14th National Conference on
Laboratory Aspects of Tuberculosis

March 10–12, 2026 | Atlanta, Georgia

Shifting Gears: Is Whole Genome Sequencing Ready to Take the Wheel in TB Drug Susceptibility Testing?

Conflict of Interest Statement

No Potential Conflicts of Interest

Speaker: Joseph Shea, Wadsworth Center, New York Department of Health

I have NO conflicts of interest related to this presentation to disclose.

Speaker: Jaime Posey, US Centers for Disease Control and Prevention

I have NO conflicts of interest related to this presentation to disclose.

Speaker: Soyeon Lippman, Washington State Department of Health

I have NO conflicts of interest related to this presentation to disclose.

Department of Health
Wadsworth Center

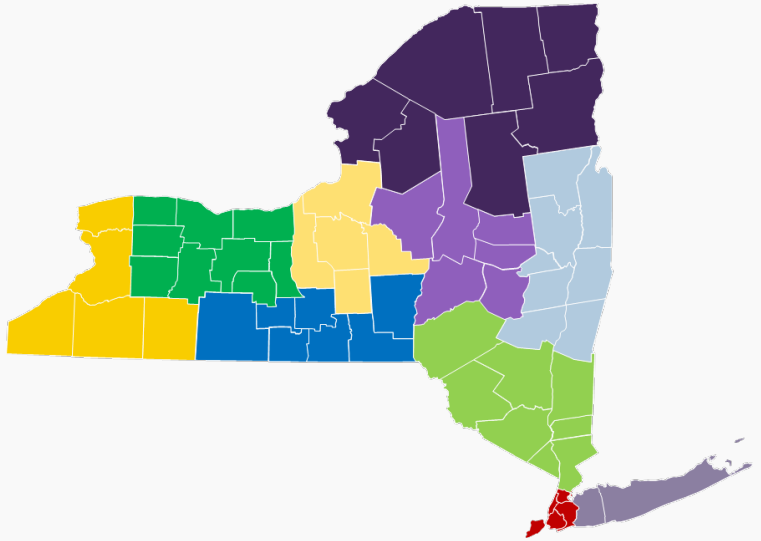
Whole Genome Sequencing and Reduced Phenotypic Drug Susceptibility Testing for *Mycobacterium tuberculosis*

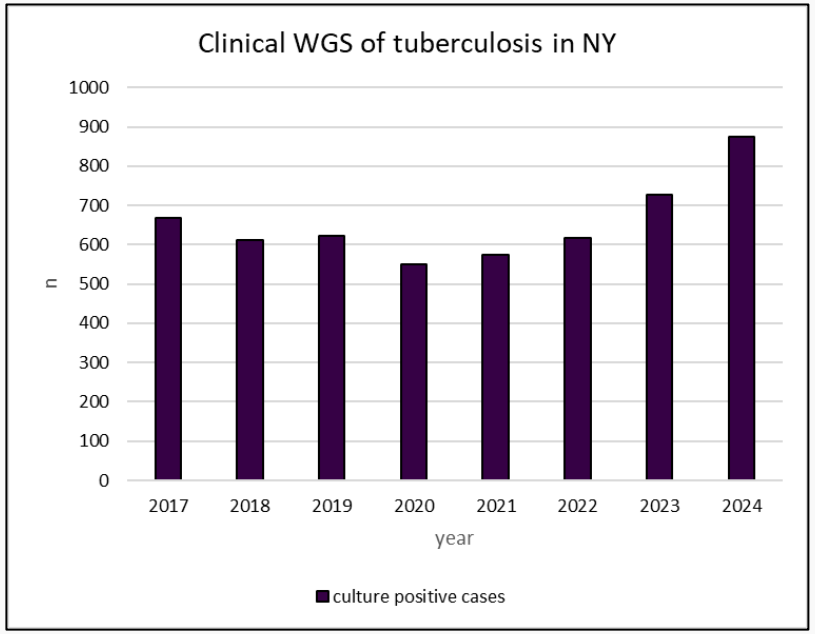
Joseph Shea, MS
Research Scientist

14th National Conference on the Laboratory Aspects of Tuberculosis

TUBERCULOSIS (TB) IN NEW YORK STATE

- Ranks 3rd by number of TB cases, annually
- New York State and New York City are distinct reporting areas at national level
- Combined incidence of 5.4 in 2024
- Receive a relatively high percentage of TB positive samples
- Whole-genome sequencing (WGS) implemented in March 2016





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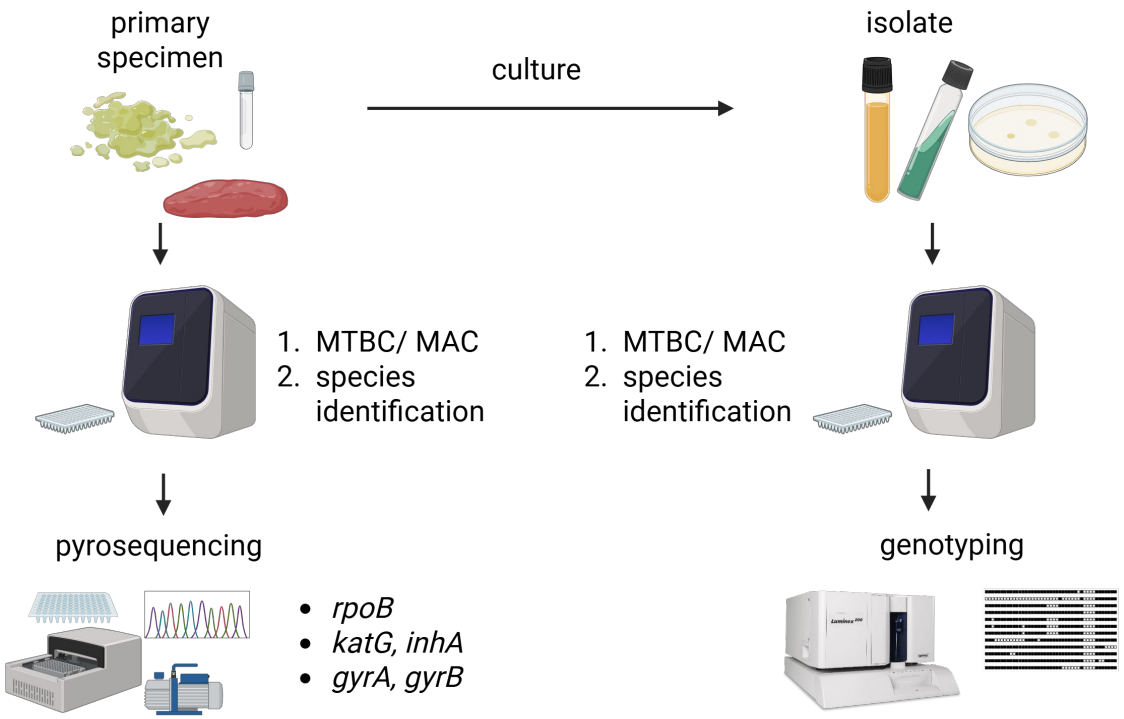
MOLECULAR TB TESTING IN 2016



MTBC: *Mycobacterium tuberculosis* complex
MAC: *Mycobacterium avium* complex

Molecular testing is performed outside of biosafety level 3(BSL-3)
All samples are heat inactivated prior to leaving BSL-3 (80C for 1h)

- Focus of molecular resistance detection on primary specimens
- Additional testing for strain characterization
- **Phenotypic drug susceptibility testing performed on all samples**

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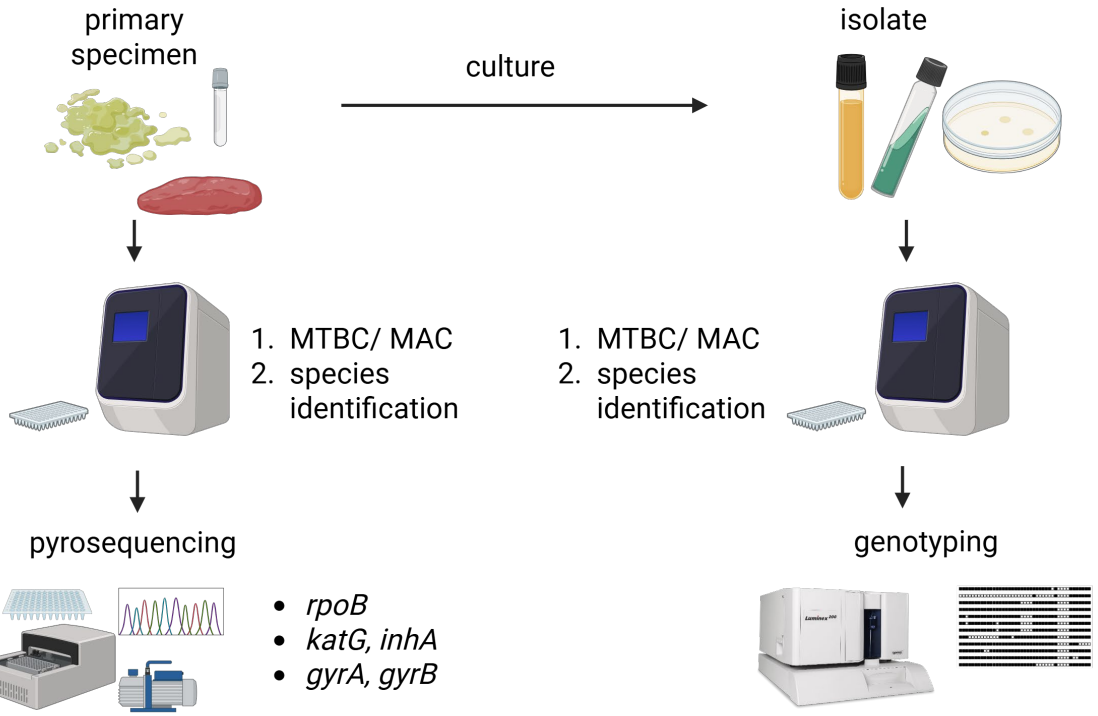
MOLECULAR TB TESTING IN 2016



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ARTICLE

Received 17 Apr 2015 | Accepted 28 Oct 2015 | Published 21 Dec 2015

DOI: 10.1038/ncomms10063 OPEN

Rapid antibiotic-resistance predictions from genome sequence data for *Staphylococcus aureus* and *Mycobacterium tuberculosis*

Phelim Bradley[1], N. Claire Gordon[2], Timothy M. Walker[2], Laura Dunn[2], Simon Heys[1], Bill Huang[1], Sarah Earle[2], Louise J. Pankhurst[2], Luke Anson[2], Mariateresa de Cesare[1], Paolo Piazza[1], Antonina A. Votintseva[2], Tanya Golubchik[2], Daniel J. Wilson[1,2], David H. Wyllie[2], Roland Diel[3], Stefan Niemann[4,5], Silke Feuerriegel[4,5], Thomas A. Kohl[4], Nazir Ismail[6,7], Shaheed V. Omar[6], E. Grace Smith[8], David Buck[1], Gil McVean[1], A. Sarah Walker[2,9], Tim E.A. Peto[2,9], Derrick W. Crook[2,9,10] & Zamin Iqbal[1]

- Cited limitations of gold standard methods (MGIT DST)
- Barriers include need for bioinformatic expertise and cost

