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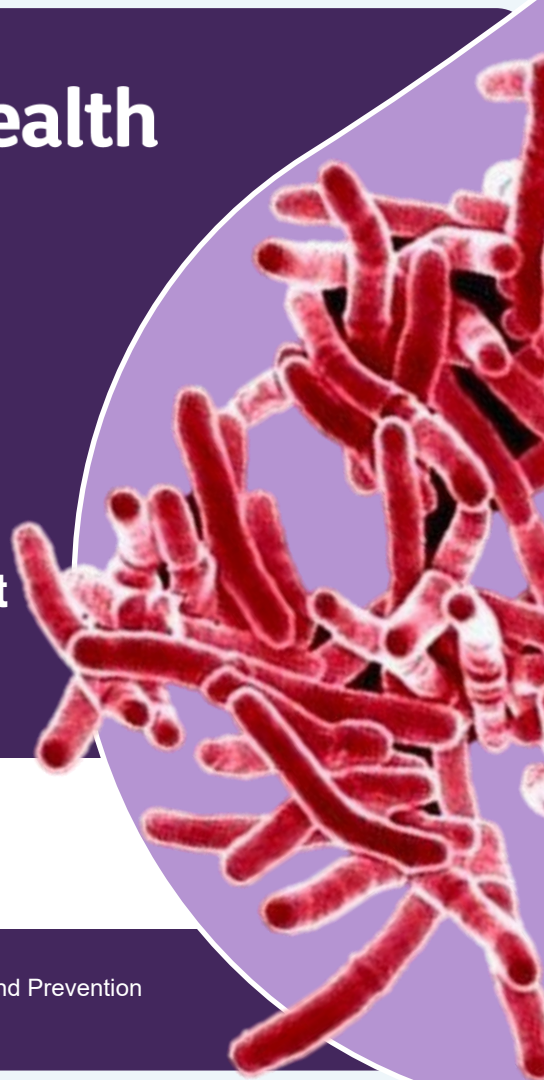
A Decade of Clinical NGS in the Mycobacteriology Lab:

Test Development to Clinical and Public Health Impact



Kimberlee Musser, PhD
Chief of Bacterial Disease
Wadsworth Center, NYSDOH

NACMID 2026 Annual Conference Sept. 21-22

Mycobacterium tuberculosis
Centers for Disease Control and Prevention
Public Health Image Library

Tuberculosis (TB) in New York

	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025
TB Cases	765	768	806	750	754	606	684	714	894	1,089	967
Rate*	3.9	4.0	4.2	3.9	3.9	3.1	3.4	3.5	4.4	5.4	4.8

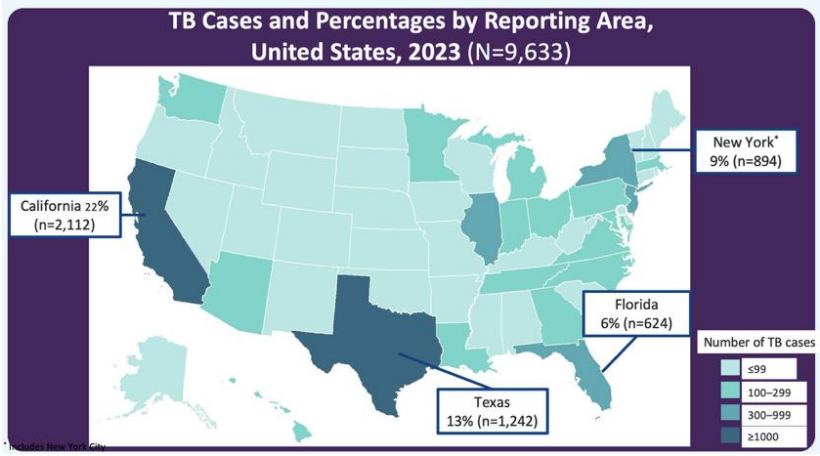
~8% Drug Resistant (DR-TB)

1-2% Multidrug Resistant (MDR-TB)

*Rate calculations are based on United States decennial Census data; per 100,000 population)

https://www.health.ny.gov/statistics/diseases/communicable/tuberculosis/docs/1960-current_cases_rates.pdf

We've come a long way!
1960 -7,075 cases (Rate 42.2)



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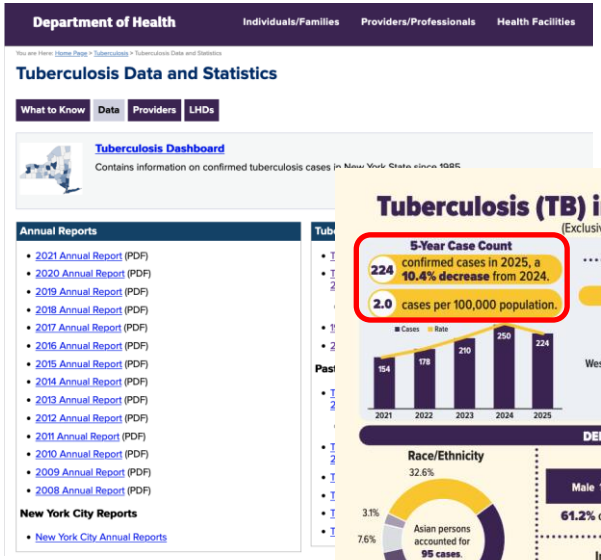
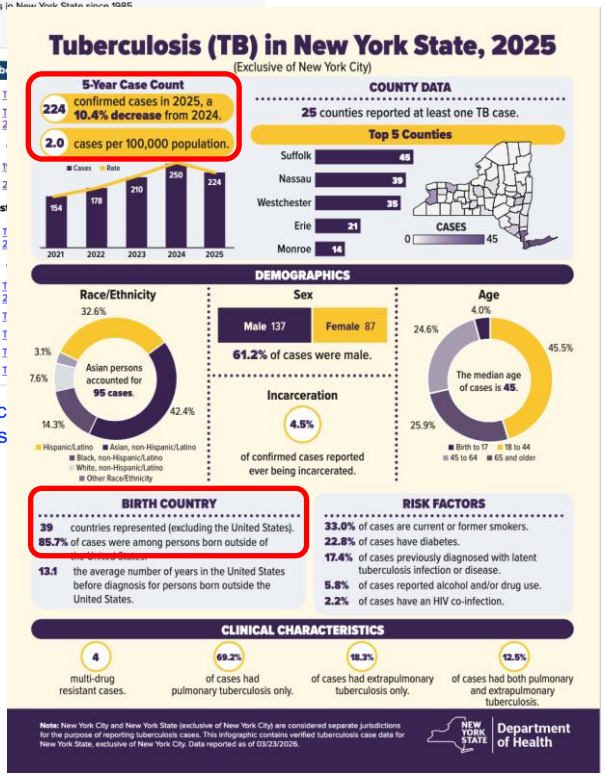
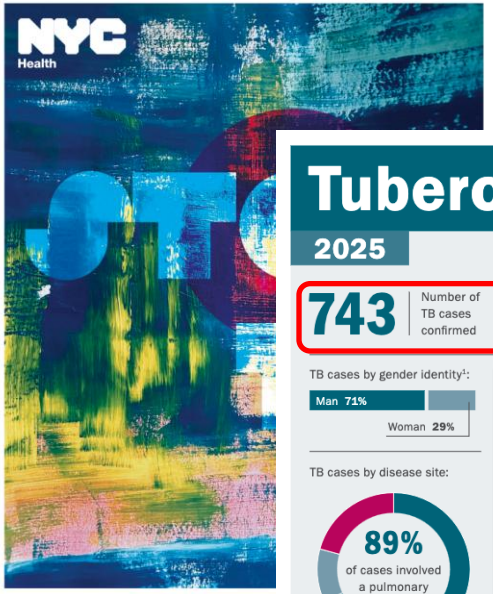
Microsoft Office Icons https://www.cdc.gov/tb/statistics/default.htm



