katG* locus resulting in wild-type serine being replaced by threonine (Ser315Thr); *inhA*, *fabG1*, and *ahpC* were wild-type (i.e., no mutations were detected).

For Isolate 2020H, 82 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **100% (17/17)** of the results when using AP
- **100% (61/61)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Fifty-five or 100% of results at the higher concentrations of INH were reported as resistant. Only 34 (56%) laboratories performing MGIT DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a second DST method.

Of the 6 molecular results reported for INH, all (100%) laboratories reported detection of a mutation with 5 laboratories specifically noting the Ser315Thr mutation.

Two of the laboratories performing Sensititre reported INH MIC values as 4 µg/ml (n=2). One additional laboratory reported an INH MIC value as 4 µg/ml (n=1).

## Ethambutol

Ethambutol (EMB) is an important first-line drug for the treatment of TB 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 [8, 9]. EMB targets the arabinosyl transferases (*embCAB* operon), thereby inhibiting the biosynthesis of the cell wall components arabinogalactan and lipoarabinomannan [15].

Issues with false-susceptibility with some growth-based methods for EMB, particularly in broth-based media, have been reported and remain a potential concern. Probable causes include the bacteriostatic nature of the drug, reduced drug activity in culture, and an organism's MIC for EMB falling too close to the critical concentration tested [16-18].

Sequence analysis of EMB-resistant clinical isolates has shown that EMB resistance is associated primarily with missense (non-synonymous) mutations within the EMB resistance determining region of the gene *embB* at codons 306, 406 and 497 [4, 15].

DNA sequence analysis of *embB* of Isolate 2020H revealed a A>G point mutation at codon 306 in the *embB* gene resulting in wild-type methionine being replaced by valine (Met306Val). Certain *embB* mutations at the 306 codon, such as Met306Val and Met306Leu, are associated with EMB resistance [4].

For Isolate 2020H, 83 EMB results were reported. This isolate was reported **resistant** to EMB by method, as follows:

- **88% (15/17)** of the results when using AP
- **16% (10/61)** of the results when using MGIT
- **100% (3/3)** of the results when using Sensititre
- **50% (1/2)** of the results when using VersaTREK

Of the 4 molecular results reported for EMB, all laboratories reported detection of a mutation and specifically noted the Met306Val mutation.

Three of the laboratories performing Sensititre reported EMB MIC values as 8 µg/ml (n=3).

## Ethionamide

As previously noted, resistance to ETA is commonly due to mutations in the *ethA* gene or mutations in *fabG1* or *inhA* resulting in cross-resistance with INH.

DNA sequencing analysis revealed a partial deletion of *ethA*; *inhA* and *fabG1* were wild-type (i.e., no mutations were detected).

For Isolate 2020H, 18 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **64% (9/14)** of the results when using AP
- **100% (3/3)** of the results when using MGIT
- **0% (0/1)** of the results when using Sensititre

Of the 2 molecular results reported for ETA, 1 (50%) laboratory reported detection of a mutation and specifically noted an *ethA* deletion.

One of the laboratories performing Sensititre reported an ETA MIC value as 2.5 µg/ml (n=1). One laboratory reported an ETA MIC value as 5 µg/ml (n=1) but as no interpretation was indicated by this laboratory, the result was excluded from Table 25.

## Streptoymycin

Streptomycin (STR) belongs to the aminoglycoside class of drugs and its primary mechanism of action is to inhibit protein synthesis by preventing the initiation of translation by binding to the 16s rRNA [8, 9]. In MTBC, the genetic basis of the majority of resistance to STR is usually due to mutations in *rrs* or *rpsL* [9, 19]. CLSI recommended testing STR as a second-line drug based on American Thoracic Society's categorization of STR as a second-line drug for treatment due to increased resistance in many parts of the world [1, 20].

DNA sequencing analysis did not reveal a mutation in *rrs* or *rpsL*; other mechanisms of resistance may exist.

Among three methods, 48 results for STR were reported for Isolate 2020H. This isolate was reported as **resistant** to STR by method, as follows:

- **76% (11/14)** of the results when using AP
- **48% (16/33)** of the results when using MGIT
- **100% (1/1)** of the results when using Sensititre

One of the laboratories performing Sensititre reported an STR MIC value as 4.0 µg/ml (n=1). A second laboratory reported a STR MIC value as 2 µg/ml (n=1) and indicated borderline resistance. A third laboratory reported STR MIC value as 4 µg/ml (n=1) but as no interpretation was indicated, the result was excluded from Table 25.

