



Susceptibility Was the Case That They Gave Me: Understanding Lab Methods & Reports in Regard to Drug Resistance

Benjamin Alpers, BA

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TB Nurse Case Management • May 6 – 8, 2025 • San Antonio, Texas



Benjamin Alpers, BA

Has the following disclosures to make:

- No conflict of interests
- No relevant financial relationships with any commercial companies pertaining to this activity





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Susceptibility Was the Case That They Gave Me

**Understanding Lab Methods and
Reports in Regard to Drug Resistance**

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WHO Classification of Drug Resistant TB



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Rifampin Resistant (RR)/MDR (INH and rifampin resistant)

Pre-XDR-TB: TB caused by *M. tuberculosis* strains that fulfill the definition of MDR/RR-TB and are also resistant to any fluoroquinolone

XDR-TB: TB caused by *M. tuberculosis* strains that fulfill the definition of MDR/RR-TB and that are also resistant to any fluoroquinolone and at least one additional Group A drug, i.e. bedaquiline or linezolid

Specimen Quality

Accurate laboratory results are directly proportional to the quality of the specimen

Sputum

- *Recently* discharged material from the bronchial tree, with minimal amounts of upper respiratory tract secretions
 - Well coached patient, collect at least 3ml
 - Label tube, form, and indicate test:
 - Initial Dx: Smear, NAAT, & Culture
 - Follow-up: Smear and Culture
 - Release from respiratory isolation?
 - Order Smear only

Transport to lab cool and quickly



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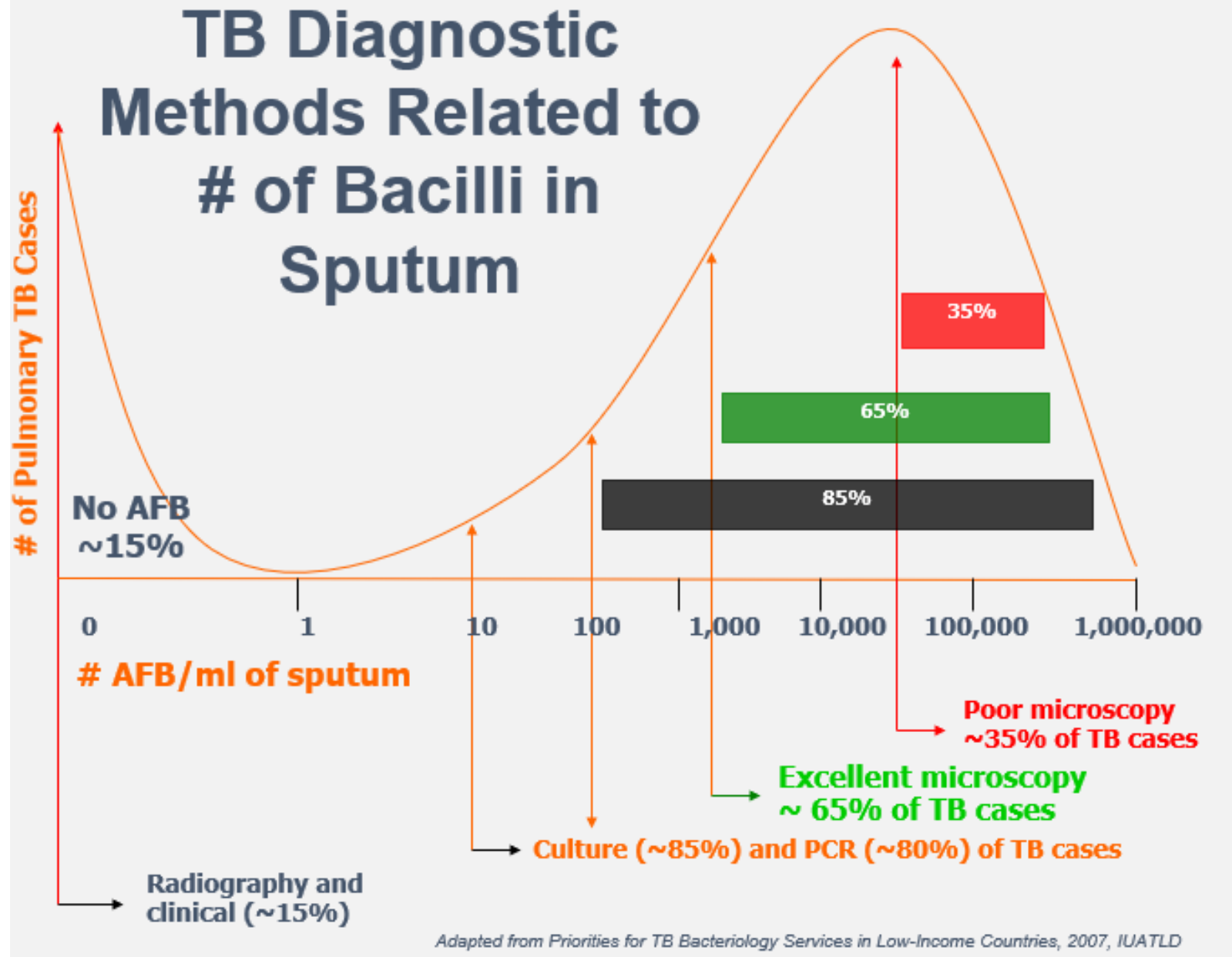
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Case #1

Typical TB presentation from patient in SE Texas:

- Fever
- Productive cough for a few weeks
- Fatigue
- Loss of appetite



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Acid Fast Bacilli Microscopy (AFB Smear)