https://www.health.ny.gov/statistic
eases/communicable/tuberculosis

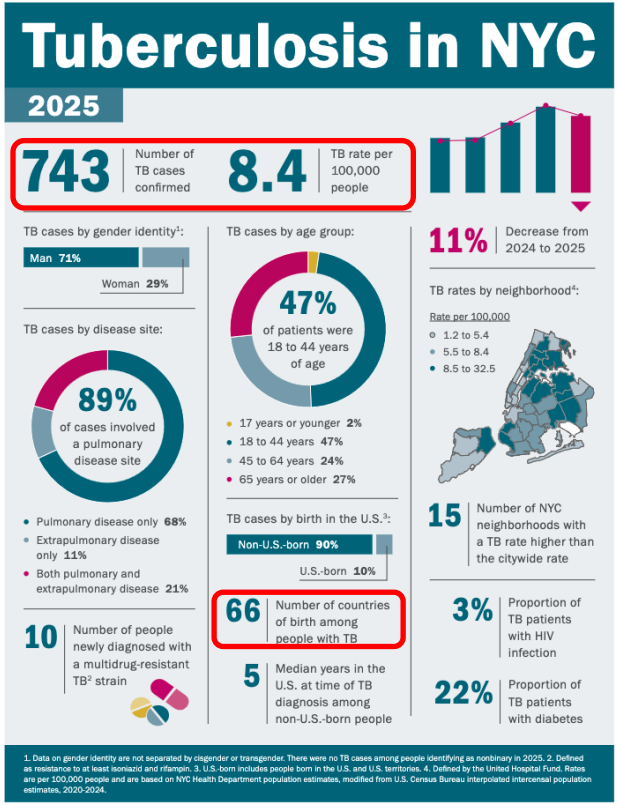


TB became a reportable disease on January 19, 1897



New York City Annual Tuberculosis (TB

https://www.nyc.gov/assets/
in-new-york-city-2025-annua



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Wadsworth Center, New York State Department of Health

Bacterial Disease Lab

- 5 Units including Mycobacteriology
- 60 Staff
- >100 Laboratory developed tests (LDTs)
- 10 Clinical next generation sequencing (NGS) tests

Notable features

- Largest US state public health laboratory
- 800 staff, 5 sites
- Basic and applied research
- Clinical Laboratory Evaluation Program (CLEP)
- In-house developed Laboratory Information Management System (CLIMS)

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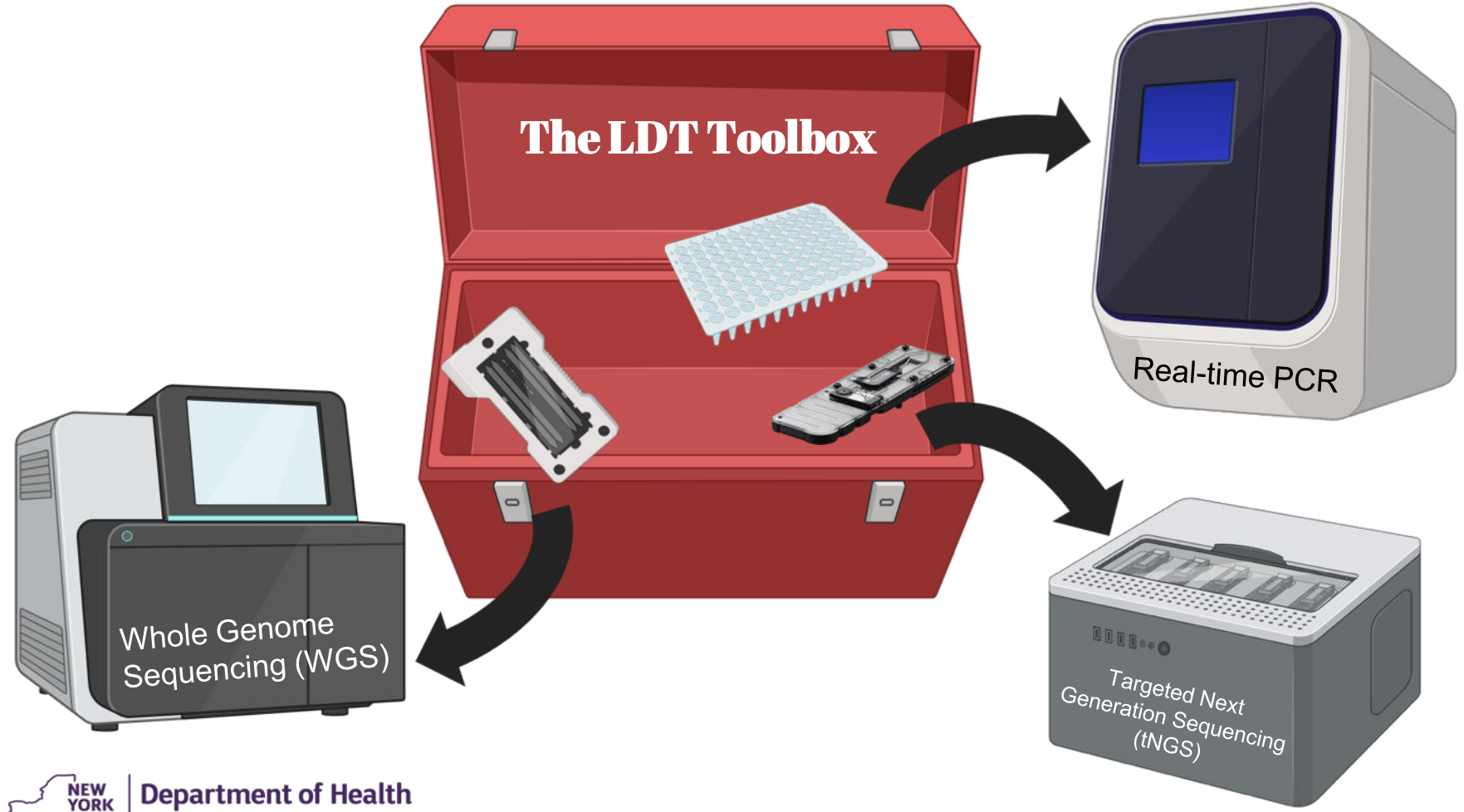
Wadsworth Center Images