*Complete first-line DST, second-line DST and molecular results submitted by all participant for Isolate 2020H are listed in Tables 19–26.*

### Table 19. Isolate 2020H—Participant Results for First-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 16 | 1 | 17 |
| Isoniazid—Low | 0 | 17 | 17 |
| Isoniazid—High | 0 | 17 | 17 |
| Ethambutol | 2 | 15 | 17 |

### Table 20. Isolate 2020H—Participant Results for First-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 62 | 0 | 62 |
| Isoniazid—Low | 0 | 61 | 61 |
| Isoniazid—High | 0 | 34 | 34 |
| Ethambutol | 51 | 10 | 61* |
| Pyrazinamide | 63 | 0 | 63 |

* One additional laboratory reported borderline for EMB by MGIT.

### Table 21. Isolate 2020H—Participant Results for First-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 3 | 0 | 3 |
| Isoniazid—Low | 0 | 2 | 2 |
| Isoniazid—High | 0 | 2 | 2 |
| Ethambutol | 0 | 3 | 3 |

### Table 22. Isolate 2020H—Participant Results for First-Line DST by VersaTREK

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 0 | 2 | 2 |
| Isoniazid—High | 0 | 2 | 2 |
| Ethambutol | 1 | 1 | 2 |
| Pyrazinamide | 1 | 0 | 1 |

### Table 23. Isolate 2020H—Participant Results for Second-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 3 | 11 | 14* |
| Ofloxacin | 10 | 0 | 10 |
| Ciprofloxacin | 5 | 0 | 5 |
| Levofloxacin | 2 | 0 | 2 |
| Moxifloxacin | 2 | 0 | 2 |
| Amikacin | 7 | 0 | 7 |
| Kanamycin | 11 | 0 | 11 |
| Capreomycin | 10 | 0 | 10 |
| Ethionamide | 5 | 9 | 14 |
| Rifabutin | 7 | 0 | 7 |
| Cycloserine | 5 | 0 | 5 |
| p-Aminosalicylic acid | 10 | 0 | 10 |

* One additional laboratory reported 'Borderline' for STR by AP.

## Table 24. Isolate 2020H—Participant Results for Second-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 17 | 16 | 33 |
| Ofloxacin | 3 | 0 | 3 |
| Ciprofloxacin | 0 | 0 | 0 |
| Levofloxacin | 2 | 0 | 2 |
| Moxifloxacin | 2 | 0 | 2 |
| Amikacin | 3 | 0 | 3 |
| Kanamycin | 1 | 0 | 1 |
| Capreomycin | 3 | 0 | 3 |
| Ethionamide | 0 | 3 | 3 |
| Rifabutin | 2 | 0 | 2 |
| Cycloserine | 0 | 0 | 0 |
| p-Aminosalicylic acid | 1 | 0 | 1 |

## Table 25. Isolate 2020H—Participant Results for Second-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 0 | 1 | 1*† |
| Ofloxacin | 1 | 0 | 1* |
| Ciprofloxacin | 0 | 0 | 0 |
| Levofloxacin | 1 | 0 | 1 |
| Moxifloxacin | 2 | 0 | 2* |
| Amikacin | 2 | 0 | 2* |
| Kanamycin | 1 | 0 | 1* |
| Capreomycin | 1 | 0 | 1 |
| Ethionamide | 1 | 0 | 1* |
| Rifabutin | 2 | 0 | 2* |
| Cycloserine | 0 | 0 | 0* |
| p-Aminosalicylic acid | 2 | 0 | 2* |

* One additional laboratory reported 'No Interpretation' for STR, OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre.
† One additional laboratory reported 'Borderline' for STR by Sensititre.