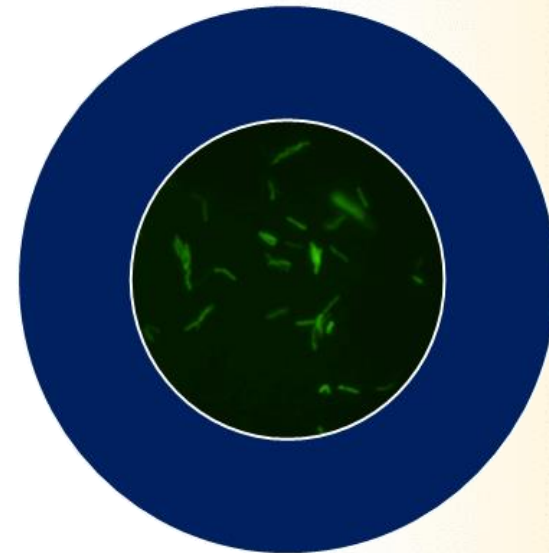
Has many qualities of an ideal diagnostic test

- Rapid & universally available
- Detects the most infectious cases
- Used to support diagnosis and identify need to isolate
- Helps monitor response to therapy
- Identifies priority cases for nucleic acid amplification (NAA)

Problems

- Not sensitive - misses ~50% of TB
- Not specific in low TB prevalence areas (e.g. Texas)
 - Positive smear may be NTM

Highly specific where TB is highly prevalent



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Smear Result

AFB Smear (Fluorochrome) **POSITIVE:**
AFB seen on direct smear, >10/field

- Strongly positive
- >1,000,000 AFB/ml sputum
- Probably very infectious
- Although, great collection!



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Nucleic Acid Amplification Tests (NAAT)

Tiny amounts of DNA/RNA are amplified (copied) until there is enough for easy detection

- Examined for both Identification and Detection of Drug Resistance

Test turnaround time measured in hours



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Nucleic Acid Amplification Test (NAAT)

Detects *M. tuberculosis* complex nucleic acids; does not distinguish between live and dead bacilli

- For initial Dx specimens only
- Not suitable for follow-up specimen or monitoring; cured patients may be NAAT + for years!

Sensitivity compared to TB culture

- >95% for AFB smear-positive
- Only 55-75% for AFB smear-negative

Does not replace culture for bacteriological Dx



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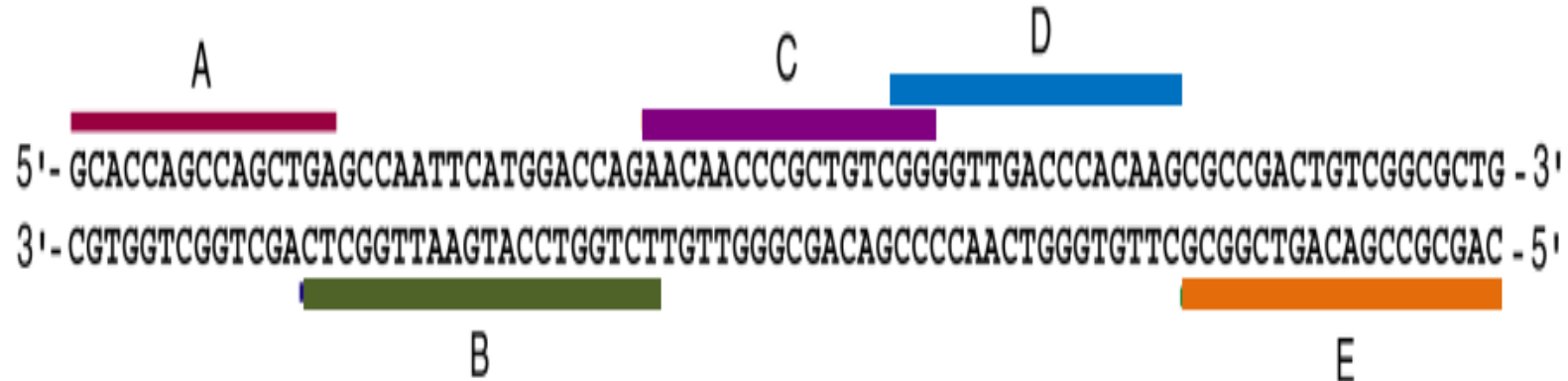
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Cepheid GeneXpert® Target Region



The MTB assay target is the 81 bp rifampin resistance determination region of the *rpoB* gene.

Approx. 10% of rifampin resistant predictions are false

(ex. Phe433Phe silent mutation)

GX Rifampin resistant results must be confirmed

NAAT Result

MTB Direct Detection, NAA **POSITIVE: M.tuberculosis complex DNA detected**

Rifampin by Direct NAA **Rifampin resistance mutation not detected; likely rifampin susceptible.**

- Note the wording—"likely" rifampin susceptible
- No mutation detected in the area of the genome probed



