Mycobacterium tuberculosis Complex Drug Susceptibility Testing Program

Model Performance Evaluation Program
Report of Results
August 2019

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

Mycobacterium tuberculosis Complex Drug Susceptibility Testing Report for August 2019 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 August 2019.

Report Content

The material in this report was developed and prepared by:

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

Acknowledged NCHHSTP, DTBE, LB contributors:
Beverly Metchock, Stephanie Johnston, Lois Diem, Mitchell Yakrus, and Angela Starks

Contact Information

Comments and inquiries regarding this report should be directed to:

TBMPEP@cdc.gov
404-639-4013
CDC TB MPEP Website

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

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 *Mycobacterium tuberculosis* MIC 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. 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), "M24: Susceptibility Testing of Mycobacteria, *Nocardiae* spp., and Other Aerobic Actinomycetes" [1].

Expected Drug Susceptibility Testing Results

Anticipated growth-based and molecular results for the panel of MTBC isolates sent to participants in August 2019 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 [2].

Table 1. Expected Growth-based Results for August 2019 Survey

Note—S=susceptible, R=resistant

Isolate	RMP	INH	EMB	PZA	Second-line Drugs Resistant to:
2019F	S	S	S	S	
2019G	S	S	S	R	
2019H	R	S	S	S	
2019I	S	S	S	S	
2019J	S	S	S	R	

Table 2. Expected Molecular Results (Mutations Detected in Loci Associated with Resistance) for August 2019 Survey

Note—Empty cell=No mutation detected

Isolate	*rpoB*	*pncA*
2019F		Ala170Val
2019G		His82Asp
2019H	His526Tyr	
2019I		Glu37Val
2019J		His57Asp

Technical Notes

The following information pertains to all of the tables and figures for the 2019 MTBC isolates F, G, H, I, and J, included in this report.

- The source of data in all tables and figures is the August 2019 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 72 (the number of participating laboratories). This report contains all results reported by participating laboratories.
- 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.
- For 25 laboratories reporting second-line drug results (with the exception of streptomycin), seven (28%) 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.
- 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

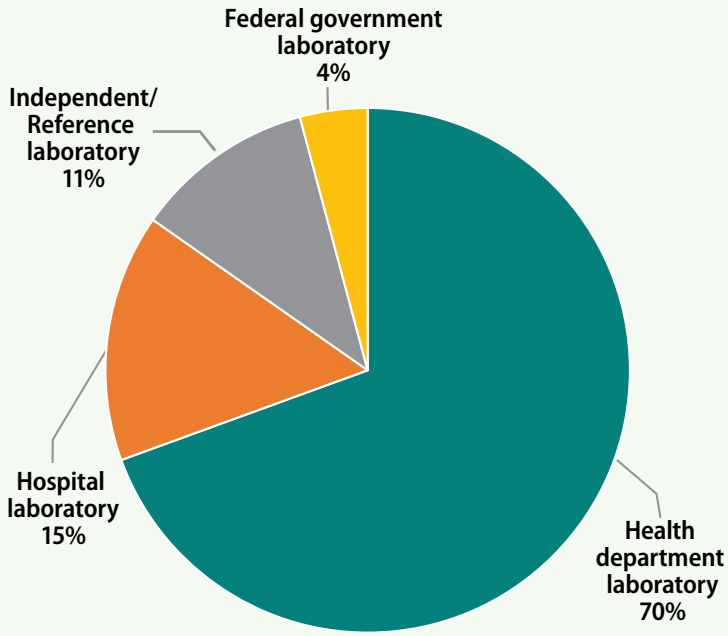
Primary Classification

This report contains DST results submitted to CDC by survey participants at 72 laboratories in 35 states.

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

- **50 (70%)**: Health department laboratory (e.g., local, county, state)
- **11 (15%)**: Hospital laboratory
- **8 (11%)**: Independent/Reference laboratory (non-hospital based)
- **3 (4%)**: Federal government laboratory