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www.Biorender.com

Mycobacterium tuberculosis Testing Algorithm

1 CLINICAL TESTING – ISOLATE PATH

Real-time PCR MTBC & MAC

MTBC-Positive

Whole Genome Sequencing (WGS)

- MTBC Species
- Mutations-Drug resistance
- Lineage Spoligotype
- SNP Relatedness

- No Mutations Detected Final Report
- Mutations Detected Report

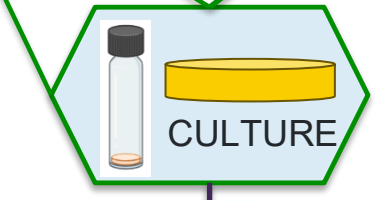
- DST 1st-Line Drugs
- DST 2nd-Line Drugs
- DST BPaL
- MIC testing

Heat-killed

2 CULTURE-BASED TESTING (ISOLATE)

Isolate

Specimen



Culture Testing:
- BACTEC MGIT™ 960
- Löwenstein-Jensen

DST Methods:
- MGIT™ DST
- MIC Testing

Processed, Decontaminated

Heat-killed

3 DIRECT SPECIMEN TESTING

Real-time PCR MTBC & MAC

MTBC-Positive

- ExtRD9 Ct ≤30 → **Targeted NGS** 13 Targets/ 9 Drugs
- ExtRD9 Ct >30 → **Targeted NGS** 5 Targets/ 3 Drugs

Mutations Detected Report

FINAL REPORTS INCLUDE

- MTBC Species Identification
- Lineage & Spoligotype
- SNP Relatedness
- Drug Resistance Mutations
- Phenotypic DST/ MIC Results

KEY FEATURES

- Integrated isolate & direct testing pathways
- WGS for comprehensive genomic insights
- Targeted NGS for rapid resistance detection
- Phenotypic DST & MIC for confirmation
- Actionable results to guide patient care & public health

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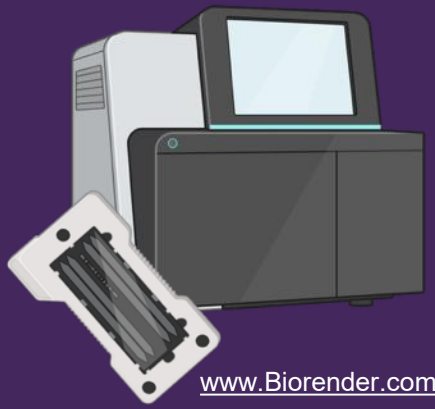
MTBC = *Mycobacterium tuberculosis* complex • MAC = *Mycobacterium avium* complex • SNP = Single Nucleotide Polymorphism
DST = Drug Susceptibility Testing • MIC = Minimum Inhibitory Concentration • BPaL regimen-bedaquiline, pretomanid, and linezolid
NGS = Next Generation Sequencing • Ct = Cycle Threshold

Background on MTBC WGS clinical testing

2013- Wadsworth Center Public Health Genomics Center (PHGC) funding announcement
2014- PHGC funding to test 60 *M. tuberculosis* isolates by whole genome sequencing (WGS)

Goals for TB WGS:

- Utilize as soon as possible in testing algorithm to impact patient treatment
- Expand molecular resistance prediction
- Provide more comprehensive results
- Mixed infections, heteroresistance, typing
- Assess costs and staff time



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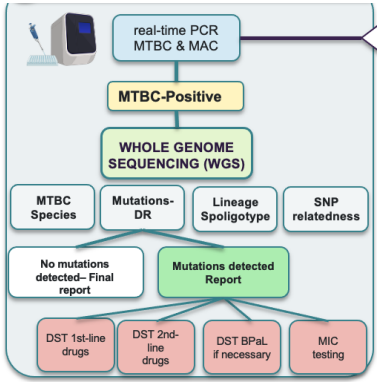
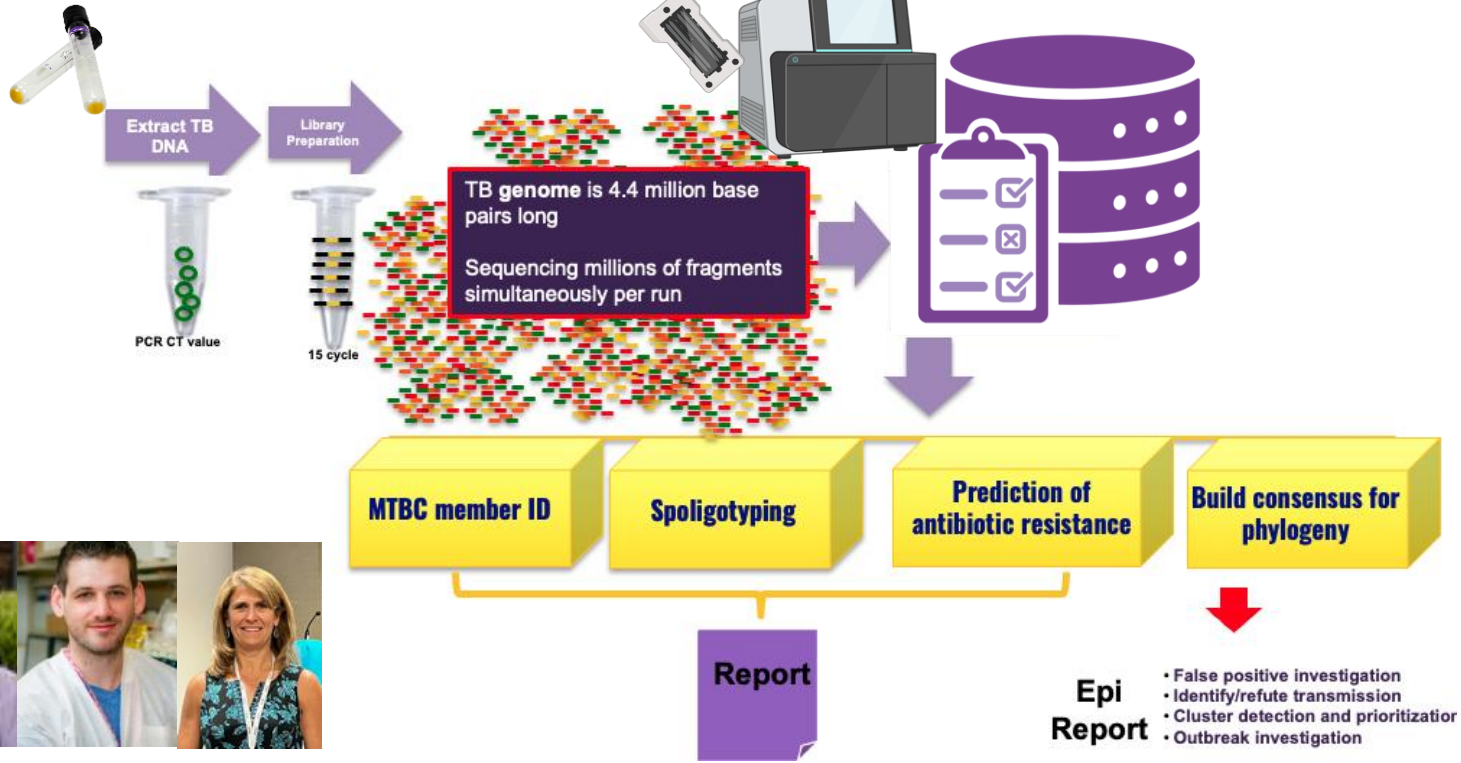
MTBC= *Mycobacterium tuberculosis* complex