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AFB Culture

More sensitive than smear

- 5,000 to 10,000 AFB/ml for smear
- ~10 viable AFB/ml for culture

Positive for only ~85% of Pulmonary TB

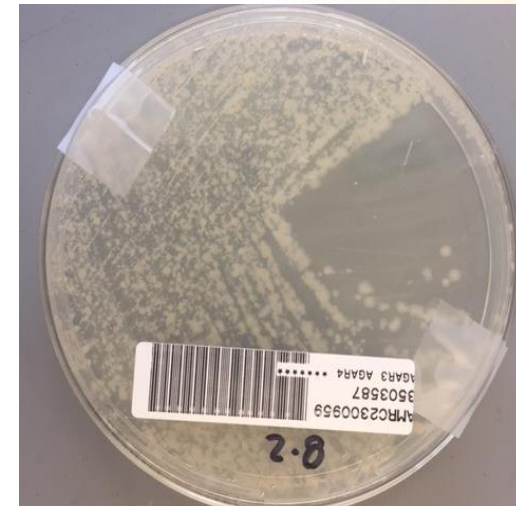
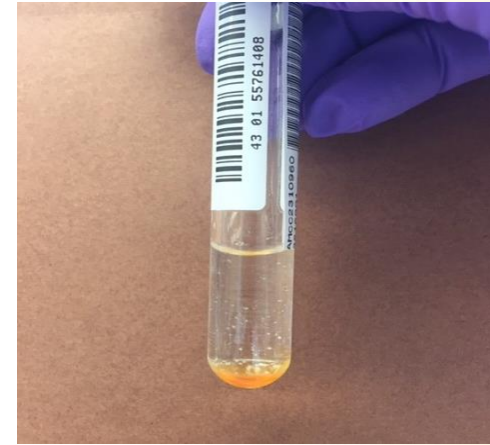
- Requires a quality specimen
- May be invalid due to contamination

Used to monitor patient response to treatment (like smear)

Required for conventional drug susceptibilities & genotype

Lengthy

- 1-6 weeks by liquid media
- 2-8 weeks by solid media



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Culture Result

Organism ID by HPLC **Mycobacterium tuberculosis complex**

- High Performance Liquid Chromatography is the primary means of organism identification at this time
- HPLC is performed on *culture*



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Conventional Drug Susceptibility Testing (DST) by Agar Proportion

A standardized suspension of *M. tuberculosis* is inoculated to quadrant plates of drug-containing Middlebrook 7H10 agar and a drug-free control.

If growth of *M. tuberculosis* on the drug quadrant is 1% or greater than the growth on the control, the drug can no longer be counted on as being effective for treatment.



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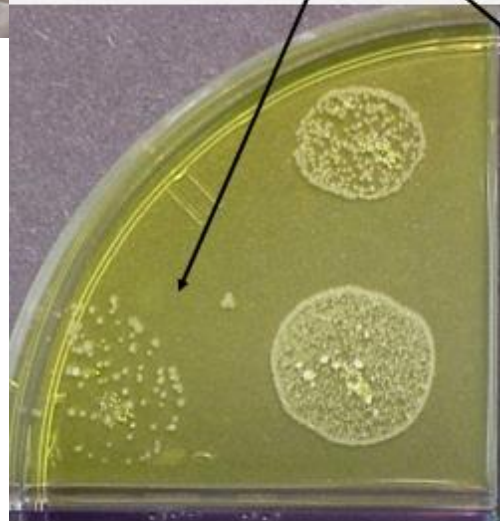
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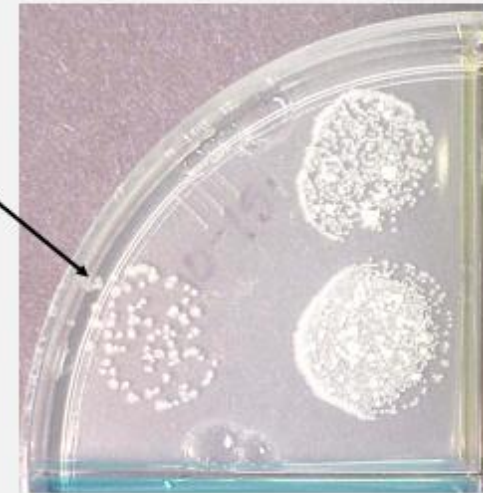
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100cfu/100cfu = 100% Resistance



Isoniazid, 1.0 mcg/ml



Drug-Free Control

Conventional DST Result

Isoniazid 0.2 mcg/ml by Agar Proportion **Resistant**
Rifampin 1.0 mcg/ml by Agar Proportion Susceptible
Ethambutol 5.0 mcg/ml by Agar Proportion Susceptible
Isoniazid 1.0 mcg/ml by Agar Proportion Susceptible
Ofloxacin 2.0 mcg/ml by Agar Proportion Susceptible



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Genotyping by Whole Genome Sequencing (WGS)

2,690 genetic loci examined and compared

Those that are 99.7% similar clustered by wgMLSType

This translates as <8 SNPs difference to at least one isolate in cluster

Phylogenetic trees can be created within clusters

Not indicative of drug resistance pattern!



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Genotyping Result

Date of Specimen Collection	Submitter Number	State Case Number	Date Received	wgMLSType
04/10/2019	AMCC1904827	2019TX201913191	04/30/2019	MTBC001328
01/15/2020	AMCC2000665	2020TX202014246	02/06/2020	MTBC001328
11/13/2021	AMCC2113031	2021TX202116989	12/16/2021	MTBC001328
02/24/2022	AMCC2202387	2022TX202219420	03/16/2022	MTBC001328
08/04/2022	AMCC2210398	2022TX202222140	09/01/2022	MTBC001328
08/16/2022	AMCC2211152	2022TX202222139	09/01/2022	MTBC001328



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Case #2

Patient is high risk for drug resistance due to contacts and place of birth (Mexico)

Submitted by local health department on the border

NAAT requested

- Will perform GeneXpert on request regardless of smear result
- One negative smear/NAAT per patient per 2 months



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Smear and NAAT Results

AFB Smear (Fluorochrome) **NEGATIVE: No AFB seen on direct smear**

- Low burden or poor collection

MTB Direct Detection, NAA **POSITIVE: M.tuberculosis complex DNA detected**

Rifampin by Direct NAA **Rifampin resistance mutation detected; likely rifampin Resistant; confirmatory testing in progress.**

- Due to limited amount of DNA will need to wait for better candidate or culture for further testing



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Culture Result

Organism ID by HPLC **Mycobacterium tuberculosis complex**

- Patient's only NAAT positive, culture positive specimen
- Suggests low-burden TB
- 2½ weeks from Xpert result to culture



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Molecular Detection of Drug Resistance (MDDR)

Can provide rapid detection of drug resistance

Both NAAT positive and culture positive specimens are candidates

Particularly useful for high-risk patients, RMP positive Xpert sediment, contaminated specimens, or those specimens that do not grow well or are non-viable in standard TB media

Examines 24 amplicons across 16 genes providing information on more than 12 antituberculosis drugs



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MDDR Report



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Rifampin (RIF)	Result	Interpretation
RIF interpretation		RIF resistant
rpoB*	Ser450Trp	
Comments and Disclaimers		
* DTBE Reference Laboratory has transitioned from the E. coli to the M. tuberculosis numbering system for reporting rpoB gene mutations.		