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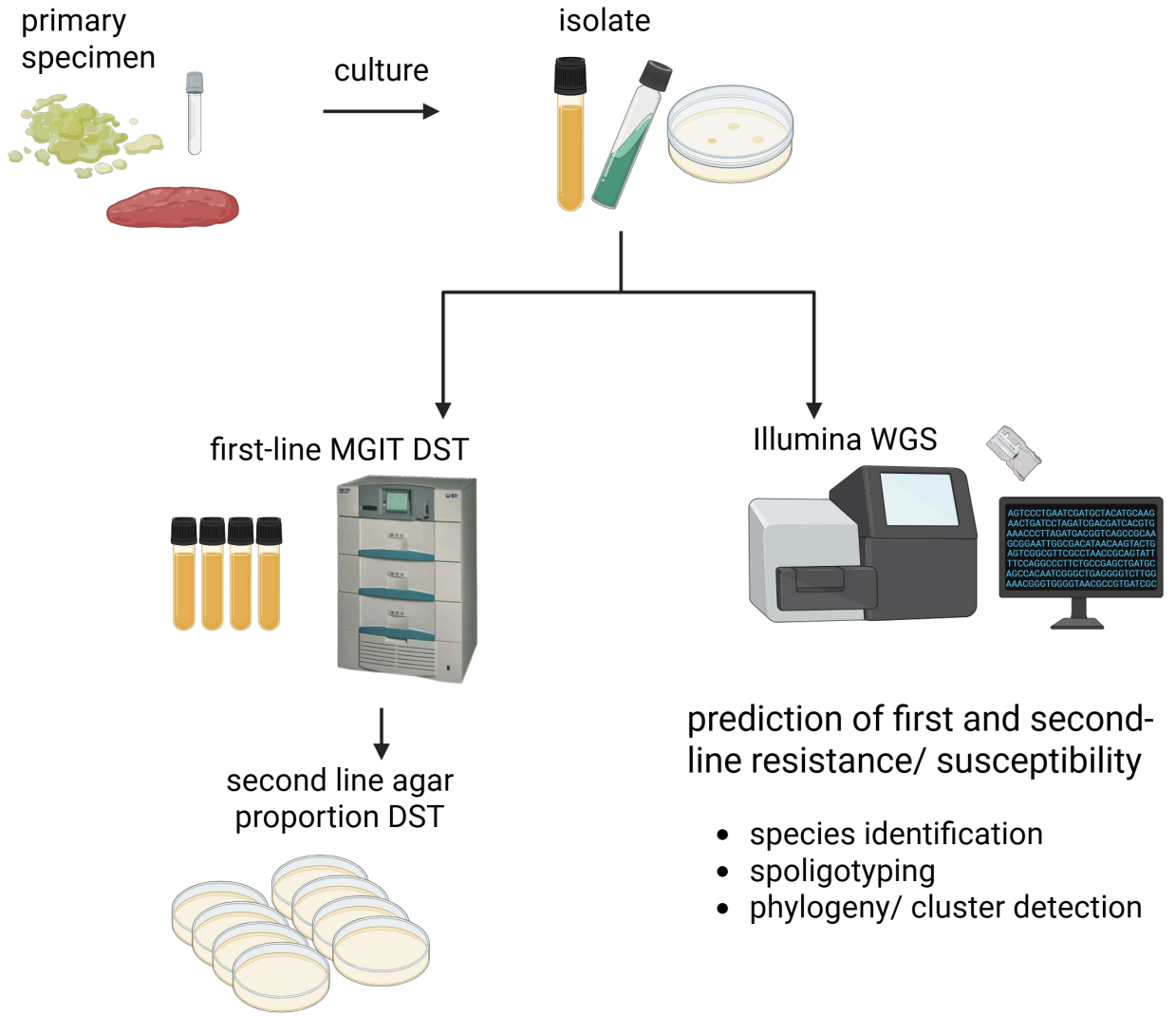
MGIT: mycobacteria growth indicator tube
DST: drug susceptibility testing

IMPLEMENTATION OF WGS AND WORKFLOW

- WGS assay designed for 1mL of positive MGIT cultures
- Extensive validation with genotypic and phenotypic methods
- Side by side testing for >2 years



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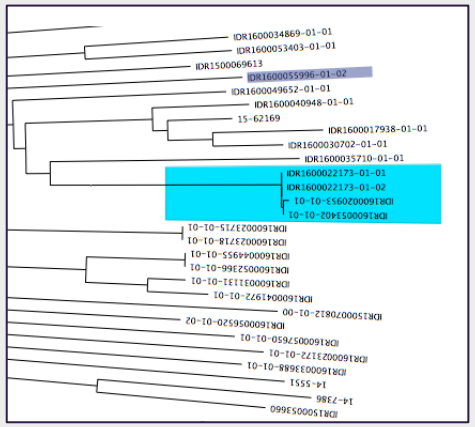
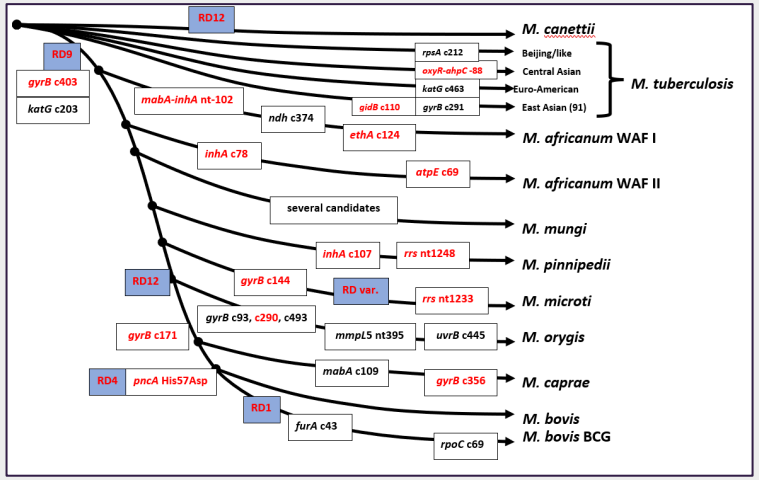
Universal phenotypic testing and WGS was not sustainable forever

WGS WAS TOO VALUABLE TO DISCONTINUE

- More complete resistance profiles for MDR and XDR strains
  - Several instances of MDR strains first identified by WGS
- Explained results of other molecular assays
  - primer mutations, target deletions
- Less common species mis-identified by previous methods
- High resolution genotyping investigations
  - resolving false positives





Predicted Resistance Profile*

Rifampin (RIF):	RESISTANT (predicted)
Isoniazid (INH):	RESISTANT (predicted)
Pyrazinamide (PZA):	RESISTANT (predicted)
Ethambutol (EMB):	RESISTANT (predicted)
Streptomycin (SM):	RESISTANT (predicted)
Kanamycin (KAN):	Susceptible (suggested)
Fluoroquinolones (FLQ):	RESISTANT (predicted)
Ethionamide (ETH):	RESISTANT (predicted)

pre-XDR case identified in 2016

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MDR: multidrug-resistant
XDR: extensively drug-resistant

Could WGS partially replace phenotypic testing?

REDUCING PHENOTYPIC TESTING

- Larger and more comprehensive studies suggested this as a possibility

- Concerns about how this would be received
  - Physicians
  - TB program partners – local, state, CDC
  - Reporting language

Whole-genome sequencing for prediction of *Mycobacterium tuberculosis* drug susceptibility and resistance: a retrospective cohort study

Timothy M Walker, Thomas A Kohl*, Shaheed V Omar*, Jessica Hedge*, Carlos Del Ojo Elias, Phelim Bradley, Zamin Iqbal, Silke Feuerriegel, Katherine E Niehaus, Daniel J Wilson, David A Clifton, Georgia Kapatai, Camilla L C Ip, Rory Bowden, Francis A Drobniewski, Caroline Allix-Béguec, Cyril Gaudin, Julian Parkhill, Roland Diel, Philip Supply, Derrick W Crook, E Grace Smith, A Sarah Walker, Nazir Ismail†, Stefan Niemann†, Tim E A Peto†, and the Modernizing Medical Microbiology (MMM) Informatics Group‡*

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Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

The CRyPTIC Consortium and the 100,000 Genomes Project

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ELSEVIER

CMI CLINICAL MICROBIOLOGY AND INFECTION

ESCMID

Review

Mycobacterium tuberculosis and whole-genome sequencing: how close are we to unleashing its full potential?

G. Satta [1,2,*], M. Lipman [3,4], G.P. Smith [5,6], C. Arnold [1,7], O.M. Kon [2,8], T.D. McHugh [1]

CONSIDERATIONS

Accuracy	Is WGS reliable to generate DST profiles and how well does it predict susceptibility?
Cost	Would WGS replace enough existing testing to offset the cost?
Turnaround Time	Can we get WGS results in a similar timeframe or faster than current methods?

ACCURACY OF WGS AT RESISTANCE PROFILING

	ethambutol	isoniazid	pyrazinamide	rifampin
Sensitivity	0.89	0.93	0.81	0.99
Specificity	1.00	1.00	0.99	0.99
Positive predictive value	0.91	0.99	0.87	0.84
Negative predictive value	0.99	0.99	0.98	1.00
Strains (n)	1516	1522	1518	1516

Data from 2018

- High levels of success predicting susceptibility

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ACCURACY OF WGS AT RESISTANCE PROFILING

Every discrepancy was investigated

- In most cases, resistance could be explained by mutations in known resistance genes
- Repeat testing performed as needed

Many, though not all, resolved

- Limitations of critical concentration testing
- Phenotype resolution with repeat testing
- Heterozygous mutations & atypical variants
- Small number of low-level INH resistant strains, often with inconsistent results

Phenotype								Genotype (WGS)							
EMB	FLQ	INH	PZA	RIF	SM	KAN	ETH	EMB	FLQ	INH	PZA	RIF	SM	KAN	ETH	
R	S	R	R	R	R	S	R	R	S	R	R	R	S	S	R	rrs A905G
S		S	S	S	S			S	S	R	S	R	S	S	R	rpoB Leu511Pro, inhA Ser94Ala
R	S	R	R	R	R	S	S	R	S	R	R	R	S	S	S	gidB frameshift nt. 88
R	S	R	R	R	R	S	S	S	S	R	R	R	R	S	S	embC-A C(-16)G
S		S	R	S	S			S	S	S	S	S	S	S	S	Lineage 1
S	S	R	S	R	R	S	S	S	S	R	S	R	S	S	S	gidB frameshift nt. 103 & rrs C559T
S		S	S	R	S			S	S	S	S	R	S	S	S	
S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
R	S	R	R	S	R	S	R	R	S	R	R	R	R	S	R	rpoB Asp516Tyr
R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	gidB partial gene deletion
S		S	S	S	S			S	S	S	S	S	S	S	S	
S		S	S	S	S			S	S	S	S	S	S	S	S	
R	S	R	S	R	R	S	S	R	S	R	S	R	S	S	S	gidB Ser149Arg
R	S	R	S	R	R	S	S	R	S	R	S	R	R	S	S	
R	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R	
R	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R	
S	S	R	S	R	R	S	S	S	S	R	S	R	R	S	S	
S	S	R	S	R	R	S	S	S	S	R	S	R	S	S	R	gidB Leu79Ser, inhA T-8C
S	S	R	S	S	S	S	R	S	S	R	S	S	S	S	R	
R	S	R	S	R	R	R	S	R	S	R	R	R	R	R	S	pncA Val163Ala
S	S	R	S	R	R	S	R	S	S	R	S	R	R	S	R	
R		R	R	R	R			R	S	R	R	R	R	S	S	
S	S	R	R	S	S	S	R	S	S	S	R	S	S	S	S	mabA Leu194Leu
S	S	R	S	S	R	S	S	S	S	R	S	S	S	S	S	gidB frameshift nt. 83
S		R	S	S	S		R	S	S	R	S	S	S	S	R	
S		S	S	S	S			S	S	S	S	S	S	S	S	
R	R	R	R	R	S	S	S	R	R	R	R	R	S	S	S	
S	S	R	S	S	S	S	S	S	S	S	S	R	S	S	S	katG Gly121Asp; rpoB Leu533Pro
S		S	S	S	S			S	S	S	S	S	S	S	S	
S	R	S	S	S	S			S	R	S	S	S	S	S	S	
S	S	R	R	R	R	S	R	R	S	R	R	R	S	S	R	embB Met306Ile gidB Ala138Val
R	S	R	R	R	S	S	R	S	S	R	R	R	S	S	R	embB Trp332Arg & embC-A C(-16)T
S	S	R	S	R	S	S	S	R	S	R	S	R	S	S	S	embB Met306Ile
S	S	R	S	S	R	S	S	S	S	R	S	S	R	S	S	
S	S	R	S	S	S	S	R	S	S	R	S	S	S	S	R	
R	S	R	R	R	R	S	R	R	S	R	R	R	S	S	R	gidB Leu79Ser
S	S	R	S	S	R	S	R	S	S	R	S	S	R	S	R	
S	S	R	S	S	R	S	S	S	S	R	S	S	R	S	S	
S	S	R	S	S	R	S	R	S	S	R	S	S	R	S	R	
S		S	S	S	S			S	S	S	S	S	S	S	S	
S		S	R	S	S			S	S	S	R	S	S	S	S	