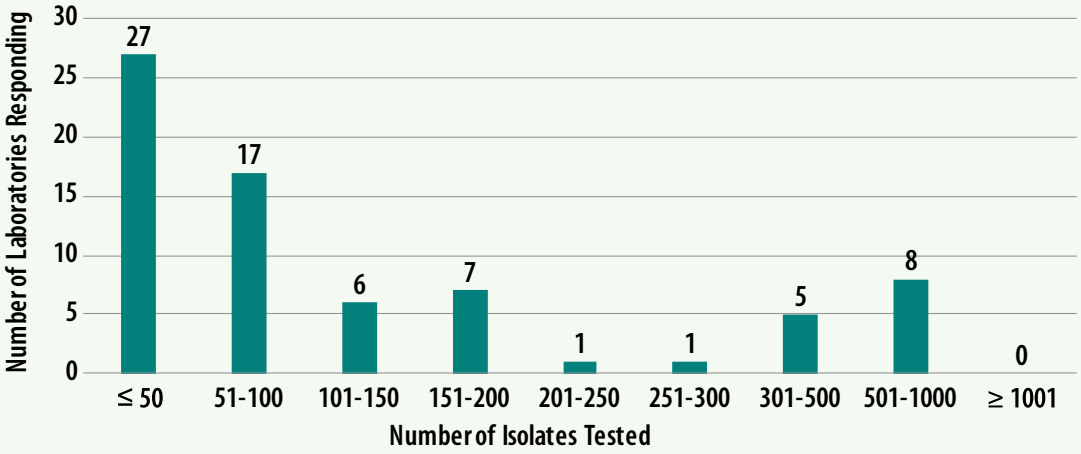
Figure 1. Primary Classification of Participating Laboratories, August 2019



Annual Number of MTBC Drug Susceptibility Tests Performed

The number of MTBC isolates tested for drug susceptibility by the 72 participants in 2018 (excluding isolates used for quality control) is shown in Figure 2. In 2018, the counts ranged from 0 to 948 tests. Participants at 27 (38%) 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].

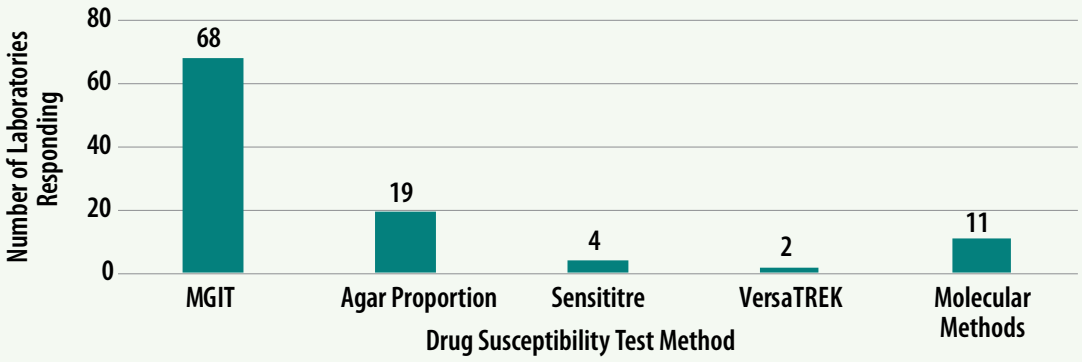
Figure 2. Distribution of the Annual Volume of MTBC Isolates Tested for Drug Susceptibility by Participants in Previous Calendar Year (n=72)



MTBC DST Methods Used by Participants

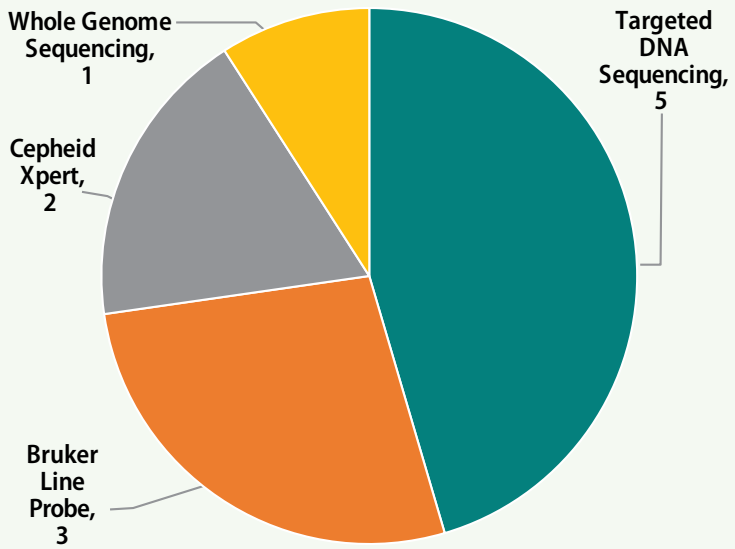
The DST methods that were used by participating laboratories for this panel of MTBC isolates are displayed in Figure 3. Furthermore, 44 (61%) laboratories reported results for only one method, 24 laboratories reported two methods, and 4 laboratories noted three susceptibility methods.