## Table 26. Isolate 2020H—Participant Results for Molecular Testing

| Drug | Mutation Detected | Mutation Not Detected | Total |
|---|---|---|---|
| **Rifampin** | 0 | 8 | 8 |
| **Isoniazid** | 6 | 0 | 6 |
| **Ethambutol** | 4 | 0 | 4 |
| **Pyrazinamide** | 1* | 1 | 2 |
| **Ofloxacin** | 0 | 3 | 3 |
| **Ciprofloxacin** | 0 | 3 | 3 |
| **Levofloxacin** | 0 | 4 | 4 |
| **Moxifloxacin** | 0 | 4 | 4 |
| **Amikacin** | 0 | 3 | 3 |
| **Kanamycin** | 0 | 3 | 3 |
| **Capreomycin** | 0 | 2 | 2 |
| **Ethionamide** | 1 | 1 | 2 |
| **Rifabutin** | 0 | 4 | 4 |

* This laboratory noted the detection of a mutation not associated with PZA resistance.

# Isolate 2020I

Expected Result: Resistant to INH at 0.2 µg/ml and ETA at 5.0 µg/ml by agar proportion

**Isolate 2020I is a duplicate of Isolate 2020F.** Therefore, laboratories should have the same results for both isolates. Overall, laboratories reported similar results for INH, ETA, and RMP. Laboratories should consider performing an internal comparison of results between these two isolates.

## Isoniazid

DNA sequence analysis for Isolate 2020I revealed a *fabG1* G>A point mutation at codon 203 resulting in the synonymous/silent mutation Leu203Leu; *inhA*, *katG*, and *ahpC* were wild-type (i.e., no mutations were detected).

The recommended critical concentration and additional higher concentrations for testing INH using the AP method are 0.2 µg/ml and 1.0 µg/ml, respectively. The equivalent concentrations for MGIT and VersaTREK are 0.1 µg/ml and 0.4 µg/ml [1].

For Isolate 2020I, 80 INH results were reported. This isolate was reported **resistant** to INH by method, as follows:

- **82% (14/17)** of the results when using AP
- **55% (32/58)** of the results when using MGIT
- **0% (0/3)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

No results were reported as **resistant** at the higher concentrations of INH. Only 29 (49%) laboratories performing MGIT DST reported a result for the higher concentration of INH, although some may have tested the higher concentration by a different method.

Of the 6 molecular results reported for INH, 3 (50%) laboratories reported detection of a mutation, specifically noting the Leu203Leu mutation.

Three of the laboratories performing Sensititre reported INH MIC values as 0.25 µg/ml (n=1), 0.50 µg/ml (n=1) and 1.2 µg/ml (n=1).

## Ethionamide

DNA sequencing analysis of *ethA* was not performed and as previously noted, sequencing of the *inhA* gene revealed wild-type (i.e., no mutations were detected). The synonymous/silent mutation Leu203Leu was detected in the *fabG1* locus for Isolate 2020I.

For Isolate 2020I, 18 ETA results were reported. This isolate was reported **resistant** to ETA by method, as follows:

- **71% (10/14)** of the results when using AP
- **67% (2/3)** of the results when using MGIT
- **0% (0/1)** of the results when using Sensititre

One of the laboratories performing Sensititre reported an ETA MIC value as 5 µg/ml (n=1). Another laboratory reported an ETA MIC value as 10 µg/ml (n=1) but as no categorical interpretation was provided, the data were not included in Table 33.

## Rifampin

DNA sequence analysis of *rpoB* in Isolate 2020I revealed a C>T point mutation in codon 447 (E. coli numbering 528) of the *rpoB* locus. However, this mutation does not result in an amino acid change; arginine remains arginine (Arg447Arg). Unlike the *fabG1* silent mutation in this isolate that was associated with INH resistance, the Arg447Arg 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. However, as noted above, the Xpert MTB/RIF assay could indicate RMP resistance for this isolate and sequencing of *rpoB* should be performed.

For Isolate 2020I, 82 results for RMP were reported. This isolate was reported as **susceptible** to RMP by method, as follows:

- **100% (17/17)** of the results when using AP
- **100% (60/60)** of the results when using MGIT
- **100% (3/3)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Of the 8 molecular results reported for RMP, 5 (63%) laboratories reported mutation detected with 4 laboratories specifically noting the Arg447Arg silent mutation. Three laboratories reported mutation not detected, however this may be due to the detection of a silent mutation not associated with resistance.