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WGS COST AND TURNAROUND TIME

TAT Comparison to Phenotypic DST		
WGS reported sooner than DST	259	82%
Same Date	30	9.5%
WGS reported later than DST	26	8.2%

- WGS reliably faster than phenotypic DST for samples <u>received as isolates</u>
- WGS turnaround time (TAT) comparable to phenotypic DST for samples received as primary specimens
- WGS always faster for second-line DST

- Cost remained a barrier, WGS was more expensive than all other testing
- WGS could replace some molecular testing, but not enough to pay for itself

Existing Testing Methods	Cost per specimen ($)	
	Reagents	Labor[a,b]
Real-time PCR-detect MTBC	3.98	10.02
Real-time PCR-detect MTBC members	4.62	10.02
Molecular DST (*rpoB, katG, inhA*)	16.78	42.06
Spoligotyping	7.91	21.52
Total costs	$33.29	$83.62
	$116.91	

Whole Genome Sequencing*	Cost per specimen ($)	
	Reagents	Labor[a,b]
DNA Extraction	3.49	11.02
MiSeq® library preparation	45.00	67.00
MiSeq® Sequencing	77.00	13.00
Total costs	$125.49	$91.02
	$216.51	

Current WGS cost $60 - $80

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Implemented universal WGS as first-line method of DST in 2018

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WGS ANALYSIS: MUTATION CATEGORIES

- High-confidence mutation

rpoB Ser450Leu

Known to be associated with RIF resistance

- Neutral mutation

katG Pro6Ser

Not associated with drug resistance

- >30 strains, all tested have INH susceptible phenotype

- Mutation of unknown significance

embB Tyr319Ser

Mutation may or may not confer drug resistance

- Found 1 previous time in a strain with EMB resistant phenotype

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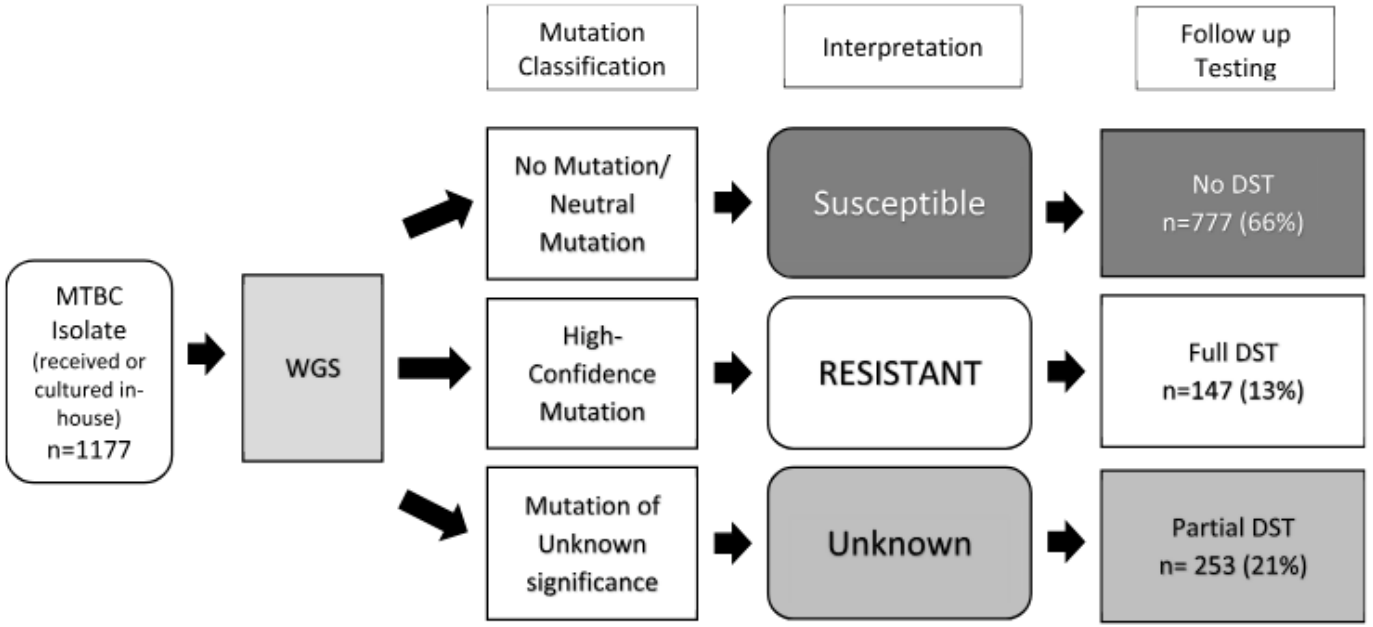
WGS INTERPRETATIONS AND DST SCHEDULING



Drug	Genes with High-Confidence Mutations for 'Resistant' predictions	Genes for 'Unknown' predictions
Rifampin (RIF)	*rpoB*	*rpoB*
Isoniazid (INH)	*katG, inhA, mabA-inhA, mabA, oxyR-ahpC*	*katG, inhA, mabA-inhA, mabA, oxyR-ahpC, ndh* & promoter, *furA-katG*
Ethambutol (EMB)	*embB, embC-embA*	*embB, embC-embA*
Pyrazinamide (PZA)	*pncA* & promoter	*pncA* & promoter, *panD*
Fluoroquinolones (FLQ)	*gyrA, gyrB*	*gyrA, gyrB*
Ethionamide (ETH)	*inhA, mabA-inhA, mabA, ethA*	*inhA, mabA-inhA, mabA, ethA*
Kanamycin (KAN)	*rrs , eis* promoter	*rrs, eis* promoter

WGS=whole genome sequencing; DST=drug susceptibility testing

Fig. 1. Updated laboratory testing algorithm overview.

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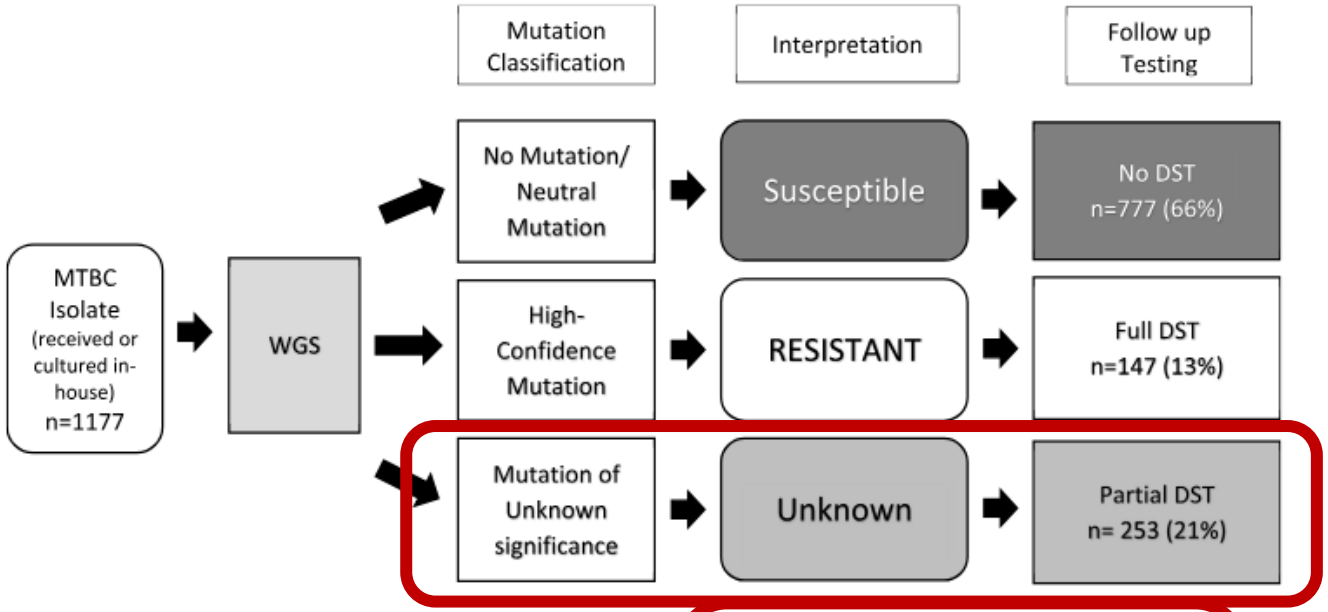
WGS INTERPRETATIONS AND DST SCHEDULING

Genes used for predictions of ‘Unknown’ are critical to avoid missing drug resistant strains

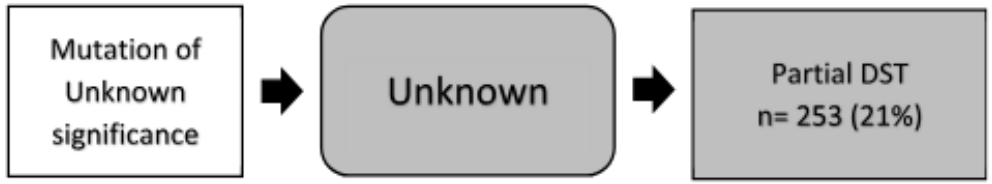


Drug	Genes with High-Confidence Mutations for ‘Resistant’ predictions	Genes for ‘Unknown’ predictions
Rifampin (RIF)	*rpoB*	*rpoB*
Isoniazid (INH)	*katG, inhA, mabA-inhA, mabA, oxyR-ahpC*	*katG, inhA, mabA-inhA, mabA, oxyR-ahpC, ndh* & promoter, *furA-katG*
Ethambutol (EMB)	*embB, embC-embA*	*embB, embC-embA*
Pyrazinamide (PZA)	*pncA* & promoter	*pncA* & promoter, *panD*
Fluoroquinolones (FLQ)	*gyrA, gyrB*	*gyrA, gyrB*
Ethionamide (ETH)	*inhA, mabA-inhA, mabA, ethA*	*inhA, mabA-inhA, mabA, ethA*
Kanamycin (KAN)	*rrs* , *eis* promoter	*rrs, eis* promoter

WGS=whole genome sequencing; DST=drug susceptibility testing

Fig. 1. Updated laboratory testing algorithm overview.