Isoniazid (INH)	Result	Interpretation
INH interpretation		INH resistant
inhA	No mutation	
fabG1	No mutation	
katG	Ser315Thr	

Ethambutol (EMB)	Result	Interpretation
EMB interpretation		Likely EMB resistant
embB	Asp354Ala	

Pyrazinamide (PZA)	Result	Interpretation
PZA interpretation		Cannot rule out PZA resistance.
pncA	No mutation	

Fluoroquinolones (FQ)	Result	Interpretation
FQ interpretation		Cannot rule out FQ resistance.
gyrA	No mutation	
gyrB	No mutation	

Amikacin, Capreomycin, and Kanamycin (AMK, CAP, and KAN)	Result	Interpretation
AMK CAP and KAN interpretation		Cannot rule out resistance to AMK, CAP, and KAN.
rrs	No mutation	
eis	No mutation	

Bedaquiline (BDQ)	Result	Interpretation
BDQ interpretation		Cannot rule out BDQ resistance.
atpE	No mutation	
rv0678	No mutation	
pepQ	No mutation	

Clofazimine (CFZ)	Result	Interpretation
CFZ interpretation		Cannot rule out CFZ resistance.
pepQ	No mutation	
rv0678	No mutation	

Linezolid (LZD)	Result	Interpretation
LZD interpretation		Cannot rule out LZD resistance.
rplC	No mutation	
rl	No mutation	

Conventional DST Result (DSHS)

Isoniazid 0.2 mcg/ml by Agar Proportion **Resistant**

Rifampin 1.0 mcg/ml by Agar Proportion **Resistant**

Ethambutol 5.0 mcg/ml by Agar Proportion **Resistant**

Isoniazid 1.0 mcg/ml by Agar Proportion **Resistant**

Ethionamide 5.0 mcg/ml by Agar Proportion Susceptible

Streptomycin 2.0 mcg/ml by Agar Proportion Susceptible

Ofloxacin 2.0 mcg/ml by Agar Proportion Susceptible

Rifabutin 2.0 mcg/ml by Agar Proportion **Resistant**

Kanamycin 5.0 mcg/ml by Agar Proportion Susceptible

Capreomycin 10.0 mcg/ml by Agar Proportion Susceptible



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Conventional DST Result (CDC)



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MTBC Agar Proportion Susceptibility*

Isoniazid 0.2 µg/mL
Isoniazid 1.0 µg/mL
Isoniazid 5.0 µg/mL
Rifampin 1.0 µg/mL
Ethambutol 5.0 µg/mL
Streptomycin 2.0 µg/mL
Streptomycin 10.0 µg/mL
Rifabutin 2.0 µg/mL
Ciprofloxacin 2.0 µg/mL
Kanamycin 5.0 µg/mL
Ethionamide 10.0 µg/mL
Capreomycin 10.0 µg/mL
PAS 2.0 µg/mL
Ofloxacin 2.0 µg/mL
Amikacin 4.0 µg/mL

% Resistant

100 %
100 %
67 %
100 %
67 %
0 %
0 %
67 %
0 %
0 %
0 %
0 %
0 %
0 %
0 %

Interpretation

Resistant
Resistant
Resistant
Resistant
Resistant
Susceptible
Susceptible
Resistant
Susceptible
Susceptible
Susceptible
Susceptible
Susceptible
Susceptible
Susceptible

Comments and Disclaimers

* Susceptibility testing method: Indirect agar proportion, 7H10 medium. Resistance is defined as >1% (growth on drug-containing medium compared to drug-free medium).
This test has not been cleared or approved by the FDA. The performance characteristics have been established by the DTBE Reference Laboratory.

MTBC Pyrazinamide Susceptibility*

Pyrazinamide 100 µg/mL[†]

Result

Not Tested

Comments and Disclaimers

[†] Test not done

This test order is unavailable until further notice.

* Susceptibility testing method: Mycobacteria Growth Indicator Tube (MGIT)

WGS (DSHS)

Sequencing performed in-house

Used primarily for molecular DST to first-line antibiotics

Currently only available means for PZA testing

Can detect low-level rifampin resistance that may not have been recognized by growth-based susceptibilities

Can detect variants with mutation associated with *M. bovis* or *bovis* BCG

- *pncA* His57Asp



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WGS Result (DSHS)

Interpretations Summary:

Drug
INH
RIF
PZA
FQ
EMB

Variant
katG_p.Ser315Thr
rpoB_p.Ser450Trp
No reportable variant detected
No reportable variant detected
embB_p.Asp354Ala

Interpretation
INH-R
RIF-R
PZA-S
FQ-S
EMB-R



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Case #3

Wife had TB disease and passed away

Patient diagnosed about one week later

Contact (wife) had resistance to critical concentration INH



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Smear and NAAT Results

AFB Smear (Fluorochrome) **NEGATIVE: No AFB seen on direct smear**

MTB Direct Detection, NAA **POSITIVE: M.tuberculosis complex DNA detected**

Rifampin by Direct NAA **Rifampin resistance mutation detected; likely rifampin Resistant; confirmatory testing in progress.**

- Low DNA, unable to send for MDDR until 1 month later (culture)



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MDDR Report



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Rifampin (RIF)

Result

Interpretation

RIF interpretation

RIF susceptible. Mutation detected is a synonymous (silent) mutation.

rpoB*

Arg447Arg

Comments and Disclaimers

* DTBE Reference Laboratory has transitioned from the E. coli to the M. tuberculosis numbering system for reporting rpoB gene mutations.