MTBC WHOLE GENOME SEQUENCING





Pascal Lapierre, Vincent Escuyer, Joseph Shea, Tanya Halse

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High-confidence and future considerations mutations

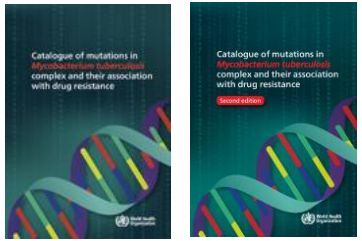
Currently

16 targets
9 drugs

Hundreds of mutations

Many more under evaluation

V6 pipeline

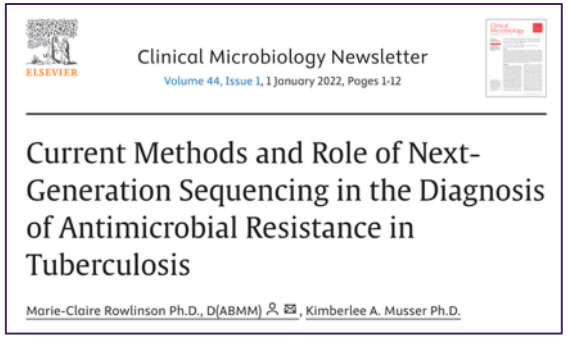


https://www.who.int/publications

Table 1. Wadsworth Center mutation list

Status	Antimicrobial/ drug/drug class	Status of mutation association with resistance		
		High confidence		Future consideration[a]
		Locus	Codon/NT change	Locus
High confidence	Rifampin (RIF)	*rpoB*	Val170Phe; Leu430Pro; Gln432Leu,Pro; Asp435Tyr,Val; Ser441Leu; His445Asn,Asp,Tyr,Arg,Leu; Ser450Leu,Trp,Cys,Phe; Leu452Pro; Ile491Phe	*rpoA, rpoC, Rv2752c*
	Isoniazid (INH)	*katG*	Gly121Asp; Trp191Gly; Gly279Asp, Ser315Ile,Asn,Thr,Arg; Thr394Pro,Ala; Gln525Pro; Any insertions/deletions	*mshA, ndh, Rv1258c, Rv2752c*
		oxyR-ahpC promoter region	C-81T	
		mabA promoter region	G-17T; C-15T; T-8A,C	
		mabA	CTG203CTA (Leu203Leu)	
		inhA	Ser94Ala	
	Pyrazinamide (PZA)	*pncA/pncA* promoter region	Any change (except Thr87Met or G-33A)	*clpC1, panD, Rv1258c, PPE35, Rv3236c*
	Ethambutol (EMB)	*embB*	Met306Leu, Val, Ile; Asp328Tyr; Asp354Ala; Gly406Ser, Asp, Ala, Cys; Gln497Arg	*embB, embC*
		embC-embA promoter region	C-12T	
	Kanamycin/amikacin (KAN/AMI)	*rrs*	A1401G	*eis, whiB7, whiB6, ccsA, fprA, aftB*
	Kanamycin (KAN)	*eis* promoter region	G-10A; G-37T	*rrs, eis, whiB7*
	Fluoroquinolones (FLQ)	*gyrA*	Ala74Ser; Gly88Cys; Ala90Val; Ser91Pro; Asp94Asn, His,Tyr, Gly, Ala	
		gyrB	Arg446Cys; Asn499Asp,Val	
	Ethionamide (ETH)	*mabA* promoter region	G-17T; C-15T; T-8A, C	*ethR, mshA, Rv3083, ndh*
		mabA	CTG203CTA	
		inhA	Ser94Ala	
		ethA	Any insertions/deletions	
Future consideration[b]	Bedaquiline (BDQ)	*pepQ, Rv0678, mmpL5, mmpS5, atpE*		
	Clofazimine (CFZ)	*pepQ, Rv0678, mmpL5, mmpS5*		
	Delamanid (DLM)	*fgd1, ddn, fbiA, fbiB, fbiC, Rv2983*		
	Linezolid (LZD)	*rplC, rrl*		

[a] Additional loci being evaluated or identified in the WHO mutation catalogue but not yet reported.
[b] Additional antimicrobials being evaluated but not yet reported.



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Received clinical approval in February 2016

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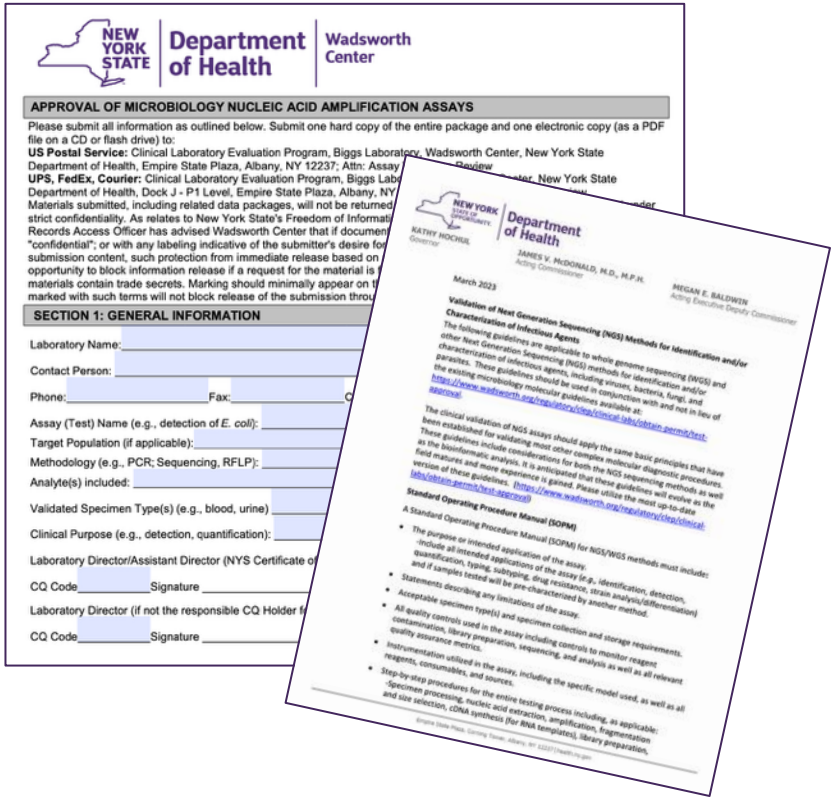
Clinical Validation and LDT Test Approval