EXAMPLE SCENARIOS



WGS result	Mutation Detected	Phenotypic Testing Scheduled	Phenotypic Result
RIF: Unknown	*rpoB* Lys157Arg	RIF MGIT	RIF: susceptible

Genes for 'Unknown' predictions

rpoB
katG, inhA, mabA-inhA, mabA, oxyR-ahpC, ndh & promoter, *furA-katG*
embB, embC-embA
pncA & promoter, *panD*
gyrA, gyrB
inhA, mabA-inhA, mabA, ethA
rrs, eis promoter

Molecular Identification and Susceptibility Testing - Whole Genome Sequencing

Molecular Identification*:	**Mycobacterium tuberculosis**

Susceptibility Profile*

Rifampin (RIF):	**Unknown**
Isoniazid (INH):	**Susceptible**
Pyrazinamide (PZA):	**Susceptible**
Ethambutol (EMB):	**Susceptible**
Streptomycin (SM):	**Susceptible**
Kanamycin/Amikacin (KAN/AMI):	**Susceptible**
Fluoroquinolones (FLQ):	**Susceptible**
Ethionamide (ETH):	**Susceptible**

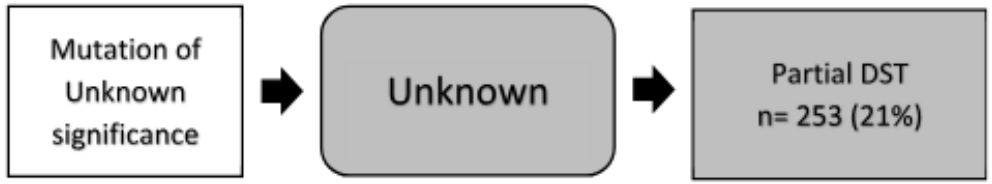
High-confidence mutations detected*

rpoB (RIF):	**No high-confidence mutation**
[Note]	A Lys157Arg rpoB mutation is present but its association with rifampin resistance is unknown. This mutation is not considered a high-confidence mutation.

Susceptibility Testing for M. tuberculosis complex (MGIT)

Rifampin [0.5 ug/ml]:	**Susceptible**

EXAMPLE SCENARIOS



WGS result	Mutation Detected	Phenotypic Testing Scheduled	Phenotypic Result
RIF: Unknown	*rpoB* Lys157Arg	RIF MGIT	RIF: susceptible
INH: Unknown	*furA-katG* A(-9)C	INH MGIT	INH: RESISTANT

A WGS analysis that does not screen the promoter region of *katG* for mutations would miss this drug resistance

Atypical and difficult to detect mutations represent the most risk

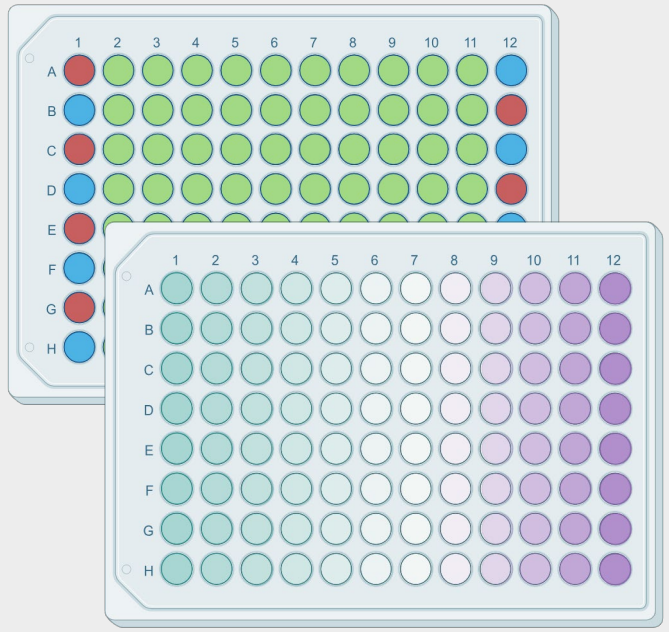
Genes for 'Unknown' predictions
rpoB
katG, inhA, mabA-inhA, mabA, oxyR-ahpC, ndh & promoter, *furA-katG*
embB, embC-embA
pncA & promoter, *panD*
gyrA, gyrB
inhA, mabA-inhA, mabA, ethA
rrs, eis promoter

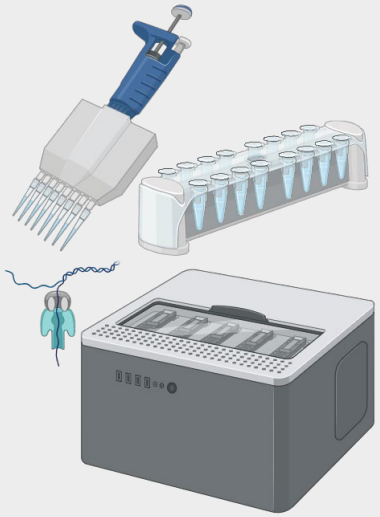
Molecular Identification and Susceptibility Testing - Whole Genome Sequencing

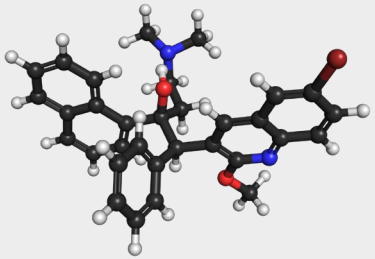
Molecular Identification*:	**Mycobacterium tuberculosis**
Susceptibility Profile*	
Rifampin (RIF):	**Susceptible**
Isoniazid (INH):	**Unknown**
Pyrazinamide (PZA):	**Susceptible**
Ethambutol (EMB):	**Susceptible**
Streptomycin (SM):	**RESISTANT (predicted)**
Kanamycin/Amikacin (KAN/AMI):	**Susceptible**
Kanamycin (KAN):	**Susceptible**
Fluoroquinolones (FLQ):	**Susceptible**
Ethionamide (ETH):	**Susceptible**
High-confidence mutations detected*	
katG (INH):	**No high-confidence mutation**
[Note]	An A(-9)C mutation is present in the furA-katG promoter region but its association with isoniazid resistance is unknown. This mutation is not considered a high-confidence mutation.
rrs 512, 513, 516, 906 (SM):	**Mutation present: A(513)C**
Susceptibility Testing for M. tuberculosis complex (MGIT)	
Isoniazid [0.1 ug/ml]:	**RESISTANT**
Isoniazid [0.4 ug/ml]:	**RESISTANT**

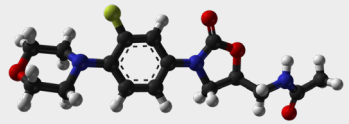
IMPACT OF REDUCED PHENOTYPIC TESTING

- WGS guided testing for resistant and challenging strains
  - Focus on relapse and suspected treatment failure
- Freed up time and resources for additional testing
  - Targeted next-generation sequencing
  - Bedaquiline, pretomanid, and linezolid phenotypic DST
  - Increased minimum inhibitory concentration testing
  - Publications and research









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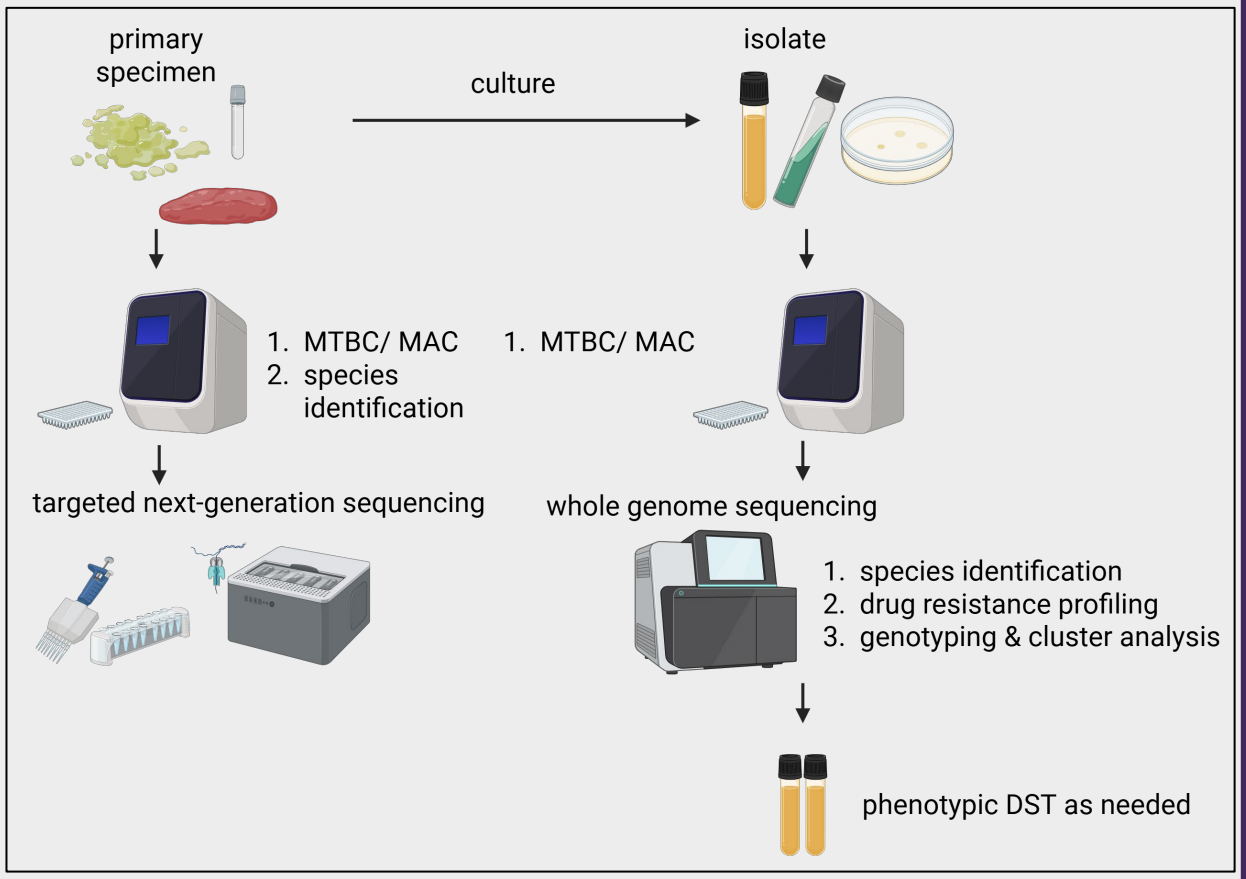
CONCLUSIONS

- WGS is capable of almost fully replacing phenotypic testing for susceptible strains
- Analysis and testing algorithm are critical to minimizing risk
- Phenotypic testing is essential for confirming resistance and understanding rare/ novel mutations