Three of the laboratories performing Sensititre reported RMP MIC values as ≤0.12 µg/ml (n=1), 0.12 µg/ml (n=1) and 0.25 µg/ml (n=1).

*Complete first-line DST, second-line DST and molecular results submitted by all participants for Isolate 2020I are listed in Tables 27–34.*

*Two laboratories noted no growth for at least one antituberculosis drug tested for Isolate 2020I.*

### Table 27. Isolate 2020I—Participant Results for First-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| **Rifampin** | 17 | 0 | 17 |
| **Isoniazid—Low** | 3 | 14 | 17 |
| **Isoniazid—High** | 17 | 0 | 17 |
| **Ethambutol** | 17 | 0 | 17 |

### Table 28. Isolate 2020I—Participant Results for First-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| **Rifampin** | 60 | 0 | 60 |
| **Isoniazid—Low** | 26 | 32 | 58* |
| **Isoniazid—High** | 29 | 0 | 29 |
| **Ethambutol** | 60 | 0 | 60 |
| **Pyrazinamide** | 60 | 1 | 61 |

* One additional laboratory reported 'Borderline' for INH by MGIT.

### Table 29. Isolate 2020I—Participant Results for First-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| **Rifampin** | 3 | 0 | 3 |
| **Isoniazid—Low** | 3 | 0 | 3 |
| **Isoniazid—High** | 1 | 0 | 1 |
| **Ethambutol** | 3 | 0 | 3 |

### Table 30. Isolate 2020I—Participant Results for First-Line DST by VersaTREK

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 2 | 0 | 2 |
| Isoniazid—Low | 0 | 2 | 2 |
| Isoniazid—High | 2 | 0 | 2 |
| Ethambutol | 2 | 0 | 2 |
| Pyrazinamide | 1 | 0 | 1 |

### Table 31. Isolate 2020I—Participant Results for Second-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 15 | 0 | 15 |
| Ofloxacin | 10 | 0 | 10 |
| Ciprofloxacin | 5 | 0 | 5 |
| Levofloxacin | 2 | 0 | 2 |
| Moxifloxacin | 2 | 0 | 2 |
| Amikacin | 7 | 0 | 7 |
| Kanamycin | 11 | 0 | 11 |
| Capreomycin | 10 | 0 | 10 |
| Ethionamide | 4 | 10 | 14 |
| Rifabutin | 7 | 0 | 7 |
| Cycloserine | 5 | 0 | 5 |
| p-Aminosalicylic acid | 10 | 0 | 10 |

### Table 32. Isolate 2020I—Participant Results for Second-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 31 | 0 | 31 |
| Ofloxacin | 3 | 0 | 3 |
| Ciprofloxacin | 0 | 0 | 0 |
| Levofloxacin | 2 | 0 | 2 |
| Moxifloxacin | 2 | 0 | 2 |
| Amikacin | 3 | 0 | 3 |
| Kanamycin | 1 | 0 | 1 |
| Capreomycin | 3 | 0 | 3 |
| Ethionamide | 1 | 2 | 3 |
| Rifabutin | 2 | 0 | 2 |
| Cycloserine | 0 | 0 | 0 |
| p-Aminosalicylic acid | 1 | 0 | 1 |

## Table 33. Isolate 2020I—Participant Results for Second-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 2 | 0 | 2* |
| Ofloxacin | 1 | 0 | 1* |
| Ciprofloxacin | 0 | 0 | 0 |
| Levofloxacin | 1 | 0 | 1 |
| Moxifloxacin | 1 | 0 | 1* |
| Amikacin | 2 | 0 | 2* |
| Kanamycin | 1 | 0 | 1* |
| Capreomycin | 1 | 0 | 1 |
| Ethionamide | 1 | 0 | 1* |
| Rifabutin | 2 | 0 | 2* |
| Cycloserine | 0 | 0 | 0* |
| p-Aminosalicylic acid | 2 | 0 | 2* |

* One additional laboratory reported 'No Interpretation' for STR, OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre.