Isoniazid (INH)

Result

Interpretation

INH interpretation

INH resistant

inhA

No mutation

fabG1

Leu203Leu

katG

No mutation

Ethambutol (EMB)

Result

Interpretation

EMB interpretation

Cannot rule out EMB resistance.

embB

No mutation

Pyrazinamide (PZA)

Result

Interpretation

PZA interpretation

Cannot rule out PZA resistance.

pncA

No mutation

Fluoroquinolones (FQ)

Result

Interpretation

FQ interpretation

Cannot rule out FQ resistance.

gyrA

No mutation

gyrB

No mutation

Amikacin, Capreomycin, and Kanamycin (AMK, CAP, and KAN)

Result

Interpretation

AMK CAP and KAN interpretation

Cannot rule out resistance to AMK, CAP, and KAN.

rrs

No mutation

eis

No mutation

Bedaquiline (BDQ)

Result

Interpretation

BDQ interpretation

Cannot rule out BDQ resistance.

atpE

No mutation

rv0678

No mutation

pepQ

No mutation

Clofazimine (CFZ)

Result

Interpretation

CFZ interpretation

Cannot rule out CFZ resistance.

pepQ

No mutation

rv0678

No mutation

Linezolid (LZD)

Result

Interpretation

LZD interpretation

Cannot rule out LZD resistance.

rplC

No mutation

rrl

No mutation

Conventional DST Results

Both DSHS and CDC agar proportion results were consistent with the molecular findings



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02/24/2022	AMCC2202387	2022TX202219420	03/16/2022	MTBC001328
08/04/2022	AMCC2210398	2022TX202222140	09/01/2022	MTBC001328
08/16/2022	AMCC2211152	2022TX202222139	09/01/2022	MTBC001328
09/17/2022	AMCC2212628	2022TX202222138	10/28/2022	MTBC001328
12/31/2022	AMCC2300105	2022TX202222518	03/29/2023	MTBC001328
03/23/2023	AMCC2304331	2023TX202324814	04/13/2023	MTBC001328
03/27/2023	AMCC2304572	2023TX202324816	04/28/2023	MTBC001328
06/01/2023	AMCC2307968	2023TX202325167	06/16/2023	MTBC001328
06/05/2023	AMCC2308160	2023TX202325010	06/30/2023	MTBC001328
06/12/2023	AMCC2308544	2023TX202325304	06/30/2023	MTBC001328
06/12/2023	AMCC2308546	2023TX202325303	07/12/2023	MTBC001328
06/12/2023	AMCC2308567	2023TX202324619	07/12/2023	MTBC001328
06/22/2023	AMCC2309111	2023TX202325168	07/21/2023	MTBC001328
06/29/2023	AMCC2309489	2023TX202325169	07/21/2023	MTBC001328
07/17/2023	AMCC2310301	2023TX202325173	08/04/2023	MTBC001328
08/08/2023	AMCC2311587	2023TX202325172	08/25/2023	MTBC001328
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10/10/2023	AMCC2315225	2023TX202325765	11/02/2023	MTBC001328
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12/28/2023	AMCC2319587	2023TX202405033	01/26/2024	MTBC001328
01/31/2024	AMCC2401756	2024TX202407656	02/16/2024	MTBC001328
02/01/2024	AMCC2401787	2024TX202408429	03/05/2024	MTBC001328
02/19/2024	AMCC2402857	2024TX202420600	03/21/2024	MTBC001328
03/04/2024	AMCC2403864	2024TX202404579	03/21/2024	MTBC001328
03/05/2024	AMCC2403865	2024TX202404133	03/21/2024	MTBC001328
03/13/2024	AMCC2404301	2024TX202402322	03/29/2024	MTBC001328
04/03/2024	AMCC2405584	2024TX202407654	05/10/2024	MTBC001328



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Genotyping Cluster Currently