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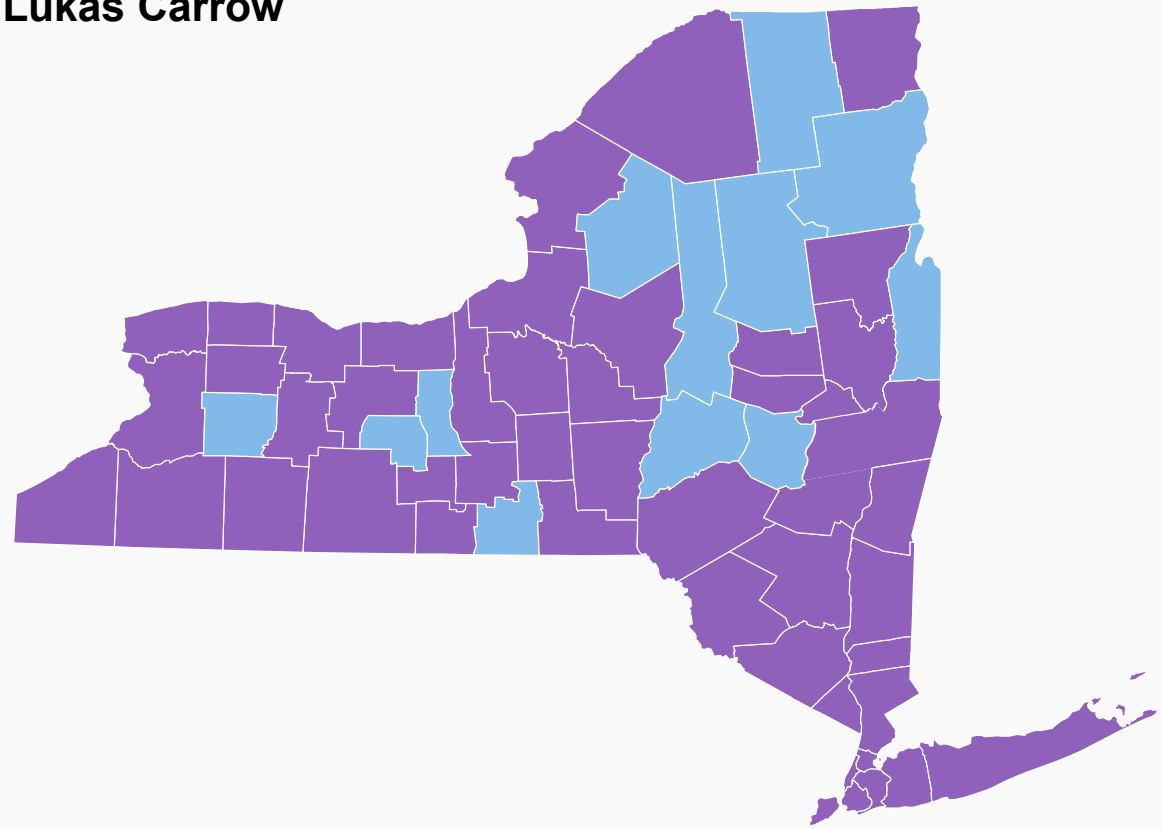
- Dr. Kimberlee Musser
- Dr. Pascal Lapierre
- Michelle Dickinson
- Donna Kohlerschmidt
- Tanya Halse
- Justine Edwards
- James Long
- Kruthika Patel
- Emily McGrath
- Carol Smith
- Skyla Carney
- Alexandra Fiero
- Amy Chiefari
- Farishta Popal
- Lukas Carrow

New York State & New York City Program Partners

- Cheryl Kearns
- Herns Modestil
- Jillian Knorr
- Jeanne Sullivan Meissner
- Namrata Madoor

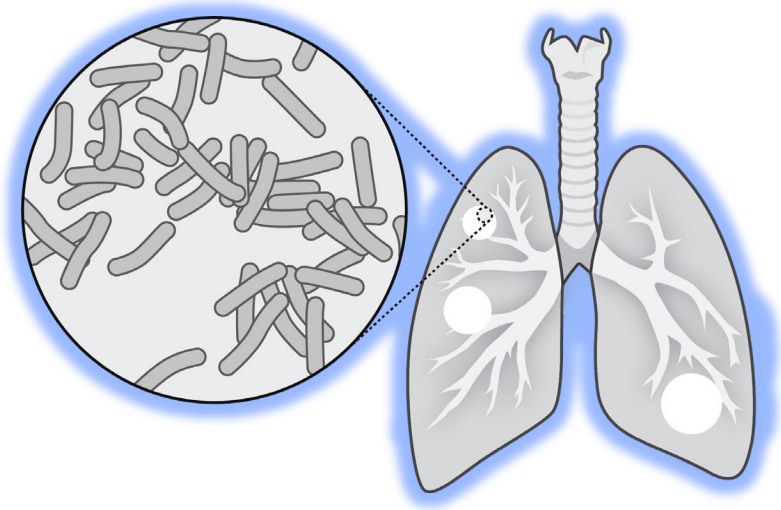
Applied Genomics Technologies Core

CLIMS Group



NEW YORK STATE | Department of Health
Wadsworth Center

Prediction of drug susceptibility using whole genome sequencing data



Jamie Posey, PhD
Lead, Applied Research Team
Division of Tuberculosis Elimination
National Center for HIV, Viral Hepatitis, STD, and TB Prevention
February 12, 2026

OBJECTIVES

- Compare the reported phenotypic results with molecular predicted susceptibilities for isoniazid, rifampin, pyrazinamide and ethambutol
- Determine the accuracy of detecting resistance
- Determine the accuracy of predicting pansusceptibility

Molecular surveillance of MTBC

- **Sequencing**
  - National TB molecular surveillance center (MI)
  - National drug susceptibility testing reference center (CA)
  - Arizona, California, Delaware, Florida and New York
- **Centralized analysis on Advanced Molecular Detection (AMD) platform for surveillance**
- **Results are merged with surveillance data and shared with partners through TB Genotyping Information Management System (GIMS)**
- **>95% culture positive cases have a sequenced isolate**
- **2018-2022 raw data with limited metadata is publicly available at NCBI**

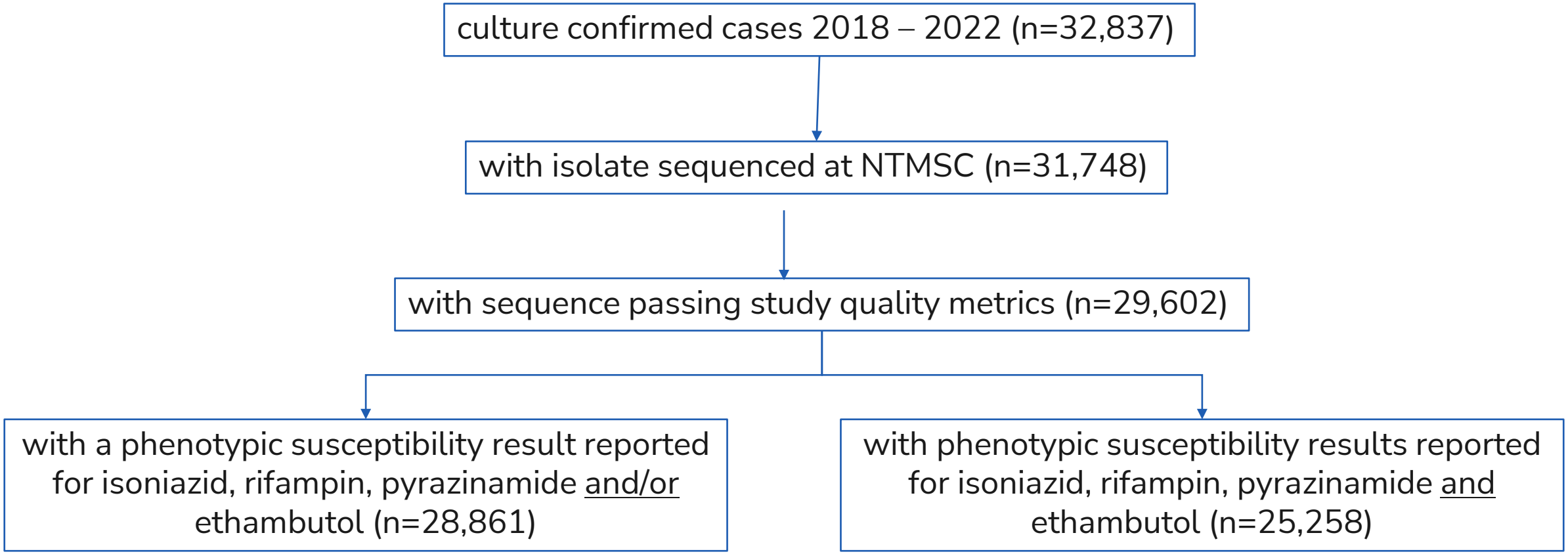
Molecular surveillance of MTBC

- **Outbreak detection**
  - Identification of clusters - isolates with closely related wgMLST patterns
  - Visualization of possible chains of transmission – whole genome SNP comparison of clustered isolates

- **Molecular prediction of antimicrobial resistance**
  - Identification and interpretation of variants in loci known to be associated with resistance

Molecular prediction of antimicrobial resistance

Sample set description



CDC pipelines for prediction of antimicrobial resistance

- **Varpipe v1.0.2 (used for this study)**
  - Prediction of isoniazid, rifampin, pyrazinamide, ethambutol and fluoroquinolone resistance
  - Interpretation based on v2 catalog and internal data
  - https://github.com/CDCgov/NCHHSTP-DTBE-Varpipe-WGS
- **Varpipe v1.1**
  - Modernized and refactored version of 1.0.2
  - More modular and incorporates NextFlow
  - Approved to share (new github site)
- **Varpipe v2.0**
  - Based on V1.1
  - Added prediction of clofazimine, bedaquiline, linezolid, ethionamide, delamanid, pretomanid and amikacin resistance

Reporting and interpretation criteria

Locus	DR_loci Coordinates	Reportable	Interpretation
gyrB	6571..6762	coding (446-507): "Non-synonymous"	✓
gyrA	7360..7583	coding (88-94): "Non-synonymous"	✓
rpoB	760307..761286	coding (170, 426-452,491): ["Non-synonymous" at codon 170] OR ["all" at codons 426 to 452] OR ["Non-synonymous" at codon 491]	✓
fabG1	1673409..1674052	only c.-17,c.-15, c.-8 and c.609 (codon 203)	✓
inhA	1674201..1675012	coding(all): "Non-synonymous"	✓
katG	2153888..2156112	coding (all): ["Non-synonymous"] OR ["synonymous" at codon 1]	✓
pncA	2288676..2289272	30 bp upstream and coding (all): ["Non-coding"] OR ["Non-synonymous"] OR ["synonymous" at codon 1]	✓
embB	4246513..4249811	coding (exclude 4246524..4246586, 4248314..4248329, 4249653..4249692): "Non-synonymous"	✓
ethA	4326003..4327474	coding (all): ["Non-synonymous"] OR ["synonymous" at codon 1]	
rrs	1473245..1473331	only c.1401,c.1402 and c.1484	
tlyA	1917939..1918747	coding (all): "Non-synonymous"	
ahpC	2726093..2726194	100 bp upstream: All	
atpE	1461044..1461290	coding (all): "Non-synonymous"	
pepQ	2859299..2860418	coding (all): "Non-synonymous"	
mmpR	778989..779487	coding (all): "Non-synonymous"	
rplC1	800808..801462	coing (all): "Non-synonymous"	
rrl	1473657..1476796	all	

Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance, 2nd ed
https://www.who.int/publications/i/item/9789240082410

Reported phenotypic results

	Reported phenotypic results		
	Susceptible	**Resistant**	**Unknown**
Isoniazid	26,136 (90.6%)	2,521 (8.7%)	204 (0.7%)
Rifampin	28,310 (98.1%)	447 (1.5%)	104 (0.4%)
Pyrazinamide	24,032 (83.3%)	1,385 (4.8%)	3,444 (11.9%)
Ethambutol	28,290 (98.0%)	333 (1.2%)	238 (0.8%)

Accuracy of WGS for the detection of resistance

	Susceptible reported phenotype			Resistant reported phenotype			Performance				
Molecular prediction	S	U	R	S	U	R	sensitivity	specificity	PPV	NPV	No prediction
Isoniazid (I)	25358	583	197	140	140	2232	94.1%	99.2%	91.9%	99.5%	2.5%
Rifampin (R)	28224	14	69	16	4	423	96.4%	99.8%	86.0%	99.9%	0.1%
Pyrazinamide (Z)	23828	140	65	643	41	693	51.9%	99.7%	91.4%	97.4%	0.7%
Ethambutol (E)	27071	1136	84	43	18	264	86.0%	99.7%	75.9%	99.8%	4.0%