SOP

- Intended use
- Limitations
- Step-by-step procedures
- Reports
- Interpretation
- Quality control (QC)
- Assay controls
- Proficiency Testing

Assay Validation

- Specificity
- Sensitivity
- Reproducibility: Intra- & Inter-assay
- Accuracy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APPROVAL OF MICROBIOLOGY NUCLEIC ACID AMPLIFICATION ASSAYS

Please submit all information as outlined below. Submit one hard copy of the entire package and one electronic copy (as a PDF file on a CD or flash drive) to:

US Postal Service: Clinical Laboratory Evaluation Program, Biggs Laboratory, Wadsworth Center, New York State Department of Health, Empire State Plaza, Albany, NY 12237; Attn: Assay Review

UPS, FedEx, Courier: Clinical Laboratory Evaluation Program, Biggs Laboratory, New York State Department of Health, Dock J - P1 Level, Empire State Plaza, Albany, NY

Materials submitted, including related data packages, will not be returned

strict confidentiality. As relates to New York State's Freedom of Information

Records Access Officer has advised Wadsworth Center that if document

"confidential"; or with any labeling indicative of the submitter's desire for

submission content, such protection from immediate release based on

opportunity to block information release if a request for the material is

materials contain trade secrets. Marking should minimally appear on t

marked with such terms will not block release of the submission throu

SECTION 1: GENERAL INFORMATION

Laboratory Name:

Contact Person:

Phone: Fax:

Assay (Test) Name (e.g., detection of *E. coli*):

Target Population (if applicable):

Methodology (e.g., PCR; Sequencing, RFLP):

Analyte(s) included:

Validated Specimen Type(s) (e.g., blood, urine)

Clinical Purpose (e.g., detection, quantification):

Laboratory Director/Assistant Director (NYS Certificate of

CQ Code Signature

Laboratory Director (if not the responsible CQ Holder f

CQ Code Signature

NEW YORK STATE | Department of Health

KATHY HOCHUL, Governor

JAMES V. McDONALD, M.D., M.P.H., Acting Commissioner

MEGAN E. BALDWIN, Acting Executive Deputy Commissioner

March 2023

Validation of Next Generation Sequencing (NGS) Methods for Identification and/or Characterization of Infectious Agents

The following guidelines are applicable to whole genome sequencing (WGS) and other Next Generation Sequencing (NGS) methods for identification and/or characterization of infectious agents, including viruses, bacteria, fungi, and parasites. These guidelines should be used in conjunction with and not in lieu of the existing microbiology molecular guidelines available at: https://www.wadsworth.org/regulatory/clep/clinical-lab/obtain-permit/test-approval

The clinical validation of NGS assays should apply the same basic principles that have been established for validating most other complex molecular diagnostic procedures. These guidelines include considerations for both the NGS sequencing methods as well as the bioinformatic analysis. It is anticipated that these guidelines will evolve as the field matures and more experience is gained. Please utilize the most up-to-date version of these guidelines. (https://www.wadsworth.org/regulatory/clep/clinical-lab/obtain-permit/test-approval)

Standard Operating Procedure Manual (SOPM)

A Standard Operating Procedure Manual (SOPM) for NGS/WGS methods must include:

- The purpose or intended application of the assay.
  - Include all intended applications of the assay (e.g., identification, detection, quantification, typing, subtyping, drug resistance, strain analysis/differentiation) and if samples tested will be pre-characterized by another method.
- Statements describing any limitations of the assay.
- Acceptable specimen type(s) and specimen collection and storage requirements.
- All quality controls used in the assay including controls to monitor reagent contamination, library preparation, sequencing, and analysis as well as all relevant quality assurance metrics.
- Instrumentation utilized in the assay, including the specific model used, as well as all reagents, consumables, and sources.
- Step-by-step procedures for the entire testing process including, as applicable: specimen processing, nucleic acid extraction, amplification, fragmentation and size selection, cDNA synthesis (for RNA templates), library preparation,

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SOP= Standard Operating Procedure

https://www.wadsworth.org/sites/default/files/WebDoc/Microbiology_NAAT_Checklist_0211.pdf

New Approach with WGS as First-line DST

Tuberculosis 142 (2023) 102380

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: www.elsevier.com/locate/tube

Mycobacterium tuberculosis complex whole-genome sequencing in New York State: Implementation of a reduced phenotypic drug susceptibility testing algorithm

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[a] *Wadsworth Center, New York State Department of Health, Albany, NY, USA*
[b] *New York City Bureau of Tuberculosis Control, New York City, NY, USA*
[c] *New York State Department of Health, Albany, NY, USA*
[d] *Public Health Laboratory, New York City Department of Health and Mental Hygiene, New York City, NY, USA*

Collaboration!

Shea *et al.*, Tuberculosis, 2023
DST= Drug Susceptibility Testing

New Approach with WGS as First-line DST

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[d] Public Health Laboratory, New York City Department of Health and Mental Hygiene, New York City, NY, USA

- Communication with State and City TB Control Epidemiologists
- Health Commerce System Advisories
- Local Health Departments

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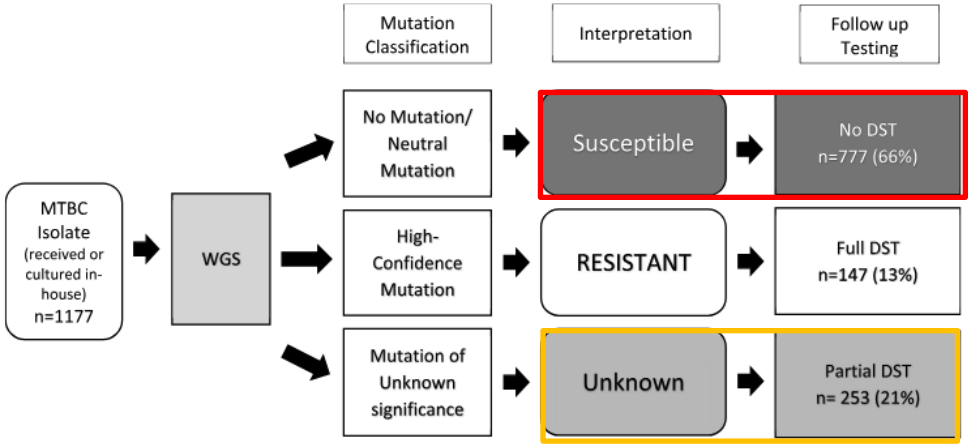