## Table 34. Isolate 2020I—Participant Results for Molecular Testing

| Drug | Mutation Detected | Mutation Not Detected | Total |
|---|---|---|---|
| Rifampin | 5 | 3 | 8 |
| Isoniazid | 3 | 3 | 6 |
| Ethambutol | 0 | 4 | 4 |
| Pyrazinamide | 0 | 2 | 2 |
| Ofloxacin | 0 | 3 | 3 |
| Ciprofloxacin | 0 | 3 | 3 |
| Levofloxacin | 0 | 4 | 4 |
| Moxifloxacin | 0 | 4 | 4 |
| Amikacin | 0 | 3 | 3 |
| Kanamycin | 0 | 3 | 3 |
| Capreomycin | 0 | 2 | 2 |
| Ethionamide | 2 | 0 | 2 |
| Rifabutin | 1 | 3 | 4 |

# Isolate 2020J

Expected Result: Resistant to RMP at 1.0 µg/ml by agar proportion

## Rifampin

DNA sequence analysis of *rpoB* in Isolate 2020J revealed a C>T point mutation in codon 450 (E. coli numbering 531) resulting in wild-type serine being replaced by leucine (Ser450Leu). Isolates with Ser450Leu (Ser531Leu in E. coli numbering system) mutations consistently test resistant to RMP in growth-based assays.

For Isolate 2020J, 81 results for RMP were reported. This isolate was reported as **resistant** to RMP by method, as follows:

- **88% (15/17)** of the results when using AP
- **98% (58/59)** of the results when using MGIT
- **100% (3/3)** of the results when using Sensititre
- **100% (2/2)** of the results when using VersaTREK

Of the 9 molecular results reported for RMP, all (100%) laboratories reported detection of a mutation. Five laboratories specifically noted the Ser450Leu mutation and two laboratories reporting results for Xpert MTB/RIF noted no signal for Probe E.

Three of the laboratories performing Sensititre reported RMP MIC values as 16 µg/ml (n=1) and >16 µg/ml (n=2).

## Rifabutin

Participant results are consistent with rifabutin (RBT) results based on the presence of the *rpoB* Ser450Leu mutation [21].

Among three methods, 11 results for RBT were reported for Isolate 2020J. This isolate was reported as **resistant** to RBT by method, as follows:

- **100% (7/7)** of the results when using AP
- **100% (2/2)** of the results when using MGIT
- **100% (2/2)** of the results when using Sensititre

Of the 4 molecular results reported specifically for RBT, 3 (75%) laboratories reported detection of a mutation.

Two of the laboratories performing Sensititre reported an RBT MIC value as 2.0 µg/ml (n=2). Another laboratory reported an RBT MIC value as 0.5 µg/ml (n=1) but as no categorical interpretation was provided, the data were excluded from Table 41.

*Complete first-line DST, second-line DST and molecular results submitted by all participants for Isolate 2020J are listed in Tables 35–42.*

*One laboratory noted no growth for at least one antituberculosis drug tested for Isolate 2020J.*

### Table 35. Isolate 2020J—Participant Results for First-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| **Rifampin** | 2 | 15 | 17 |
| **Isoniazid—Low** | 17 | 0 | 17 |
| **Isoniazid—High** | 17 | 0 | 17 |
| **Ethambutol** | 17 | 0 | 17 |

### Table 36. Isolate 2020J—Participant Results for First-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 1 | 58 | 59 |
| Isoniazid—Low | 59 | 0 | 59 |
| Isoniazid—High | 23 | 0 | 23 |
| Ethambutol | 59 | 0 | 59 |
| Pyrazinamide | 63 | 0 | 63 |

### Table 37. Isolate 2020J—Participant Results for First-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 0 | 3 | 3 |
| Isoniazid—Low | 3 | 0 | 3 |
| Isoniazid—High | 2 | 0 | 2 |
| Ethambutol | 3 | 0 | 3 |

### Table 38. Isolate 2020J—Participant Results for First-Line DST by VersaTREK

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Rifampin | 0 | 2 | 2 |
| Isoniazid—Low | 2 | 0 | 2 |
| Isoniazid—High | 2 | 0 | 2 |
| Ethambutol | 2 | 0 | 2 |
| Pyrazinamide | 1 | 0 | 1 |