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Ethambutol (E)	27071	**1136**	**84**	**43**	**18**	264	86.0%	99.7%	75.9%	99.8%	4.0%

Isoniazid discordance

	Susceptible reported phenotype			Resistant reported phenotype			Performance				
Molecular prediction	S	U	R	S	U	R	sensitivity	specificity	PPV	NPV	No prediction
Isoniazid (I)	25358	583	**197**	**140**	140	2232	94.1%	99.2%	91.9%	99.5%	2.5%

- **156 of 197 samples reported isoniazid susceptible were predicted to be resistant based on variants that are associated with low level resistance**
  - fabG1 upstream_c.-17G>T (n=11), fabG1 upstream_c.-15C>T (n=75), fabG1 upstream_c.-8T>A/C/G (n=15), fabG1_p.Leu203Leu (n=55)
- **140 samples reported isoniazid resistant were predicted to be susceptible**

Rifampin discordance

	Susceptible reported phenotype			Resistant reported phenotype			Performance				
Molecular prediction	S	U	R	S	U	R	sensitivity	specificity	PPV	NPV	No prediction
Rifampin (R)	28224	14	69	16	4	423	96.4%	99.8%	86.0%	99.9%	0.1%

- **57 of 69 samples reported rifampin susceptible were predicted to be resistant based on variants that are associated with low level resistance**
  - rpoB p.Leu430Pro (n=21), p.Asp435Tyr (n=9), p.His445Asn/Leu (n=14), p.Leu452Pro (n=7), p.Ile491Phe (n=6)
- **16 samples reported rifampin resistant were predicted to be susceptible**

Pyrazinamide discordance

	Susceptible reported phenotype			Resistant reported phenotype			Performance				
Molecular prediction	S	U	R	S	U	R	sensitivity	specificity	PPV	NPV	No prediction
Pyrazinamide (Z)	23828	140	65	**643**	41	693	51.9%	99.7%	91.4%	97.4%	0.7%
Lineage 1	5207	42	6	**558**	15	30	**5.1%**	99.9%	83.3%	90.3%	1.0%
Lineage 2	4146	25	9	26	10	108	80.6%	99.8%	92.3%	99.4%	0.8%
Lineage 3	1628	5	1	8	5	15	65.2%	99.9%	93.8%	99.5%	0.6%
Lineage 4	12650	68	5	44	11	84	65.6%	100.0%	94.4%	99.7%	0.6%

- **643 samples with reported pyrazinamide resistance were predicted to be susceptible**
  - 558 of the 643 samples with reported pyrazinamide resistance predicted to be susceptible were Lineage 1
  - 446 of the 536 samples with reported mono-pyrazinamide resistance predicted to be susceptible were Lineage 1

Ethambutol discordance

	Susceptible reported phenotype			Resistant reported phenotype			Performance				
Molecular prediction	S	U	R	S	U	R	sensitivity	specificity	PPV	NPV	No prediction
Ethambutol (E)	27071	**1136**	**84**	**43**	**18**	264	86.0%	99.7%	75.9%	99.8%	4.0%

- Prevalence of phenotypic ethambutol resistance was 1.2%
- Subset of *embB* variants confer lower-level resistance to ethambutol
- No prediction could be made for 4% of the samples in this study
- 98% of samples with no prediction are phenotypically susceptible

Ethambutol discordance

	Susceptible reported phenotype			Resistant reported phenotype			Performance				
Molecular prediction	S	U	R	S	U	R	sensitivity	specificity	PPV	NPV	No prediction
Ethambutol (E)	27071	1136	84	43	18*	264	86.0%	99.7%	75.9%	99.8%	4.0%
mDST-U=pDST-S	**28207**		84	**61**		264	**81.2%**	**99.7%**			

*9/18 mDST-U with ethambutol resistance had nonsynonymous variant within codons 228-410

Prediction of pansusceptible

	Pansusceptible reported phenotype			Any resistance reported phenotype			Performance	
Molecular prediction	panS	unknown	R	panS	Unknown	R	Accuracy	No prediction
All samples	20219	1446	233	611	194	2539	97.1%	6.5%
Lineage 1	4177	403	67	458	71	646	90.1%	8.1%
Lineage 2 - 4	15930	978	124	150	117	1431	99.1%	5.8%

Pansusceptible reported phenotype: reported as susceptible to I,R,Z and E
Any resistance reported phenotype: reported as resistant to I,R,Z and/or E
panS molecular prediction: predicted to be susceptible to I,R,Z and E
R molecular prediction: predicted to be resistant to I,R,Z and/or E

Prediction of pansusceptible

	Pansusceptible reported phenotype			Any resistance reported phenotype			Performance	
Molecular prediction	panS	unknown	R	panS	Unknown	R	Accuracy	No prediction
All samples	20219	1446	**233**	**611**	194	2539	97.1%	6.5%
Lineage 1	4177	403	67	458	71	646	90.1%	8.1%
Lineage 2 - 4	15930	978	124	150	117	1431	99.1%	5.8%

- **233 samples with reported pansusceptibility were predicted to be resistant**
  - 136 (58%) panS samples predicted to be resistant had a prediction based on variants associated with low level isoniazid resistance or borderline rifampin resistance
- **611 samples with reported resistance were predicted to be pansusceptible**
  - 419 (68%) were mono-pyrazinamide Lineage 1

Conclusions

- **WGS accurately detected resistance to first-line drugs**
  - Based on national surveillance data (except NY)
  - Lineage 1 effect was observed for pyrazinamide
  - The gold standard is not perfect

- **WGS accurately predicted susceptible phenotypes to first-line drugs**
  - Low prevalence of drug resistance
  - Could reduce the need for phenotypic testing (~75%) for first-line drugs

- **WGS can be used for surveillance of any drug if molecular mechanism is well documented**

New drugs
Bedaquiline and Pretomanid

Surveillance of new drugs

- **Loss of function variants (LOF)**
  - Indels, frameshifts, loss of start codon, early stop codon
  - Does not include other nonsynonymous variants
- **Bedaquiline**
  - 31 isolates have a Rv0678 LOF variant
  - 6/31 (19%) also have a *mmpL5* LOF variant
- **Delamanid and pretomanid**
  - LOF variants >75% allele frequency
  - *ddn* (48), *fgd1* (4), *fbiA* (4), *fbiB* (1), *fbiC* (11), *fbiD* (4)
  - Mostly found in RIF-S strains

Thank you

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 https://www.cdc.gov/
Follow us on social **@CDCgov**

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U. S. Centers for Disease Control and Prevention.