Figure 3. MTBC Drug Susceptibility Test Method Used by Participants (n=104)



Molecular methods reported by 11 participants are shown in Figure 4. The method used most frequently by laboratories (5) was targeted DNA sequencing (45%), including pyrosequencing and Sanger sequencing. Three laboratories reported use of line probe assays Genotype MTBDRplus and MTBDRsl by Bruker, two reported results for the Cepheid Xpert MTB/RIF assay, and one reported results from whole genome sequencing.

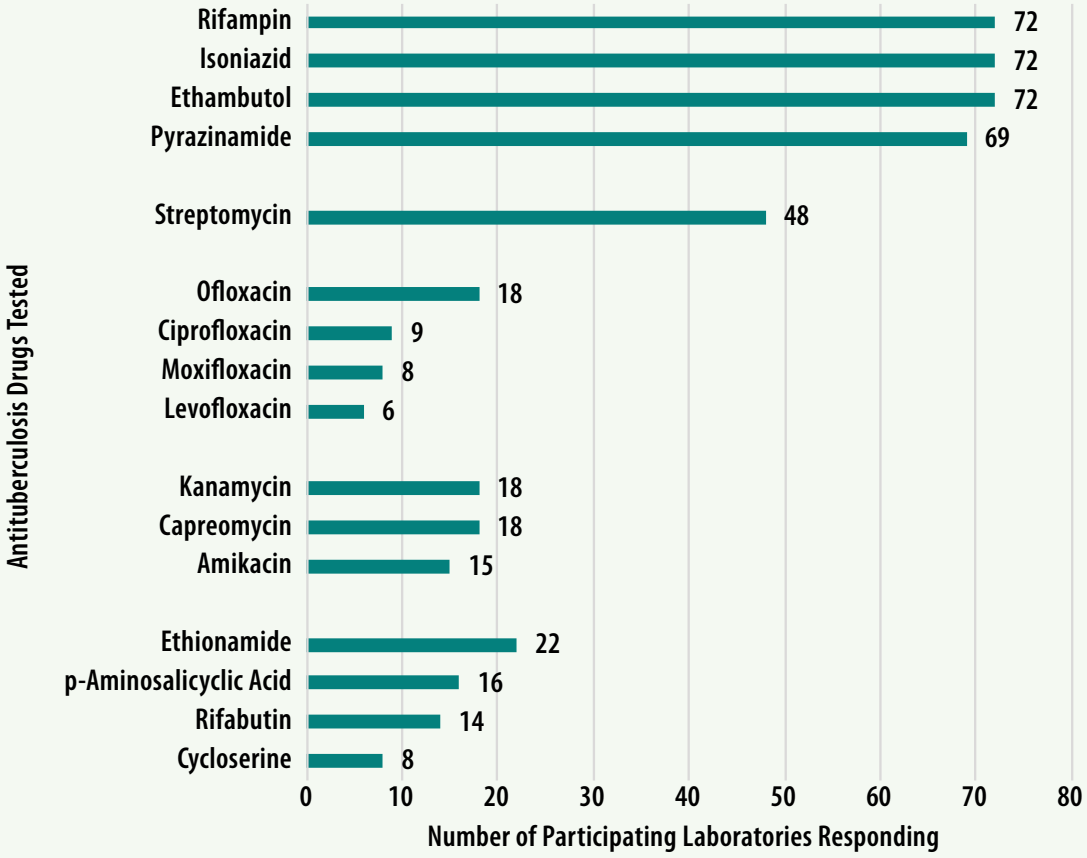
Figure 4. Molecular Method Reported (n=11)



Antituberculosis Drugs Tested by Participants

The number of participating laboratories that reported testing each antituberculosis drug in the August 2019 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 69 (96%) also reported results for PZA by growth-based DST methods.

Figure 5. Antituberculosis Drugs Tested by Participants



Isolate 2019F

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

Isolate 2019F is susceptible to all first- and second-line drugs.

Most (99%) results were reported susceptible for this isolate across all methods.