### Table 39. Isolate 2020J—Participant Results for Second-Line DST by AP

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 15 | 0 | 15 |
| Ofloxacin | 10 | 0 | 10 |
| Ciprofloxacin | 5 | 0 | 5 |
| Levofloxacin | 1 | 0 | 1 |
| Moxifloxacin | 1 | 0 | 1 |
| Amikacin | 7 | 0 | 7 |
| Kanamycin | 11 | 0 | 11 |
| Capreomycin | 10 | 0 | 10 |
| Ethionamide | 14 | 0 | 14 |
| Rifabutin | 0 | 7 | 7 |
| Cycloserine | 5 | 0 | 5 |
| p-Aminosalicylic acid | 10 | 0 | 10 |

## Table 40. Isolate 2020J—Participant Results for Second-Line DST by MGIT

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 32 | 0 | 32 |
| Ofloxacin | 3 | 0 | 3 |
| Ciprofloxacin | 0 | 0 | 0 |
| Levofloxacin | 2 | 0 | 2 |
| Moxifloxacin | 2 | 0 | 2 |
| Amikacin | 3 | 0 | 3 |
| Kanamycin | 1 | 0 | 1 |
| Capreomycin | 3 | 0 | 3 |
| Ethionamide | 3 | 0 | 3 |
| Rifabutin | 0 | 2 | 2 |
| Cycloserine | 1 | 0 | 1 |
| p-Aminosalicylic acid | 1 | 0 | 1 |

## Table 41. Isolate 2020J—Participant Results for Second-Line DST by Sensititre

| Drug | Susceptible | Resistant | Total |
|---|---|---|---|
| Streptomycin | 2 | 0 | 2* |
| Ofloxacin | 1 | 0 | 1* |
| Ciprofloxacin | 0 | 0 | 0 |
| Levofloxacin | 1 | 0 | 1 |
| Moxifloxacin | 1 | 0 | 1* |
| Amikacin | 2 | 0 | 2* |
| Kanamycin | 1 | 0 | 1* |
| Capreomycin | 1 | 0 | 1 |
| Ethionamide | 1 | 0 | 1* |
| Rifabutin | 0 | 2 | 2* |
| Cycloserine | 1 | 0 | 1* |
| p-Aminosalicylic acid | 2 | 0 | 2* |

* One additional laboratory reported 'No Interpretation' for STR, OFL, MOX, AMK, KAN, ETA, RBT, CYC, and PAS by Sensititre.

## Table 42. Isolate 2020J—Participant Results for Molecular Testing

| Drug | Mutation Detected | Mutation Not Detected | Total |
|---|---|---|---|
| Rifampin | 9 | 0 | 9 |
| Isoniazid | 0 | 6 | 6 |
| Ethambutol | 0 | 4 | 4 |
| Pyrazinamide | 0 | 2 | 2 |
| Ofloxacin | 0 | 3 | 3 |
| Ciprofloxacin | 0 | 3 | 3 |
| Levofloxacin | 0 | 4 | 4 |
| Moxifloxacin | 0 | 4 | 4 |
| Amikacin | 0 | 3 | 3 |
| Kanamycin | 0 | 3 | 3 |
| Capreomycin | 0 | 2 | 2 |
| Ethionamide | 0 | 2 | 2 |
| Rifabutin | 3 | 1 | 4 |

# Equivalent Critical Concentrations

(Concentrations listed as µg/ml)

## Agar Proportion

| First-line Drugs | 7H10 agar | 7H11 agar |
|---|---|---|
| **Isoniazid** | 0.2 and 1.0* | 0.2 and 1.0* |
| **Rifampin** | 1.0† | 1.0 |
| **Ethambutol** | 5.0 | 7.5 |
| **Pyrazinamide** | Not recommended | Not recommended |

NOTE—Critical concentrations as indicated in CLSI M24 document [1]

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

† CLSI critical concentrations for RMP differ from revised WHO recommendation of 0.5 µg/ml published in 2021 [2].

| Second-line Drugs | 7H10 agar | 7H11 agar |
|---|---|---|
| **Streptomycin** | 2.0 | 2.0 |
| **Amikacin** | 4.0† | Not determined* |
| **Capreomycin** | 10.0 | 10.0 |
| **Kanamycin** | 5.0¥ | 6.0¥ |
| **Levofloxacin** | 1.0 | Not determined* |
| **Moxifloxacin** | 0.5 | 0.5 |
| **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]

*Breakpoints for establishing susceptibility have not be determined.