Date of Specimen Collection	Submitter Number	State Case Number	Date Received	wgMLSType
04/10/2019	AMCC1904827	2019TX201913191	04/30/2019	MTBC001328
04/15/2020	AMCC2000665	2020TX202014246	02/06/2020	MTBC001328
1/13/2021	AMCC2113031	2021TX202116989	12/16/2021	MTBC001328
02/24/2022	AMCC2202387	2022TX202219420	03/16/2022	MTBC001328
08/04/2022	AMCC2210398	2022TX20222140	09/01/2022	MTBC001328
08/16/2022	AMCC2211152	2022TX202222139	09/01/2022	MTBC001328
09/17/2022	AMCC2212628	2022TX202222138	10/28/2022	MTBC001328
12/31/2022	AMCC2300105	2023TX2023225118	03/29/2023	MTBC001328
03/23/2023	AMCC2304331	2023TX202324814	04/13/2023	MTBC001328
03/27/2023	AMCC2304572	2023TX202324816	04/28/2023	MTBC001328
06/01/2023	AMCC2307968	2023TX202325167	06/16/2023	MTBC001328
06/05/2023	AMCC2308160	2023TX202325010	06/30/2023	MTBC001328
06/12/2023	AMCC2308544	2023TX202325304	06/30/2023	MTBC001328
06/12/2023	AMCC2308546	2023TX202325303	07/12/2023	MTBC001328
06/12/2023	AMCC2308567	2023TX202324619	07/12/2023	MTBC001328
06/22/2023	AMCC2309111	2023TX202325168	07/21/2023	MTBC001328
06/29/2023	AMCC2309489	2023TX202325169	07/21/2023	MTBC001328
07/17/2023	AMCC2310301	2023TX202325173	08/04/2023	MTBC001328
08/08/2023	AMCC2311587	2023TX202325172	08/25/2023	MTBC001328
08/21/2023	AMCC2312221	2023TX202325161	09/15/2023	MTBC001328
10/10/2023	AMCC2315225	2023TX202325765	11/02/2023	MTBC001328
11/15/2023	AMCC2317439	2023TX202326516	12/06/2023	MTBC001328
11/28/2023	AMCC2318063	2023TX202326518	12/20/2023	MTBC001328
12/11/2023	AMCC2318275	2023TX202326698	01/11/2024	MTBC001328
12/28/2023	AMCC2319587	2023TX202405033	01/26/2024	MTBC001328
01/31/2024	AMCC2401756	2024TX202407656	02/16/2024	MTBC001328
02/01/2024	AMCC2401787	2024TX202408428	03/05/2024	MTBC001328
02/19/2024	AMCC2402857	2024TX202420600	03/21/2024	MTBC001328
03/04/2024	AMCC2403864	2024TX202404579	03/21/2024	MTBC001328
03/05/2024	AMCC2403865	2024TX202404133	03/21/2024	MTBC001328
03/13/2024	AMCC2404301	2024TX202402322	03/29/2024	MTBC001328
04/10/2024	AMCC2405953	2024TX202411241	05/10/2024	MTBC001328
05/05/2024	AMCC2408258	2024TX202408595	06/10/2024	MTBC001328
05/26/2024	AMCC2408701	2024TX202407701	06/13/2024	MTBC001328
05/29/2024	AMCC2408776	2024TX202407680	06/21/2024	MTBC001328
07/23/2024	AMCC2411818	2024TX202404951	08/22/2024	MTBC001328
08/05/2024	AMCC2412459	2024TX202415236	08/22/2024	MTBC001328
08/22/2024	AMCC2413592	2024TX202410760	09/10/2024	MTBC001328
10/11/2024	AMCC2416688	2024TX202420003	11/14/2024	MTBC001328
12/10/2024	AMCC2419407	2024TX202415480	01/03/2025	MTBC001328
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01/30/2025	AMCC2501492	2025TX202531031	02/21/2025	MTBC001328
02/25/2025	AMCC2502034		03/19/2025	MTBC001328
10/29/2017	AMRC1800894	2017TX201710835	05/04/2018	MTBC001328
07/16/2018	AMRC1802020	2018TX201801689	09/06/2018	MTBC001328
10/29/2018	AMRC1802811	2018TX201812286	12/28/2018	MTBC001328
11/29/2018	AMRC1900359	2018TX201812230	02/20/2019	MTBC001328
04/03/2019	AMRC1901233	2019TX201913157	06/18/2019	MTBC001328
05/21/2019	AMRC1901318	2019TX201913076	07/17/2019	MTBC001328
06/03/2019	AMRC1901553	2019TX201913151	08/06/2019	MTBC001328
08/18/2019	AMRC1902027	2019TX201913823	10/22/2019	MTBC001328
10/09/2019	AMRC1902328	2019TX201913856	12/18/2019	MTBC001328
11/30/2019	AMRC1902475	2019TX201913838	01/08/2020	MTBC001328
11/10/2019	AMRC1902485	2019TX201913837	01/08/2020	MTBC001328
01/08/2020	AMRC2000393	2020TX202015390	03/12/2020	MTBC001328
08/21/2020	AMRC2001234	2020TX202015932	09/23/2020	MTBC001328
08/10/2020	AMRC2001235	2020TX202015935	09/23/2020	MTBC001328
04/06/2021	AMRC2100443	2021TX202116438	05/18/2021	MTBC001328
04/01/2021	AMRC2100444	2021TX202116431	05/18/2021	MTBC001328
05/25/2021	AMRC2100689	2021TX2021216599	06/29/2021	MTBC001328
07/01/2021	AMRC2100871	2021TX202116735	08/19/2021	MTBC001328
07/28/2021	AMRC2100940	2021TX202116725	08/19/2021	MTBC001328
07/18/2021	AMRC2100960	2021TX202116727	08/19/2021	MTBC001328
09/17/2021	AMRC2101343	2021TX202117061	12/02/2021	MTBC001328
10/19/2021	AMRC2101407	2021TX202117097	12/16/2021	MTBC001328
11/19/2021	AMRC2101409	2021TX202117077	12/16/2021	MTBC001328
10/20/2021	AMRC2101425	2021TX202117005	12/21/2021	MTBC001328
10/23/2021	AMRC2101426	2021TX202117048	12/21/2021	MTBC001328
01/05/2022	AMRC2200267	2022TX202219231	03/04/2022	MTBC001328
03/11/2022	AMRC2200511	2022TX202219822	04/20/2022	MTBC001328
03/18/2022	AMRC2200530	2022TX202220630	04/26/2022	MTBC001328
03/11/2022	AMRC2200649	2022TX202219546	05/19/2022	MTBC001328
04/18/2022	AMRC2200721	2022TX202220544	06/21/2022	MTBC001328
04/26/2022	AMRC2200742	2022TX202220142	06/21/2022	MTBC001328
05/08/2022	AMRC2200838	2022TX202220563	07/15/2022	MTBC001328
08/18/2022	AMRC2201204	2022TX202222413	10/05/2022	MTBC001328
05/15/2022	AMRC2201225	2022TX202220094	10/11/2022	MTBC001328
09/21/2022	AMRC2201516	2022TX202225045	01/05/2023	MTBC001328
12/07/2022	AMRC2300115	2023TX202325217	02/08/2023	MTBC001328
10/19/2022	AMRC2300115	2023TX202325217	02/10/2023	MTBC001328
02/13/2023	AMRC2300468	2023TX202324896	03/29/2023	MTBC001328
02/11/2023	AMRC2300551	2023TX202324864	04/13/2023	MTBC001328
01/12/2023	AMRC2300678	2023TX202325778	05/12/2023	MTBC001328
03/14/2023	AMRC2300692	2023TX202324813	05/12/2023	MTBC001328
05/20/2023	AMRC2300918	2023TX202324814	07/12/2023	MTBC001328
05/18/2023	AMRC2300968	2023TX202324583	07/21/2023	MTBC001328
07/02/2023	AMRC2301303	2023TX202326627	09/29/2023	MTBC001328
08/10/2023	AMRC2301332	2023TX202325162	10/06/2023	MTBC001328
10/31/2023	AMRC2301845	2023TX202326333	01/11/2024	MTBC001328
11/22/2023	AMRC2304006	2023TX202326586	01/26/2024	MTBC001328
11/21/2023	AMRC2400021	2024TX202420600	01/26/2024	MTBC001328
12/22/2023	AMRC2400359	2024TX202412995	03/21/2024	MTBC001328
07/18/2024	AMRC2401411	2024TX202420202	10/30/2024	MTBC001328
07/23/2024	AMRC2401414	2024TX202404951	10/16/2024	MTBC001328
09/01/2024	AMRC2401458	2024TX202411737	10/23/2024	MTBC001328
09/11/2024	AMRC2401537	2024TX202409575	11/14/2024	MTBC001328
10/14/2024	AMRC2401689	2024TX202417120	12/18/2024	MTBC001328
10/24/2024	AMRC2500048	2024TX202417120	02/06/2025	MTBC001328
12/28/2024	AMRC2500226	2024TX202532768	02/28/2025	MTBC001328