Pyrazinamide

Pyrazinamide (PZA) is an important first-line drug for treatment of TB and is used with INH and RIF. The addition of this drug shortens TB treatment from the previous 9–12 months to 6 months because it kills a population of persistent bacilli in acidic pH environments within the lesions that are not killed by other drugs [4]. PZA is a prodrug that requires conversion to its active form, pyrazinoic acid, by the pyrazinamidase encoded by the *pncA* gene of *M. tuberculosis*. PZA-resistant *M. tuberculosis* strains lose pyrazinamidase activity, and resistance to PZA is usually caused by nucleotide changes scattered throughout the *pncA* gene. However, there may be additional mechanisms of resistance to PZA that are still unknown [5].

DNA sequence analysis of *pncA* in Isolate 2019F revealed a C>T point mutation in codon 170 resulting in wild-type alanine being replaced by valine (Ala170Val). Isolates with the non-synonymous Ala170Val mutation have been reported to test susceptible to PZA in growth-based assays [6].

Isolate 2019F was expected to be susceptible to PZA and among MGIT and VersaTREK responses, 97% (66/68) of results for PZA were reported as susceptible.

Of the 2 molecular results reported for PZA, both (100%) laboratories reported detection of a mutation with both laboratories specifically noting the Ala170Val mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2019F are listed in Tables 3–10.

Table 3. Isolate 2019F—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	16	0	16
Isoniazid—Low	16	0	16
Isoniazid—High	16	0	16
Ethambutol	16	0	16

Table 4. Isolate 2019F—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	66	0	66
Isoniazid—Low	65	1	66
Isoniazid—High	24	0	24
Ethambutol	66	0	66
Pyrazinamide	65	2	67

Table 5. Isolate 2019F—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	4	0	4
Isoniazid—High	4	0	4
Ethambutol	4	0	4

Table 6. Isolate 2019F—Participant Results for First-Line DST by VersaTREK

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

Table 7. Isolate 2019F—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	16	0	16
Ofloxacin	11	0	11
Ciprofloxacin	6	0	6
Levofloxacin	1	0	1
Moxifloxacin	2	0	2
Amikacin	8	0	8
Kanamycin	12	0	12
Capreomycin	12	0	12
Ethionamide	14	0	14
Rifabutin	7	0	7
Cycloserine	5	0	5
p-Aminosalicylic acid	10	0	10

Table 8. Isolate 2019F—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	34	0	34
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	2	1	3
Moxifloxacin	3	0	3
Amikacin	3	0	3
Kanamycin	2	0	2
Capreomycin	4	0	4
Ethionamide	4	0	4
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0

Table 9. Isolate 2019F—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	0	0	0
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 2019F—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	11	11
Isoniazid	0	9	9
Ethambutol	1*	2	3
Pyrazinamide	2	0	2
Ofloxacin	1†	4	5
Ciprofloxacin	1†	3	4
Levofloxacin	1†	5	6
Moxifloxacin	1†	6	7
Amikacin	0	6	6
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	0	2	2
Rifabutin	0	4	4

* This laboratory noted the detection of an *emb*B mutation not associated with resistance.
† This laboratory noted the detection of a mutation not associated with fluoroquinolone resistance.

Isolate 2019G

Expected Result: Resistant to PZA at 100 µg/ml by MGIT

Pyrazinamide

DNA sequence analysis of *pncA* in Isolate 2019G revealed a C>G point mutation in codon 82 resulting in wild-type histidine being replaced by aspartate (His82Asp).

Among two methods, 67 results for PZA were reported for Isolate 2019G. This isolate was reported as **resistant** to PZA by method, as follows:

- **85% (56/66)** of the results when using MGIT
- **0% (0/1)** of the results when using VersaTREK

Of the 2 molecular results reported for PZA, both (100%) laboratories reported detection of a mutation, specifically noting the His82Asp mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2019G are listed in Tables 11–18.

Table 11. Isolate 2019G—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	15	0	15*
Isoniazid—Low	15	0	15*
Isoniazid—High	15	0	15*
Ethambutol	15	0	15*

* One additional laboratory reported no growth for RMP, INH, and EMB by AP.