Washington State Department of HEALTH

WGS-DST AT WA PHL

Soyeon Lippman

Story of WGS-DST at WA PHL

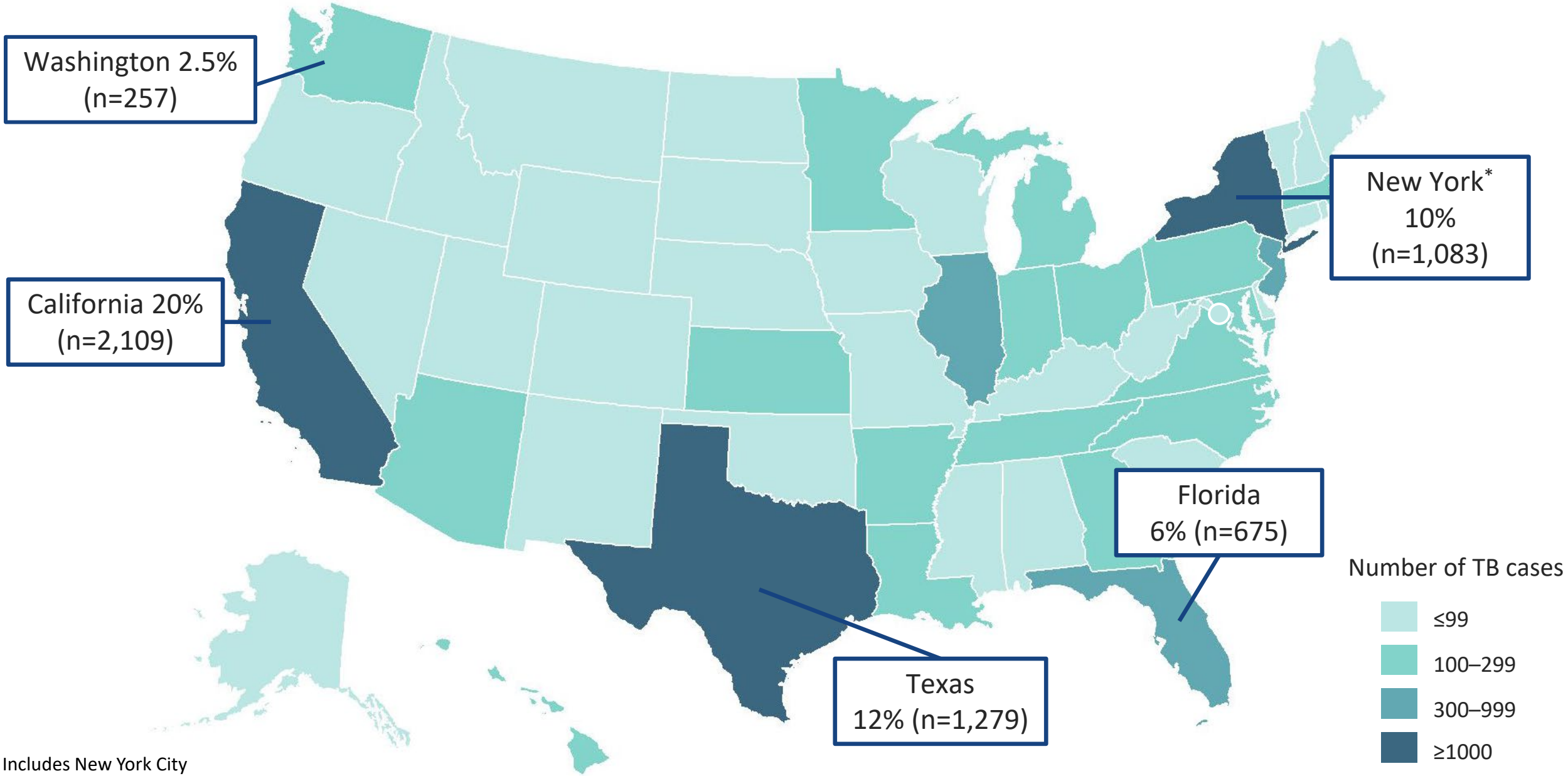
TB in Washington, Meet the People

Past – Why & How

Current – Workflow

Future – What's Next

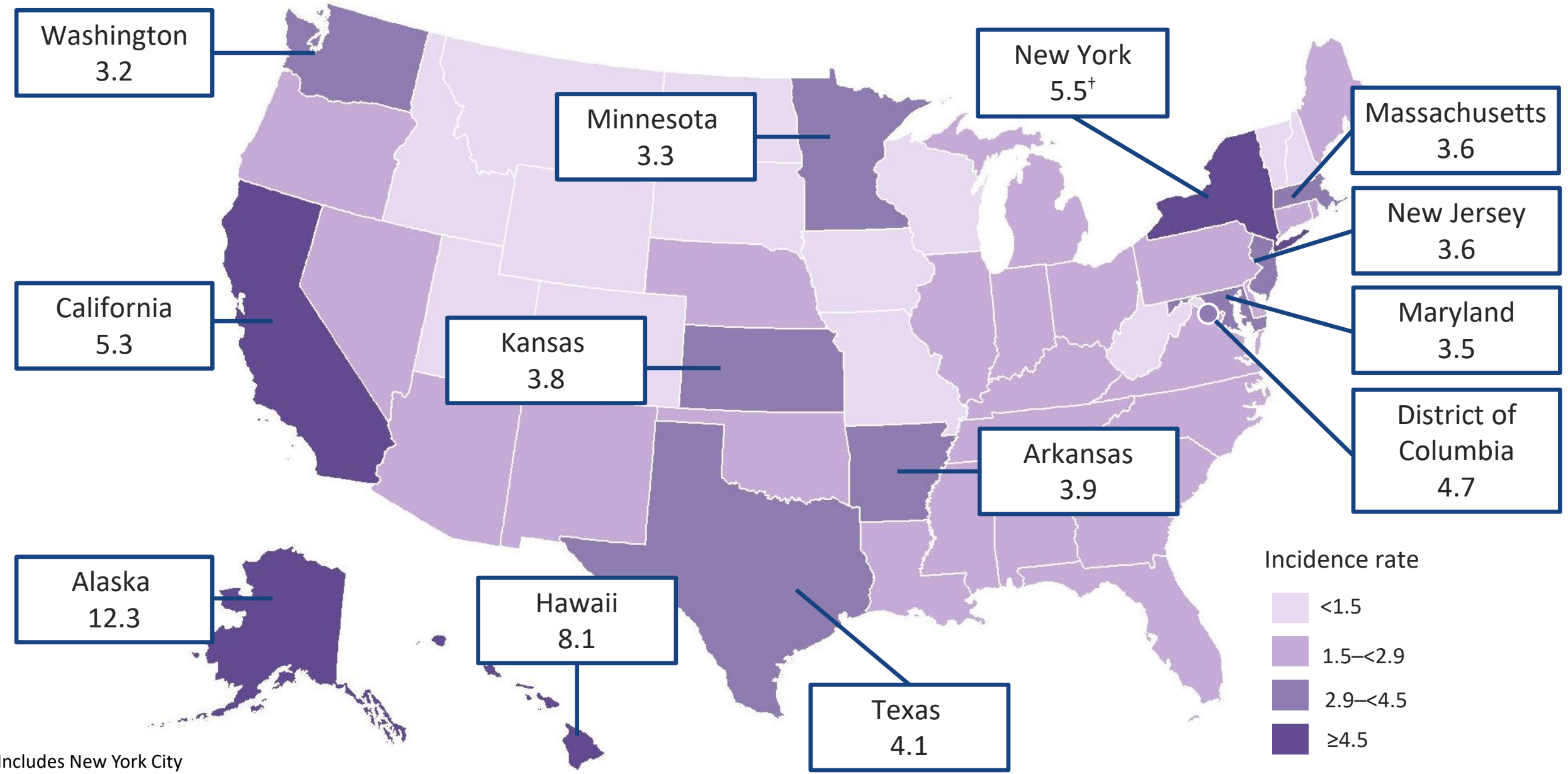
TB Cases and Percentages by Reporting Area, United States, 2024 (N=10,388)



* Includes New York City

Centers for Disease Control and Prevention (CDC). *Reported Tuberculosis in the United States, 2024.* Atlanta, GA: US Department of Health and Human Services, CDC; 2025

TB Incidence Rates by Reporting Area, United States, 2024 (per 100,000)



†Includes New York City

Centers for Disease Control and Prevention (CDC). *Reported Tuberculosis in the United States, 2024.* Atlanta, GA: US Department of Health and Human Services, CDC; 2025

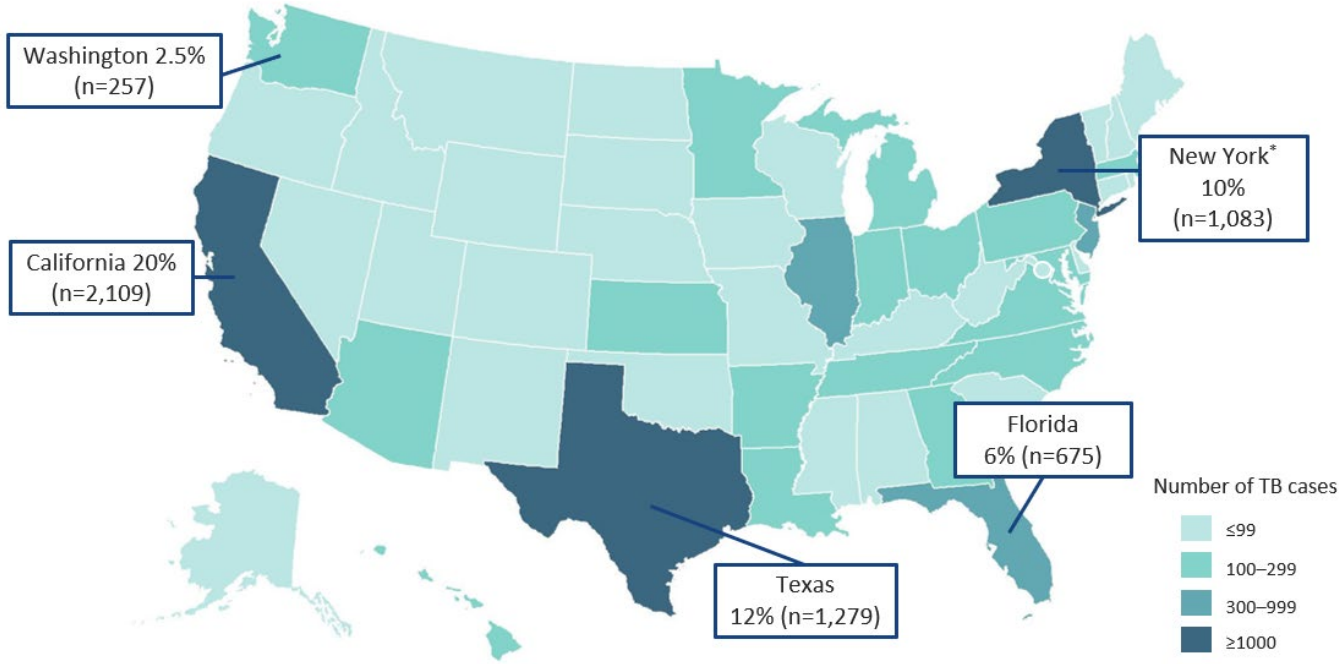
TB Laboratory Workload, 2022

	Washington State	National (Range)
Total number of clinical specimens processed for smear and culture	1,797	161,772 (5-17,214)
Number of individual patients (from above)	388	65,049 (3-9,952)

TB Laboratory Workload, 2022

	Washington State	National (Range)
Total number of clinical specimens processed for smear and culture	1,797	161,772 (5-17,214)
Number of individual patients (from above)	388	65,049 (3-9,952)

Number of Clinical Specimens Processed	Number of PHLs (% of Total)
1-1,000	16 (27.6%)
1,001-2,000	17 (29.3%)
2,001-4,000	13 (22.4%)
4,001-8,000	8 (13.8%)
>8,000	4 (6.9%)



WA Public Health Laboratory – TB Testing Service

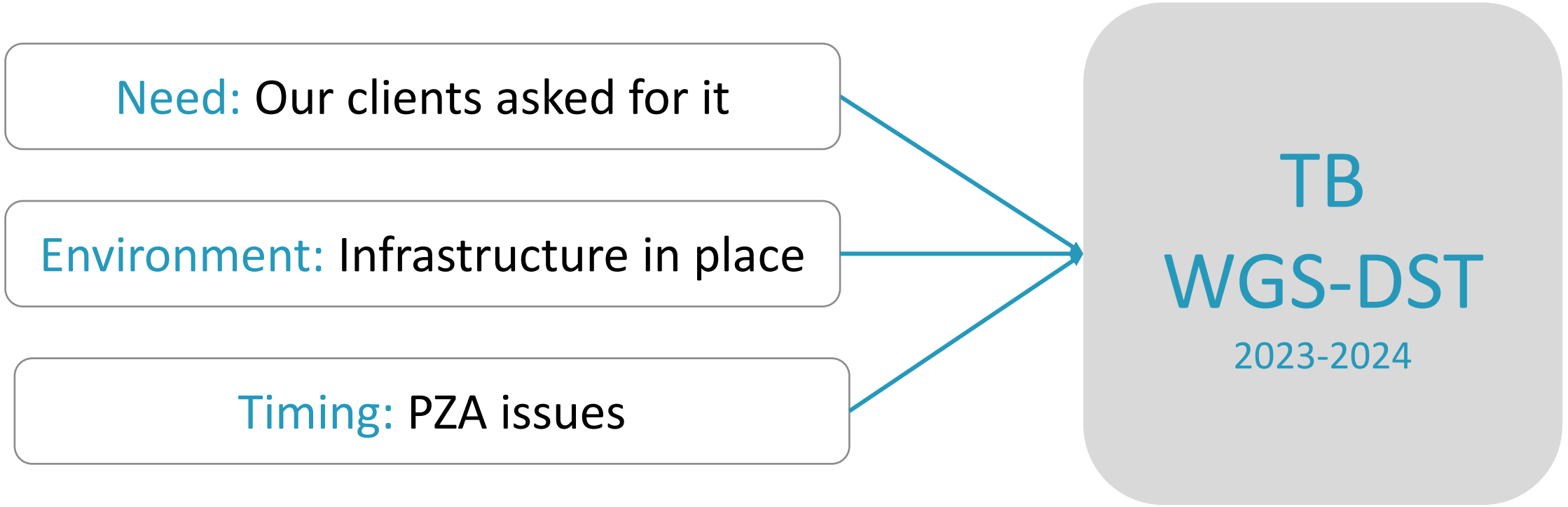
WA Public Health Laboratory – TB Testing Service

TB Lab (Microbiology)

Advanced Molecular Detection (AMD) Lab (Sequencing)

Computational (Bioinformatics)

Why Did We Implement TB WGS-DST?



WGS DST Validation

Selected 93 clinical isolates with variety of DST results:

Isoniazid (INH), Rifampin (RIF), Ethambutol (EMB), Pyrazinamide (PZA), Fluoroquinolones (FQ)

CDC analyzed existing* WGS for mutations in 19 loci:

ahpC	*embB*	*gyrA*	*mmpR*	*rrl*
eis	*ethA*	*gyrB*	*pepQ*	*rrs*
fabG1	*fabG1*	*inhA*	*pncA*	*tlyA*
eis	*atpE*	*katG*	*rpoB*	

*performed for genotyping

Grew isolates in-house, DNA extracted, sequenced, analyzed the 19 loci for mutations

WGS DST Validation

1) Comparison of all mutations observed per gene allele
2) Comparison of interpretations for drug susceptibility or resistance

Grew isolates in-house, DNA extracted, sequenced, analyzed the 19 loci for mutations

WGS DST Validation (cont)

Sequencing

- Illumina DNA Prep
- MiSeq platform using v3 600 cycles

Bioinformatics Analysis

- varpipe_wgs_1.02 (CDC)
- Sequence mapping
- Variant calling

Develop Report

- Variants of clinical significance
- Variant interpretation
- Engagement with submitters

Illumina Sequencing Run Metrics	
Cluster Density	V3 (1200-1900)
Recommended avg. Q30 (per organism)	>= 75%
Cluster passing filter	>= 80%

Bioinformatic Quality Assessment	
Minimum base quality	Q15
Average genome coverage depth	>= 30x
% reads mapping to reference genome	>=90%
% reference genome coverage	>=90%

WGS DST Validation (cont): How did we do in detecting?