Drug	Genes with High-Confidence Mutations for 'Resistant' predictions	Genes for 'Unknown' predictions
Rifampin (RIF)	*rpoB*	*rpoB*
Isoniazid (INH)	*katG, inhA, mabA-inhA, mabA, oxyR-ahpC*	*katG, inhA, mabA-inhA, mabA, oxyR-ahpC, ndh* & promoter, *furA-katG*
Ethambutol (EMB)	*embB, embC-embA*	*embB, embC-embA*
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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Shea *et al.*, Tuberculosis, 2023

REPORT

Clinical Mycobacteriology Laboratory
Phone: (518) 474-4158 Fax: (518) 408-2264

Testing performed at CLIA# 33D2005937

Specimen Id: IDR1800000381

Direct Molecular Detection - Real-time PCR

Mycobacterium tuberculosis complex DNA by real-time PCR*:	**DETECTED**
Mycobacterium avium complex DNA by real-time PCR*:	**Not Detected**

Molecular Identification and Susceptibility Testing - Whole Genom

Molecular Identification*:	**Mycobacterium**

Susceptibility Profile*

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

High-confidence mutations detected*

rpoB (RIF):	**Mutation preser**
katG (INH):	**Mutation preser**

Updated Report following reduced DST

Susceptibility Profile*

Rifampin (RIF):	**Susceptible**	11/12/2019
Isoniazid (INH):	**Unknown**	11/12/2019
Pyrazinamide (PZA):	**Susceptible**	11/12/2019
Ethambutol (EMB):	**Susceptible**	11/12/2019
Streptomycin (SM):	**Susceptible**	11/12/2019
Kanamycin/Amikacin (KAN/AMI):	**Susceptible**	11/12/2019
Kanamycin (KAN):	**Susceptible**	11/12/2019
Fluoroquinolones (FLQ):	**Susceptible**	11/12/2019
Ethionamide (ETH):	**Susceptible**	11/12/2019

High-confidence mutations detected*

katG (INH):	**No high-confidence mutation**	11/12/2019
[Note]	An Asp729Asn mutation is present but its association with isoniazid resistance is unknown. This mutation is not considered a high-confidence mutation.	

Susceptibility Testing for M. tuberculosis complex (MGIT)

	Isoniazid [0.1 ug/ml]:	**PENDING**
new	Isoniazid [0.4 ug/ml]:	**PENDING**

* The performance characteristics of this test were determined by the Wadsworth Center. It has not been cleared or approved by the U.S. Food and Drug Administration.

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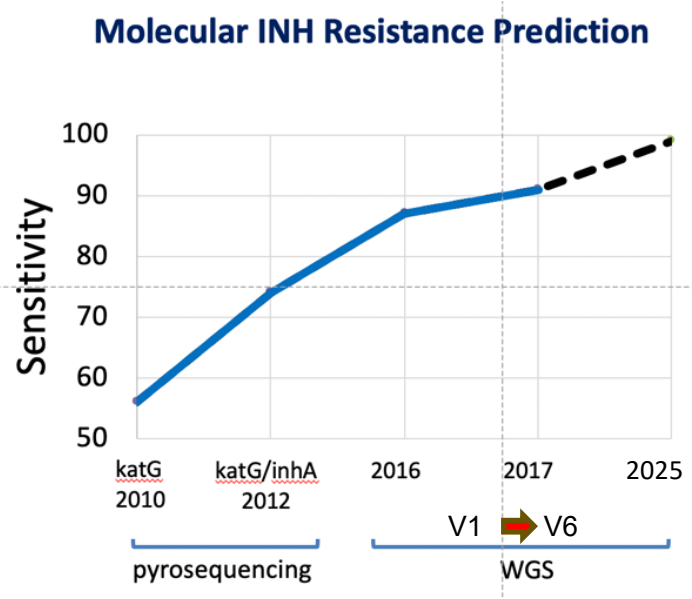
MTBC WGS Performance Characteristics with Culture-based DST as the Gold Standard

	EMB	FLQ	INH	PZA	RIF	SM	KAN	ETH	ALL
Specificity	1.00	1.00	1.00	0.98	0.98	1.00	1.00	0.98	0.99
Susceptibility-PV	1.00	0.99	0.98	0.98	1.00	0.92	0.99	0.91	0.98
n	1961	625	1993	2021	1996	1463	501	581	11141

Wadsworth Center Data

Almost 8000 MTBC Genomes to date



EMB= ethambutol
FLQ= fluoroquinolones
INH= isoniazid
PZA= pyrazinamide
RIF= rifampin
SM= streptomycin
KAN/AMI= kanamycin/amikacin
ETH= ethionamide

DST= Drug Susceptibility Testing

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Wadsworth Center Data

10 years of Wadsworth Center MTBC WGS

What can we learn?

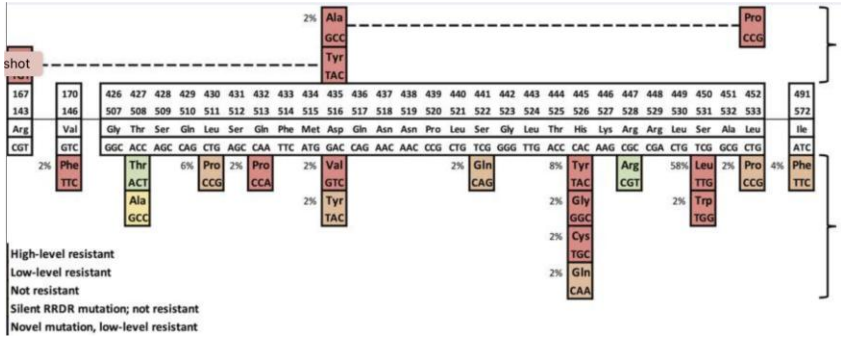
- Improved understand of drug resistance mutations
- Improved data on low-level drug resistance
- Advances in interpreting SNP thresholds for relatedness
- Assessments of drug resistance prevalence and trends over time
- Evaluations of Clusters of TB disease over time
- Improved understanding of TB lineage and sublineage on drug resistance and relatedness

Microsoft Office Icons

Low-level Rifampin Resistance

Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP 2020):

"...isolates with mutations conferring low-level RMP resistance may test as susceptible with growth-based drug susceptibility methods.