Table 12. Isolate 2019G—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	64	0	64*
Isoniazid—Low	63	1	64*
Isoniazid—High	23	0	23
Ethambutol	64	0	64*
Pyrazinamide	10	56	66†

* One additional laboratory reported no growth for RMP, INH, and EMB by MGIT.
† One additional laboratory reported contaminated for PZA by MGIT.

Table 13. Isolate 2019G—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	4	0	4
Isoniazid—High	4	0	4
Ethambutol	4	0	4

Table 14. Isolate 2019G—Participant Results for First-Line DST by VersaTREK

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

Table 15. Isolate 2019G—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	2	0	2
Amikacin	8	0	8*
Kanamycin	12	0	12
Capreomycin	12	0	12
Ethionamide	10	2	12*†
Rifabutin	7	0	7
Cycloserine	5	0	5
p-Aminosalicylic acid	9	0	9*

* One additional laboratory reported no growth for STR, OFL, AMK, ETA, and PAS by AP.
† One additional laboratory reported borderline for ETA by AP.

Table 16. Isolate 2019G—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	32	0	32*
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0

* One additional laboratory reported no growth for STR by MGIT.

Table 17. Isolate 2019G—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 2019G—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	11	11
Isoniazid	0	9	9
Ethambutol	0	3	3
Pyrazinamide	2	0	2
Ofloxacin	0	5	5
Ciprofloxacin	0	4	4
Levofloxacin	0	6	6
Moxifloxacin	0	7	7
Amikacin	0	6	6
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	0	2	2
Rifabutin	0	4	4

Isolate 2019H

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

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 [7]. The primary mechanism of resistance is a mutation within the 81-bp central region of the *rpo*B gene that encodes the β-subunit of the bacterial DNA-dependent RNA polymerase [8]. Mutations in codons 531, 526, and 516 (E. coli numbering system corresponding to 450, 445, and 435 in MTBC) are among the most frequent mutations in RMP-resistant isolates and serve as predictors of RMP resistance [7, 8]. The activity of RMP on isolates with *rpo*B 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 *rpo*B [9]. 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 [10]. Sequencing of *rpo*B will allow for clarification of the result and understanding of possible discordance between rapid molecular and growth-based testing results.

DNA sequence analysis of *rpo*B in Isolate 2019H revealed a C>G point mutation in codon 526 resulting in wild-type histidine being replaced by tyrosine (His526Tyr). Isolates with His526Tyr (His445Tyr in MTBC numbering system) mutations consistently test resistant to RMP in growth-based assays.

Among four methods, 88 results for RMP were reported for Isolate 2019H. This isolate was reported as **resistant** to RMP by method, as follows:

- **100% (18/18)** of the results when using AP
- **98% (64/65)** 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 11 molecular results reported for RMP, all (100%) laboratories reported detection of a mutation with 6 laboratories specifically noting the His526Tyr mutation.

The three laboratories performing Sensititre reported RMP MIC values as >16 µg/ml.

Pyrazinamide

For Isolate 2019H, DNA sequencing of the *pncA* gene did not reveal a mutation. There may be additional mechanisms of resistance to PZA besides nucleotide changes in the *pncA* gene that are still unknown [5]. Issues with false-resistance to PZA have been reported as well [11] and remain a potential concern.

Isolate 2019H was expected to be susceptible to PZA; however, of those testing PZA, **resistant** was reported by:

- **60% (39/65)** of the results when using MGIT
- **0% (0/1)** of the results when using VersaTREK

Complete first-line DST, second-line DST, and molecular results submitted by all participant for Isolate 2019H are listed in Tables 19–26.

Table 19. Isolate 2019H—Participant Results for First-Line DST by AP

Drug	Susceptible	Resistant	Total
Rifampin	0	18	18
Isoniazid—Low	18	0	18
Isoniazid—High	18	0	18
Ethambutol	18	0	18

Table 20. Isolate 2019H—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	1	64	65
Isoniazid—Low	63	2	65
Isoniazid—High	24	0	24
Ethambutol	65	0	65
Pyrazinamide	26	39	65*

* One additional laboratory reported contaminated for PZA by MGIT.

Table 21. Isolate 2019H—Participant Results for First-Line DST by Sensititre

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

* One additional laboratory reported contaminated for RMP, INH, and EMB by Sensititre.