†CLSI critical concentration for AMK differ from revised WHO recommendation of 2.0 µg/ml published in 2018 [3].

¥CLSI critical concentration for KAN differ from revised WHO recommendation of 4.0 µg/ml for 7H10. WHO recommended the withdrawal of the current KAN critical concentration for 7H11 published in 2018 [3].

## Broth Based Media

| First-line Drugs | MGIT | VersaTREK |
|---|---|---|
| **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 |

NOTE—Critical concentrations as indicated in applicable manufacturer package inserts

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

†CLSI critical concentrations for RMP differ from revised WHO recommendation of 0.5 µg/ml published in 2021 [2].

| Second-line Drug | MGIT† | VersaTREK |
|---|---|---|
| **Streptomycin** | 1.0 (and 4.0*) | Not available |

NOTE—Critical concentrations as indicated in applicable manufacturer package inserts

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

†Revised WHO recommendations provide LEV and MOX critical concentrations for MGIT published in 2018 [3].

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# Appendix 1: Accessible Explanations of Figures

**Figure 1.** The primary classification of the 69 laboratories participating in the September 2020 MPEP survey is shown in this pie chart. The largest slice, at 73%, represents 50 laboratories that have self-classified as a health department laboratory. The next major slice signifies 9 hospital laboratories. The remaining three slices of the pie chart represent 7 independent laboratories, 2 federal government laboratories, and 1 laboratory self-identified as a medical manufacturer. (page 8)

**Figure 2.** The annual volume of MTBC isolates tested for drug susceptibility by participating laboratories (N=69) in 2019 is displayed in this vertical bar graph. The vertical y–axis is the number of laboratories responding and ranges from 0 to 30 using increments of 5. Along the horizontal x-axis are eight vertical bars representing the number of isolates tested per year. From left to right, 28 laboratories tested less than or equal to 50 isolates per year; 13 laboratories tested between 51 to 100 isolates per year; 6 laboratories tested between 101 to 150 isolates per year; 7 laboratories tested between 151 to 200 isolates per year; 2 laboratories tested between 201 to 300 isolates per year; 5 laboratories tested between 301 to 500 isolates per year; 6 laboratories tested between 501 to 1000 isolates per year, and 2 laboratories tested greater than or equal to 1001 isolates per year. (page 9)

**Figure 3.** The drug susceptibility testing methods used by MPEP participants (N=97) is displayed in this vertical bar graph. The vertical y-axis is the number of laboratories reporting with ranges from 0 to 70, by increments of 10, and the horizontal x- axis lists the susceptibility testing methods. Each bar represents the number of reporting laboratories performing a particular drug susceptibility test method. From left to right: 64 used MGIT, 17 used agar proportion, 4 used Sensititre, 2 used VersaTREK, and 10 used molecular methods. (page 10)

**Figure 4.** The molecular methods used by MPEP participants (N=10) are displayed in this pie chart. The largest slice represents the 5 laboratories that perform targeted DNA sequencing. The next three slices represent 3 laboratories that use the Cepheid Xpert MTB/RIF assay, 1 laboratory that uses Bruker line probe assays and 1 laboratory that uses whole genome sequencing. (page 10)

**Figure 5.** The anti*tuberculosis* drugs tested by MPEP participants is displayed in a horizontal bar graph. The vertical y -axis contains a list of each drug tested and the horizontal x-axis contains the number of laboratories with ranges from 0 to 80, by increments of 10. There are 16 horizontal bars with each bar representing the number of laboratories reporting a result for a particular drug for susceptibility testing. 69 laboratories tested rifampin; 69 laboratories tested isoniazid; 69 laboratories tested *eth*Ambutol; 64 laboratories tested pyrazinamide; 44 laboratories tested streptomycin; 13 laboratories tested ofloxacin; 6 laboratories tested moxifloxacin; 5 laboratories tested ciprofloxacin; 5 laboratories tested levofloxacin; 14 laboratories tested capreomycin; 13 laboratories tested kanamycin; 12 laboratories tested amikacin; 18 laboratories tested ethionamide; 13 laboratories tested PAS; 11 laboratories tested rifabutin; and 7 laboratories tested cycloserine. (page 11)

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