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MYCOBACTERIOLOGY AND AEROBIC ACTINOMYCETES

Low-Level Rifampin Resistance and *rpoB* Mutations in *Mycobacterium tuberculosis*: an Analysis of Whole-Genome Sequencing and Drug Susceptibility Test Data in New York

Joseph Shea,[a] Tanya A. Halse,[a] Donna Kohlerschmidt,[a] Pascal Lapierre,[a] Herns A. Modestil,[b] Cheryl H. Kearns,[d]

WHO announces updated critical concentrations for susceptibility testing to rifampicin

5 February 2021 | Departmental news | Geneva |Reading time: Less than a minute (233 words)

The critical concentrations for culture-based phenotypic drug susceptibility testing (DST) to first-line anti-TB drugs have been revised by the the World Health Organization (WHO). Critical concentrations for rifampicin have been lowered while those for isoniazid have been maintained at the present level. This update helps address the discordance observed between phenotypic and molecular methods to detect rifampicin resistance and improves the accuracy of DST. As a result patients with TB will have a more accurate diagnosis.

The revision was the outcome of a Technical Expert Group meeting convened by WHO in 2020 to assess the results of a systematic review of published literature on critical concentrations for DST of the most important first-line anti-TB drugs, isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine). New evidence showed that critical concentrations used for phenotypic methods to detect rifampicin resistance may incorrectly classify strains with certain mutations. The following media were considered: Löwenstein-Jensen (LJ), Middlebrook 7H10 (7H10), Middlebrook 7H11 (7H11) and BACTEC™ Mycobacterial Growth Indicator Tube™ 960 (MGIT).

Isoniazid Resistance

Predicting Isoniazid Resistance in *Mycobacterium tuberculosis* Complex in New York State using Whole Genome Sequencing

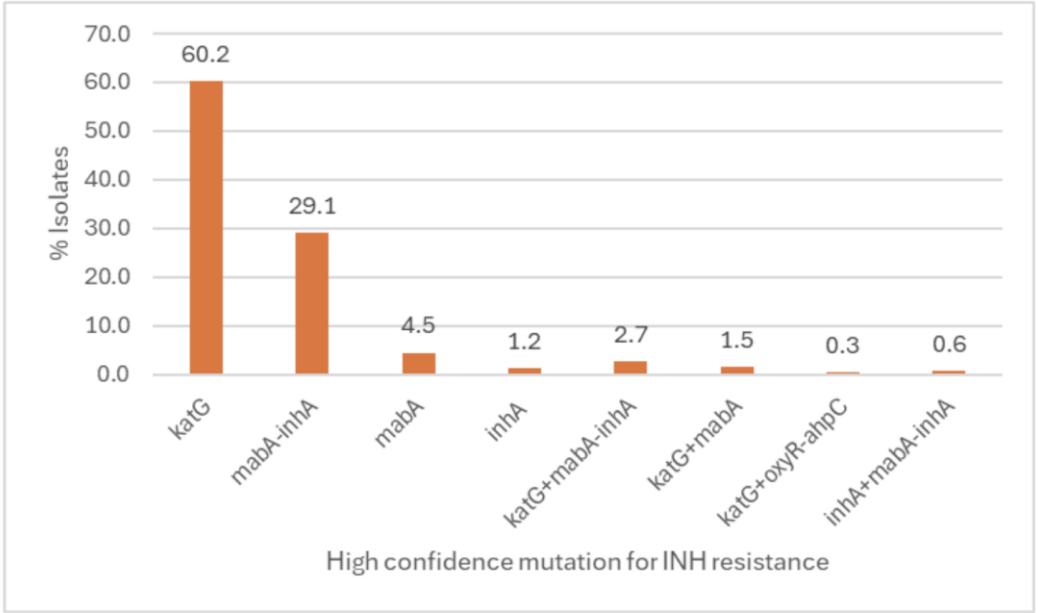
Kruthika Patel, Joseph Shea, Pascal Lapierre, Tanya A. Halse, Donna Kohlerschmidt, Michelle Dickinson, Vincent Escuyer, Kimberlee A. Musser

doi: https://doi.org/10.1101/2025.10.30.685518

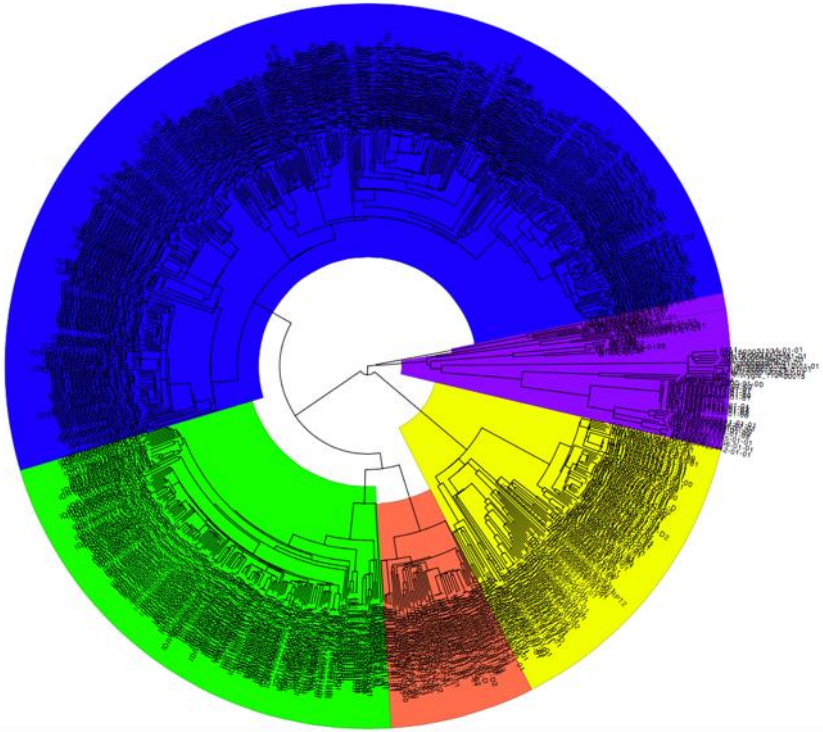
Table 7: Isoniazid Resistance and Mutation Pre

Lineage	No. of Strains (%)
L1	531 (14.4)
L2	526 (22.3)
L3	312 (8.4)
L4	1892 (51.2)
M. bovis BCG	52 (0.01)
M. bovis	37 (0.01)
M. africanum	34 (0.009)
M. orygis	6 (0.002)
L7	1 (0.0003)
La4	1 (0.0003)
Mix of 2 Lineages	1 (0.0003)
M. caprae	1 (0.0003)
Total	3696

Figure 1: Proportion of resistance mutations in each gene/gene region in INH-resistant MTBC isolates (n=337)



MTBC WGS -Added Bonus-Relatedness Analysis



Euro-American (53%)

Beijing (23%)

Central Asian Strain (CAS) (6.3%)

East African-Indian (EAI) (13.3%)

Other species (4.4%)