Table 22. Isolate 2019H—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 23. Isolate 2019H—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	18	0	18
Ofloxacin	12	0	12
Ciprofloxacin	7	0	7
Levofloxacin	1	0	1
Moxifloxacin	2	0	2
Amikacin	9	0	9
Kanamycin	14	0	14
Capreomycin	13	0	13
Ethionamide	16	0	16
Rifabutin	0	7	7
Cycloserine	6	0	6
p-Aminosalicylic acid	12	0	12

Table 24. Isolate 2019H—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	33	0	33
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	0	3	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0

Table 25. Isolate 2019H—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	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 contaminated for STR, OFL CIP, LEV, MOX, AMK, KAN, CAP, ETA, RBT, CYC, and PAS by Sensititre.

Table 26. Isolate 2019H—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	11	0	11
Isoniazid	0	9	9
Ethambutol	0	3	3
Pyrazinamide	0	2	2
Ofloxacin	1*	4	5
Ciprofloxacin	1*	3	4
Levofloxacin	1*	5	6
Moxifloxacin	1*	6	7
Amikacin	0	6	6
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	0	2	2
Rifabutin	4	0	4

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

Isolate 2019I

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

Isolate 2019I is susceptible to all first- and second-line drugs.

Most (97%) results were reported susceptible for this isolate across all methods.

Pyrazinamide

Isolate 2019I was expected to be susceptible to PZA. DNA sequence analysis of *pncA* in Isolate 2019I revealed an A>T point mutation in codon 37 resulting in wild-type glutamate being replaced by valine (Glu37Val). However, isolates with the non-synonymous Glu37Val mutation have been reported to test susceptible to PZA in growth-based assays [6]. As noted with Isolate 2019H, issues with false-resistance to PZA have been reported [11] and remain a concern.

Of those testing PZA for Isolate 2019I, **resistant** was reported by:

- **23% (15/65)** of the results when using MGIT
- **0% (0/1)** of the results when using VersaTREK

Of the 2 molecular results reported for PZA, both (100%) laboratories reported detection of a mutation, specifically noting the Glu37Val mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2019I are listed in Tables 27–34.

Table 27. Isolate 2019I—Participant Results for First-Line DST by AP

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

Table 28. Isolate 2019I—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	65	0	65
Isoniazid—Low	63	1	64
Isoniazid—High	22	0	22
Ethambutol	65	0	65
Pyrazinamide	50	15	65*

* One additional laboratory reported borderline for PZA by MGIT.

Table 29. Isolate 2019I—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	4	0	4
Isoniazid—High	4	0	4
Ethambutol	4	0	4

Table 30. Isolate 2019I—Participant Results for First-Line DST by VersaTREK

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

Table 31. Isolate 2019I—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	17	0	17
Ofloxacin	11	0	11
Ciprofloxacin	6	0	6
Levofloxacin	1	0	1
Moxifloxacin	2	0	2
Amikacin	9	0	9
Kanamycin	13	0	13
Capreomycin	13	0	13
Ethionamide	14	1	15
Rifabutin	7	0	7
Cycloserine	5	1	6
p-Aminosalicylic acid	11	0	11

Table 32. Isolate 2019I—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	33	0	33
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0

Table 33. Isolate 2019I—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	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.

Table 34. Isolate 2019I—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	11	11
Isoniazid	0	9	9
Ethambutol	0	3	3
Pyrazinamide	2	0	2
Ofloxacin	1*	4	5
Ciprofloxacin	1*	3	4
Levofloxacin	1*	5	6
Moxifloxacin	1*	6	7
Amikacin	0	6	6
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	0	2	2
Rifabutin	0	4	4

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

Isolate 2019J

Expected Result: *Mycobacterium bovis*; Resistant to PZA at 100 µg/ml by MGIT

Pyrazinamide

Unlike *M. tuberculosis*, *M. bovis* has an inherent resistance to PZA caused by a characteristic single point mutation of C>G at nucleotide position 169 of the *pncA* gene resulting in aspartic acid replacing histidine at codon 57 (His57Asp). This substitution causes defective pyrazinamidase activity and confers natural PZA resistance in *M. bovis* strains, including BCG substrains [12, 13]. DNA sequence analysis of *pncA* in Isolate 2019J confirmed the His57Asp mutation.

Among two methods, 68 results for PZA were reported for Isolate 2019J. This isolate was reported as **resistant** to PZA by method, as follows:

- **99% (66/67)** of the results when using MGIT
- **100% (1/1)** of the results when using VersaTREK

Of the four molecular results reported for PZA, all (100%) laboratories reported detection of a mutation with three laboratories specifically noting the His57Asp mutation.