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Case #4

Father of son and daughter with pre-XDR
TB five years prior

Refused evaluation then

Now symptomatic



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Smear and NAAT Results

AFB Smear (Fluorochrome) **POSITIVE: AFB seen on direct smear, >10/field**

MTB Direct Detection, NAA **POSITIVE: M.tuberculosis complex DNA detected**

Rifampin by Direct NAA **Rifampin resistance mutation not detected; likely rifampin susceptible.**

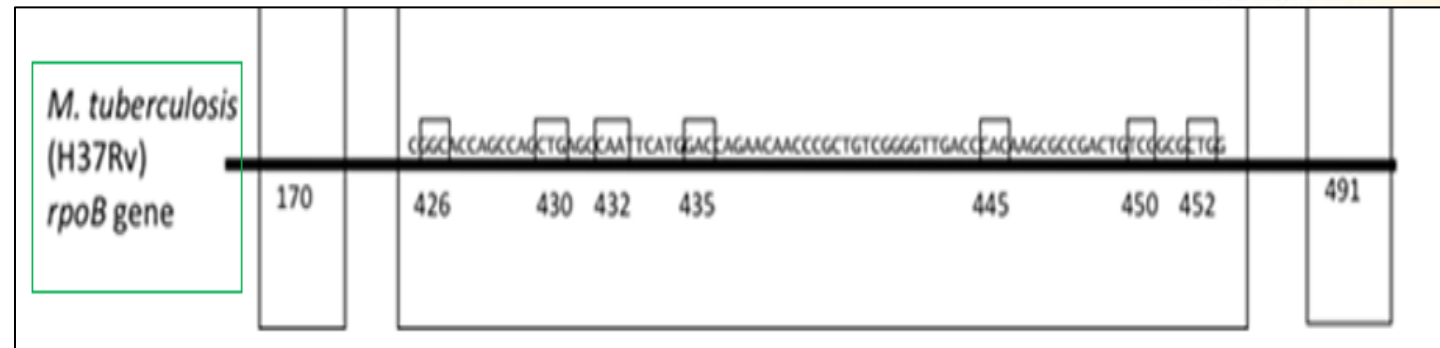
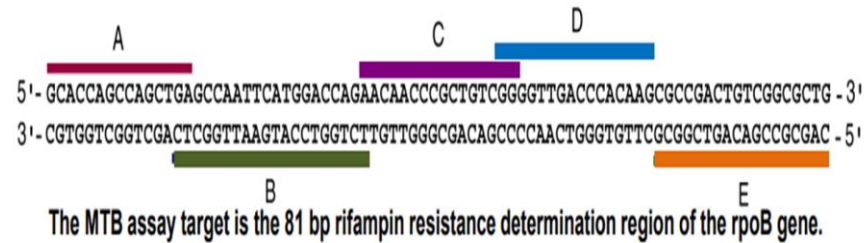
- Although rifampin susceptible by Xpert, patient still high risk and urgency to develop drug regimen



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Xpert vs MDDR *rpoB* Evaluation



MDDR sequences 2 codons outside of RRDR known to be associated with rifampin resistance

MDDR can detect as low as 10% rifampin resistant population while Xpert limit of detection is 30%



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Rifampin (RIF)

RIF interpretation

rpoB*

Comments and Disclaimers

* DTBE Reference Laboratory has transitioned from the E. coli to the M. tuberculosis numbering system for reporting rpoB gene mutations.