Accuracy: Variant calling WAPHL vs CDC (indels, SNPs)

- True positive (CDC-R, WAPHL-R): all
- False positive (CDC-S, WAPHL-R): 2
- True negative (CDC-S, WAPHL-S): all
- False negative (CDC-R, WAPHL-S): 0

- Overall 99.8%
- Estimated sequencing error rate: 0.0002 (2/(113 sites x 93 isolates))

Sensitivity, Specificity

- [TP/(TP+FN)] X 100
- [TN/(TN+FP)] X 100

DRUG	GENE	SENSITIVITY	SPECIFICITY
Isoniazid	*ahpC* upstream	100	99.44%
	inhA	100	100%
	katG	100	100%
	fabG1	100	100%
	fabG1 upstream	100	100%
Ethionamide	*ethA*	100	100%
Ethambutol	*embB*	100	99.95%
Rifampin	*rpoB*	100	100%
Pyrazidamide	*pncA*	100	100%
Fluoroquinolones	*gyrA*	100	100%
	gyrB	100	100%
Second-line Injectables	*rrl*	100	100%
	rrs	100	100%
	tlyA	100	100%
	eis	100	100%
	eis upstream	100	100%
Bedaquiline	*mmpR*	100	100%
	pepQ	100	100%

WGS DST Validation (cont): How did we do in interpreting?

DRUG	CATEGORICAL AGREEMENT	PERCENTAGE AGREEMENT	mE	ME	VME	ERROR RATE, n%
Ethambutol	92	98.9	1	0	0	1.1
Fluoroquinolones	93	100	0	0	0	0
Isoniazid	92	98.9	1	0	0	1.1
Pyrazinamide	93	100	0	0	0	0
Rifampin	93	100	0	0	0	0

Categorical Agreement (CA): Total Susceptible, Resistant, Unknown

% Categorical Agreement: CA results / Total results

Errors: minor, major, very major

- Minor Error (mE): Of result difference due to Unknown call
- Major Error (ME): CDC=S, WAPHL=R (false resistance)
- Very Major Error (VME): CDC=R, WAPHL=S (false susceptible)

Reproducibility: 97.2% (*katG,* INH)

The Report

M. tuberculosis WGS Detection of Drug Resistance Report

Washington State Department of Health Public Health Laboratories
1610 NE 150th Street, Shoreline, WA 98155
Phone: 206-418-5400 | Email: StateTBlab@doh.wa.gov

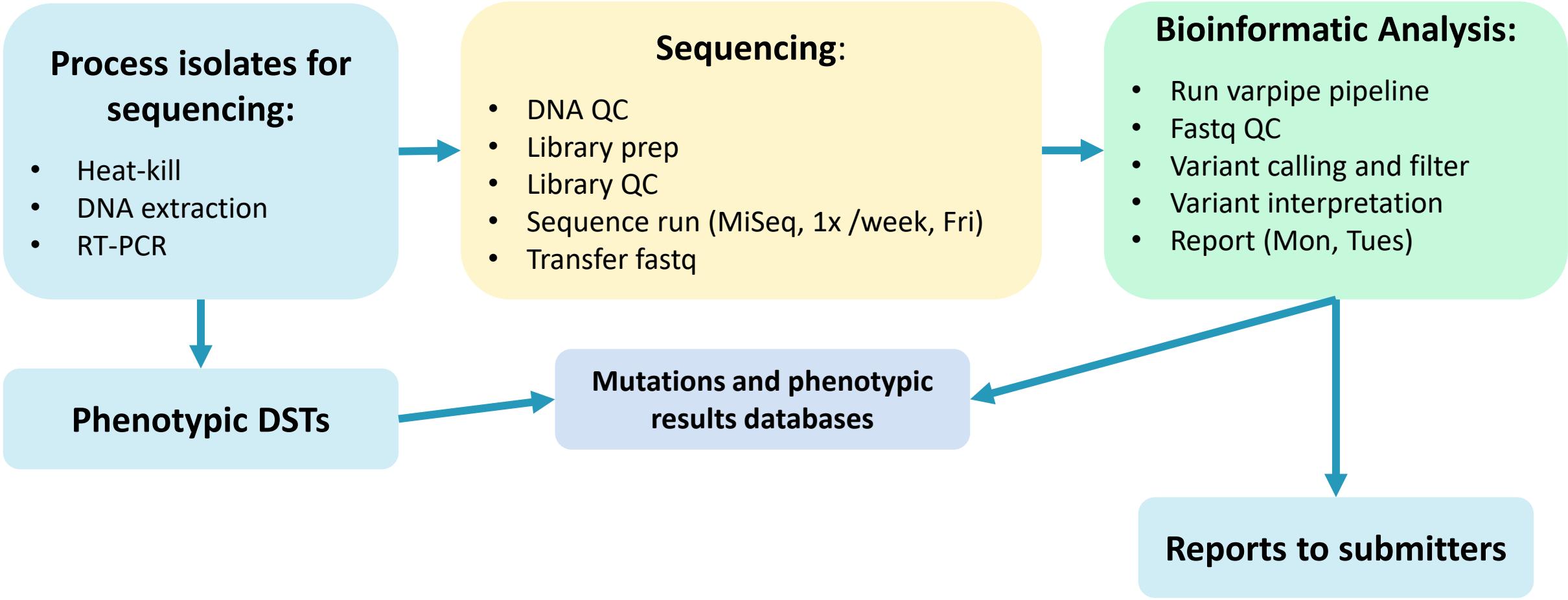
Report Date	Sample ID	Pipeline version
2025-10-31	WA1044808A	Varpipe_wgs_1.0.2

Patient First Name	Patient Last Name	DOB	Sample Collection Date	Specimen Source	LHJ	Submitter Name
Wile E.	Coyote	1983-05-07	1999-12-31	Sputum	Acme	Dr. Strangelove

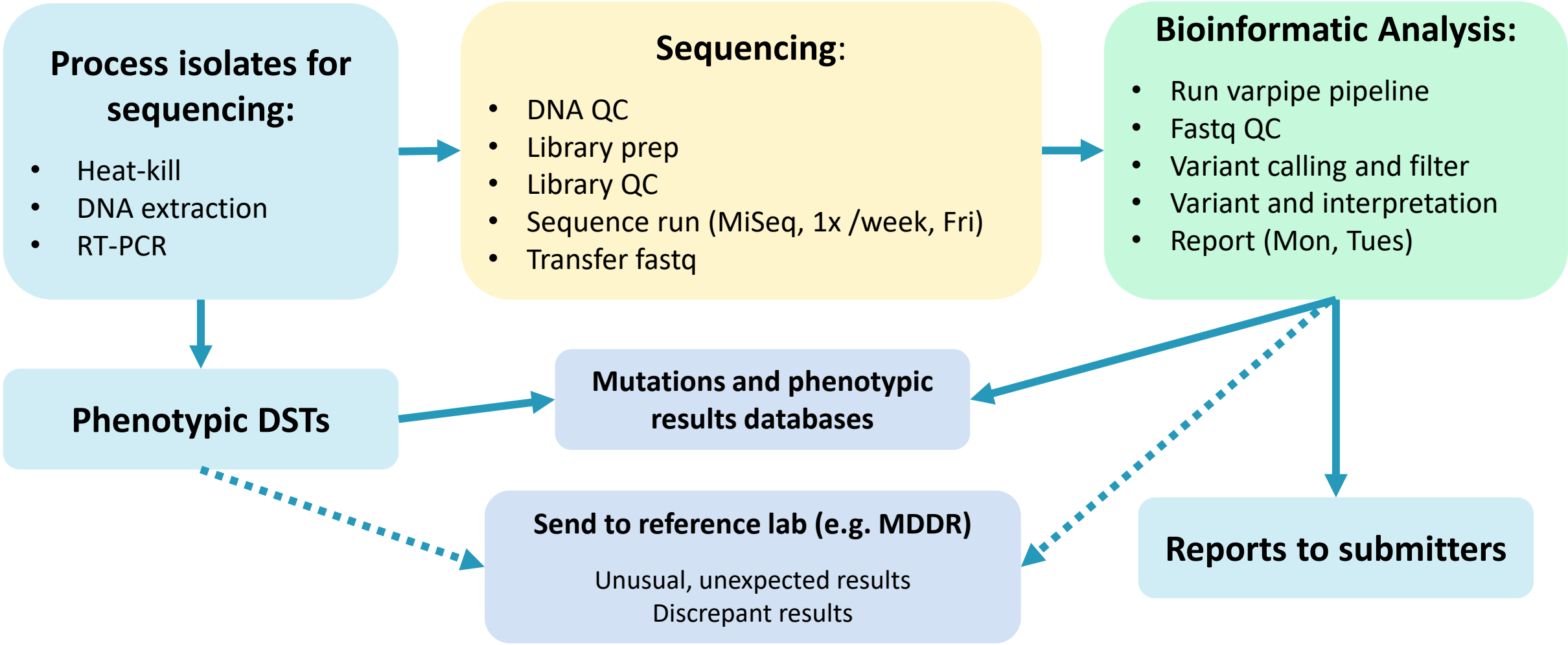
Drug	Loci Analyzed	Result	Drug Resistance Prediction
Ethambutol	embB	No reportable variant detected	Result does not confer resistance to Ethambutol.
Fluoroquinolone	**gyrA; gyrB**	**gyrA_p.Asp94Gly**	**Mutation expected to show resistance to Fluoroquinolone.**
Isoniazid	katG; inhA; fabG1; ahpC	No reportable variant detected	Result does not confer resistance to Isoniazid.
Pyrazinamide	pncA	No reportable variant detected	Result does not confer resistance to Pyrazinamide.
Rifampicin	rpoB	No reportable variant detected	Result does not confer resistance to Rifampicin.

This report was generated by whole genome sequencing analysis. Interpretations of mutations are based on published resistance data from the 2021 WHO catalogue of *Mycobacterium tuberculosis* complex mutations associated with drug resistance and from CDC Molecular Detection of Drug Resistance Service in Division of TB Elimination Laboratory Branch. Results reflect mutations that are associated with drug resistances.Expected reference range is "No reportable variant detected". Antibiotic susceptibility testing of isolates combined with whole genome sequencing results improves the accuracy of detecting antibiotic resistances. This test was adopted, and its performance characteristics determined by the WA PHL. It has not been approved by the FDA. Results are for infection control and epidemiological purposes and should not be used as the sole means for clinical diagnosis or patient management.

WGS DST Workflow



WGS DST Workflow



WGS-DST as Primary Method

Why?

- Turnaround-time
- Genotyping surveillance
- Scientific knowledge on resistance mechanisms (inc. MDR in WA)

WGS-DST as Primary Method

What would it take?

Statistics:

- Sufficient N to achieve 98.9%, ~700 isolates with variety of DST results

Cost:

- Reagents, staff

Infrastructure:

- Maintain pDST capability
- Maintain databases (mutations, phenotypic), paired with clinical treatment and outcomes
- Data storage, reporting, sharing across partners

Assessment:

- Methods, workflow
- Needs of clients

Collaboration:

- Engagement with clients for clinical decision-making and relevance (e.g. discrepancy between sequence-based and phenotypic; between labs)
- Standardization across labs

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WGS-DST as Primary Method

What would it take?

Results for Molecular Detection of Drug Resistance (Complete Panel); Conventional Drug Susceptibility Test in progress.

Drug	Locus *	Result	Interpretation
Rifampin	rpoB	No mutation	Probably Rifampin susceptible. (97% of RMP-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)
Isoniazid	inhA	No mutation	Cannot rule out INH resistance. (86% of INH-R isolates in our in-house evaluation of 550 clinical isolates have a mutation in locus inhA or locus katG.)
	katG	No mutation	
	fabG1	No mutation	

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Thank you Alaska State PHL – TB Lab!

2025 U.S. TB Elimination Champions