Complete first-line DST, second-line DST, and molecular results submitted by all participants for Isolate 2019J are listed in Tables 35–42.

Table 35. Isolate 2019J—Participant Results for First-Line DST by AP

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

Table 36. Isolate 2019J—Participant Results for First-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Rifampin	63	0	63*
Isoniazid—Low	62	1	63*
Isoniazid—High	22	1	23
Ethambutol	63	0	63*
Pyrazinamide	1	66	67

* One additional laboratory reported no growth for RMP, INH, and EMB by MGIT.

Table 37. Isolate 2019J—Participant Results for First-Line DST by Sensititre

Drug	Susceptible	Resistant	Total
Rifampin	4	0	4
Isoniazid—Low	3	1	4
Isoniazid—High	3	1	4
Ethambutol	4	0	4

Table 38. Isolate 2019J—Participant Results for First-Line DST by VersaTREK

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

Table 39. Isolate 2019J—Participant Results for Second-Line DST by AP

Drug	Susceptible	Resistant	Total
Streptomycin	17	0	17
Ofloxacin	11	0	11
Ciprofloxacin	6	0	6
Levofloxacin	1	0	1
Moxifloxacin	2	0	2
Amikacin	9	0	9
Kanamycin	13	0	13
Capreomycin	13	0	13
Ethionamide	12	2	14
Rifabutin	7	0	7
Cycloserine	4	2	6
p-Aminosalicylic acid	11	0	11

Table 40. Isolate 2019J—Participant Results for Second-Line DST by MGIT

Drug	Susceptible	Resistant	Total
Streptomycin	31	0	31*
Ofloxacin	4	0	4
Ciprofloxacin	1	0	1
Levofloxacin	3	0	3
Moxifloxacin	3	0	3
Amikacin	2	0	2
Kanamycin	1	0	1
Capreomycin	3	0	3
Ethionamide	3	0	3
Rifabutin	3	0	3
Cycloserine	0	0	0
p-Aminosalicylic acid	0	0	0

* One additional laboratory reported no growth for STR by MGIT.

Table 41. Isolate 2019J—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	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.

Table 42. Isolate 2019J—Participant Results for Molecular Testing

Drug	Mutation Detected	Mutation Not Detected	Total
Rifampin	0	11	11
Isoniazid	0	9	9
Ethambutol	1	2	3
Pyrazinamide	4	0	4
Ofloxacin	1*	4	5
Ciprofloxacin	1*	3	4
Levofloxacin	1*	5	6
Moxifloxacin	1*	6	7
Amikacin	0	6	6
Kanamycin	0	6	6
Capreomycin	0	5	5
Ethionamide	0	2	2
Rifabutin	0	4	4

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

Equivalent Critical Concentrations

(Concentrations listed as µg/ml)

Agar Proportion

First-line Drugs	7H10 agar	7H11 agar
Rifampin	1.0	1.0
Ethambutol	5.0	7.5
Pyrazinamide	Not recommended	Not recommended
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.

Second-line Drugs	7H10 agar	7H11 agar
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
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.

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.

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.