Result

Ile491Phe

Interpretation

Low-level RIF resistance;
May test susceptible by
phenotypic method.

Amikacin, Capreomycin, and Kanamycin (AMK, CAP, and KAN)

AMK CAP and KAN interpretation

rrs

eis

Result

No mutation

No mutation

Interpretation

Cannot rule out resistance
to AMK, CAP, and KAN.

Isoniazid (INH)

INH interpretation

inhA

fabG1

katG

Result

No mutation

No mutation

Ser315Thr

Interpretation

INH resistant

Bedaquiline (BDQ)

BDQ interpretation

atpE

rv0678

pepQ

Result

No mutation

No mutation

No mutation

Interpretation

Cannot rule out BDQ
resistance.

Ethambutol (EMB)

EMB interpretation

embB

Result

Asp354Ala

Interpretation

Likely EMB resistant

Clofazimine (CFZ)

CFZ interpretation

pepQ

rv0678

Result

No mutation

No mutation

Interpretation

Cannot rule out CFZ
resistance.

Pyrazinamide (PZA)

PZA interpretation

pncA

Result

No mutation

Interpretation

Cannot rule out PZA
resistance.

Linezolid (LZD)

LZD interpretation

rplC

rri

Result

No mutation

No mutation

Interpretation

Cannot rule out LZD
resistance.

Fluoroquinolones (FQ)

FQ interpretation

gyrA

gyrB

Result

Ser91Pro

No mutation

Interpretation

FQ resistant

Conventional DST Results

Both DSHS and CDC agar proportion results were consistent with the molecular findings

- DSHS found isolate to be 10% resistant to rifampin
- CDC found 8% resistance



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Minimum Inhibitory Concentration (MIC) Testing

Lowest concentration of a drug which prevents detectable growth in vitro when tested in a series of concentrations

Only available at specialized laboratories such as CAHD and Wadsworth Center NYHD

Performed only by special request on select antibiotics

Can be tested by MGIT, AP, or Broth Micro-dilution (BMD)



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MIC Result

California Health Department performed,
moxifloxacin MIC=1.0 ug/ml

- Intermediate resistance can be overcome with higher dose



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Genotyping Result

Date of Specimen Collection	Submitter Number	State Case Number	Date Received	wgMLSType
11/12/2024	AMCC2418101		12/12/2024	MTBC001079
07/30/2024	AMCC2412158	2024TXBN2426175	08/14/2024	MTBC001079
06/07/2019	AMCC1908001	2019TXBN1913136	07/02/2019	MTBC001079



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Case #5

Patient is a 71 y/o male from Mexico
visiting family in Texas including a 2 y/o

Non-productive cough

Fatigue

Weight loss for a year

Xpert positive for rifampin resistance

MDDR shows isolate to be XDR (old
definition)



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Conventional DST Result



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Case #6

Eighty y/o male visiting from Mexico

Poor health

Rifampin resistant by Xpert



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Rifampin (RIF)	<u>Result</u>	<u>Interpretation</u>
RIF interpretation		RIF resistant
rpoB*	His445Tyr	
Comments and Disclaimers		
* DTBE Reference Laboratory has transitioned from the E. coli to the M. tuberculosis numbering system for reporting rpoB gene mutations.		
Isoniazid (INH)	<u>Result</u>	<u>Interpretation</u>
INH interpretation		INH resistant
inhA	No mutation	
fabG1	No mutation	
katG	Ser315Thr	
Ethambutol (EMB)	<u>Result</u>	<u>Interpretation</u>
EMB interpretation		Effect of mutation unknown. Cannot rule out EMB resistance.
embB	Trp290Cys	
Pyrazinamide (PZA)	<u>Result</u>	<u>Interpretation</u>
PZA interpretation		Effect of mutation unknown. Cannot rule out PZA resistance.
pncA	Ile6frameshift	
Fluoroquinolones (FQ)	<u>Result</u>	<u>Interpretation</u>
FQ interpretation		Cannot rule out FQ resistance.
gyrA	No mutation	
gyrB	No mutation	

Amikacin, Capreomycin, and Kanamycin (AMK, CAP, and KAN)	<u>Result</u>	<u>Interpretation</u>
AMK CAP and KAN interpretation		Cannot rule out resistance to AMK, CAP, and KAN.
rrs	No mutation	
eis	No mutation	
Bedaquiline (BDQ)	<u>Result</u>	<u>Interpretation</u>
BDQ interpretation		Effect of mutation unknown. Cannot rule out BDQ resistance.
atpE	No mutation	
rv0678	Asp15Glu	
pepQ	No mutation	
Clofazimine (CFZ)	<u>Result</u>	<u>Interpretation</u>
CFZ interpretation		Effect of mutation unknown. Cannot rule out CFZ resistance.
pepQ	No mutation	
rv0678	Asp15Glu	
Linezolid (LZD)	<u>Result</u>	<u>Interpretation</u>
LZD interpretation		Effect of mutation unknown. Cannot rule out LZD resistance.
rplC	No mutation	
rli	C2070A, C2130A	

Conventional DST Result (Wadsworth Center NYHD)

BPaL Susceptibility testing for M. tuberculosis complex (MGIT)

Bedaquiline [1.0 ug/ml]: **Susceptible**

Clofazimine [1.0 ug/ml]: **Susceptible**

Linezolid [1.0 ug/ml]: **Susceptible**

Very scary situation.



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Summary

Each of these testing methods have individual benefits and disadvantages

Understanding these characteristics can reconcile seeming discordance

Integrating these methods provides a clearer understanding of patient's situation and appropriate treatment especially with the rise of resistant and complex cases

If unsure how to interpret results, ask!



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Thank You!



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