References

1. CLSI, *Susceptibility Testing of Mycobacteria, Nocardiae spp., and Other Aerobic Actinomycetes, in 3rd Ed. CLSI Standard M24*. 2018, Clinical and Laboratory Standards Institute: Wayne, PA.
2. Campbell, P.J., et al., *Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis*. Antimicrob Agents Chemother, 2011. 55(5): p. 2032-41.
3. APHL, *TB Drug Susceptibility Testing Expert Panel Meeting Summary Report*. 2007, Association of Public Health Laboratories: Washington, D.C.
4. Zhang, Y. and D. Mitchison, *The curious characteristics of pyrazinamide: a review*. Int J Tuberc Lung Dis, 2003. 7(1): p. 6-21.
5. Ramirez-Busby, S.M. and F. Valafar, *Systematic Review of Mutations in Pyrazinamidase Associated with Pyrazinamide Resistance in Mycobacterium tuberculosis Clinical Isolates*. Antimicrob Agents Chemother, 2015. 59(9): p. 5267-77.
6. Whitfield, M.G., et al., *Mycobacterium tuberculosis pncA Polymorphisms That Do Not Confer Pyrazinamide Resistance at a Breakpoint Concentration of 100 Micrograms per Milliliter in MGIT.* Journal of clinical microbiology, 2015. 53(11): p. 3633-3635.
7. Almeida Da Silva, P.E. and J.C. Palomino, *Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs*. J Antimicrob Chemother, 2011. 66(7): p. 1417-30.
8. Zhang, Y. and W.W. Yew, *Mechanisms of drug resistance in Mycobacterium tuberculosis*. Int J Tuberc Lung Dis, 2009. 13(11): p. 1320-30.
9. *Availability of an assay for detecting Mycobacterium tuberculosis, including rifampin-resistant strains, and considerations for its use—United States, 2013*. MMWR Morb Mortal Wkly Rep, 2013. 62(41): p. 821-7.
10. Van Deun, A., et al., *Rifampin drug resistance tests for tuberculosis: challenging the gold standard*. J Clin Microbiol, 2013. 51(8): p. 2633-40.
11. Chedore, P., et al., *Potential for erroneous results indicating resistance when using the Bactec MGIT 960 system for testing susceptibility of Mycobacterium tuberculosis to pyrazinamide*. J Clin Microbiol, 2010. 48(1): p. 300-1.
12. Scorpio, A., et al., *Rapid differentiation of bovine and human tubercle bacilli based on a characteristic mutation in the bovine pyrazinamidase gene*. J Clin Microbiol, 1997. 35(1): p. 106-10.
13. Somoskovi, A., et al., *Sequencing of the pncA gene in members of the Mycobacterium tuberculosis complex has important diagnostic applications: Identification of a species-specific pncA mutation in "Mycobacterium canettii" and the reliable and rapid predictor of pyrazinamide resistance*. J Clin Microbiol, 2007. 45(2): p. 595-9.

Appendix 1: Accessible Explanations of Figures

Figure 1. The primary classification of the 72 laboratories participating in the August 2019 MPEP survey is shown in this pie chart. The largest slice, at 70%, represents 50 laboratories that have self-classified as a health department laboratory. The next major slice signifies 11 hospital laboratories. The remaining two slices of the pie chart represent 8 independent laboratories and 3 federal government laboratories. (page 7)

Figure 2. The annual volume of MTBC isolates tested for drug susceptibility by participating laboratories (N=72) in 2018 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 nine vertical bars representing the number of isolates tested per year. From left to right, 27 laboratories tested less than or equal to 50 isolates per year; 17 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; 1 laboratory tested between 201 to 250 isolates per year; 1 laboratory tested between 251 to 300 isolates per year; 5 laboratories tested between 301 to 500 isolates per year; 8 laboratories tested between 501 to 1000 isolates per year, and 0 laboratories tested greater than or equal to 1001 isolates per year. (page 8)

Figure 3. The drug susceptibility testing methods used by MPEP participants (N=104) is displayed in this vertical bar graph. The vertical y-axis is the number of laboratories reporting with ranges from 0 to 80, by increments of 20, 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: 68 used MGIT, 19 used agar proportion, 4 used Sensititre, 2 used VersaTREK, and 11 used molecular methods. (page 9)

Figure 4. The molecular methods used by MPEP participants (N=11) 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 Bruker line probe assays, 2 laboratories that use the Cepheid Xpert MTB/RIF assay, and 1 laboratory that uses whole genome sequencing. (page 9)

Figure 5. The antituberculosis 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. 72 laboratories tested rifampin; 72 laboratories tested isoniazid; 72 laboratories tested ethambutol; 69 laboratories tested pyrazinamide; 48 laboratories tested streptomycin; 18 laboratories tested ofloxacin; 9 laboratories tested ciprofloxacin; 8 laboratories tested moxifloxacin; 6 laboratories tested levofloxacin; 18 laboratories tested kanamycin; 18 laboratories tested capreomycin; 15 laboratories tested amikacin; 22 laboratories tested ethionamide; 16 laboratories tested PAS; 14 laboratories tested rifabutin; and 8 laboratories tested cycloserine. (page 10)

For more information please contact

Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 33029-4027

Telephone: 1-800-CDC-INFO (232-4636)

MPEP Telephone: 404-639-4013

MPEP Email: TBMPEP@cdc.gov

MPEP Web: www.cdc.gov/tb/topic/laboratory/mpep/default.htm

Publication date: January 2020

CS 314164-A