Cluster= 2 or more TB WGS that are ~~≤10~~ SNPs <=10

→ Epi Report

≤ 5 SNP= Recent Transmission

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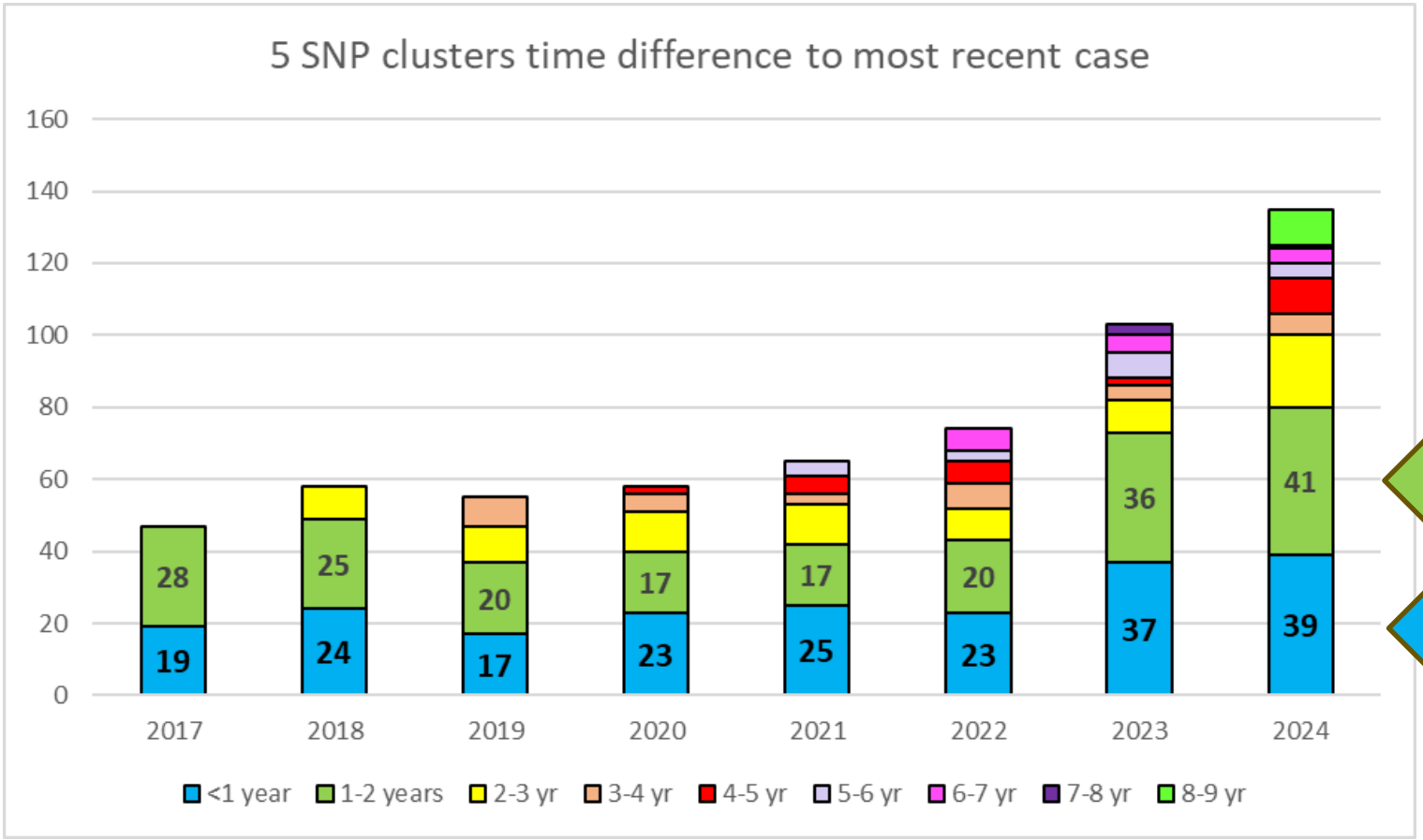
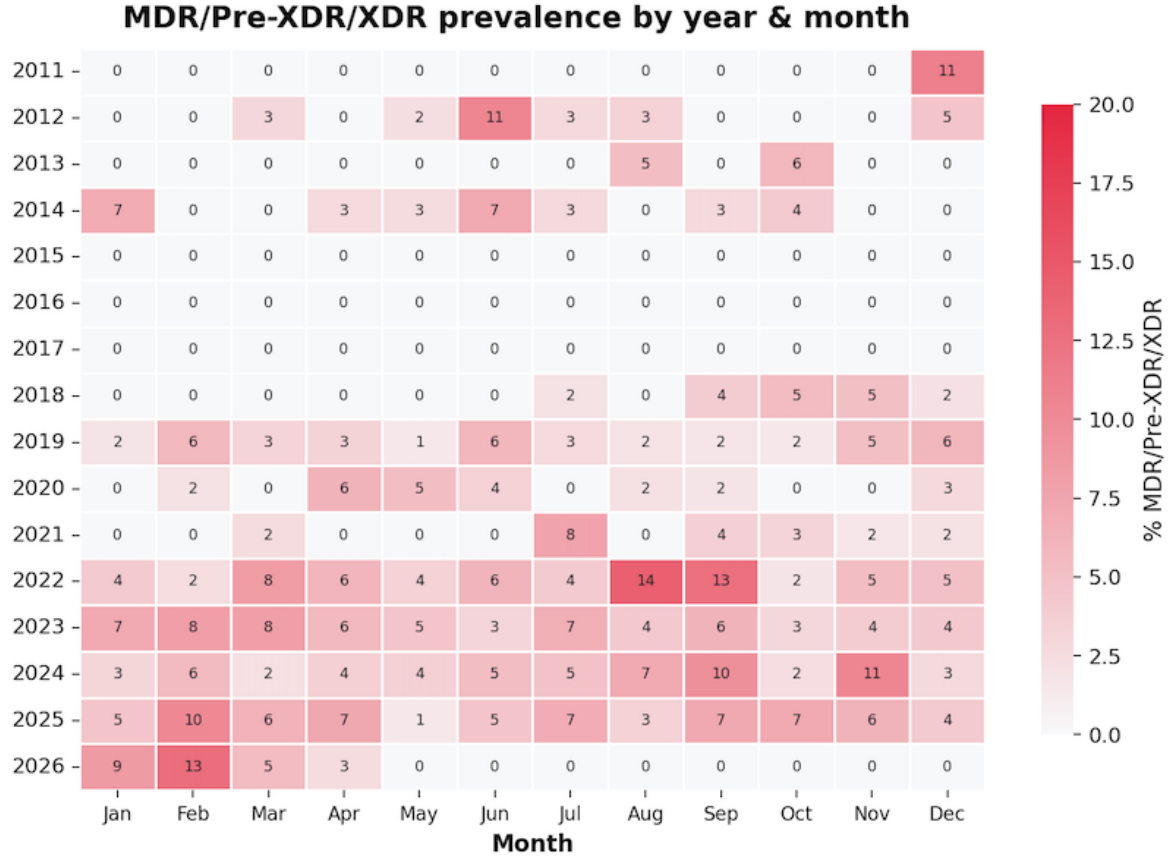
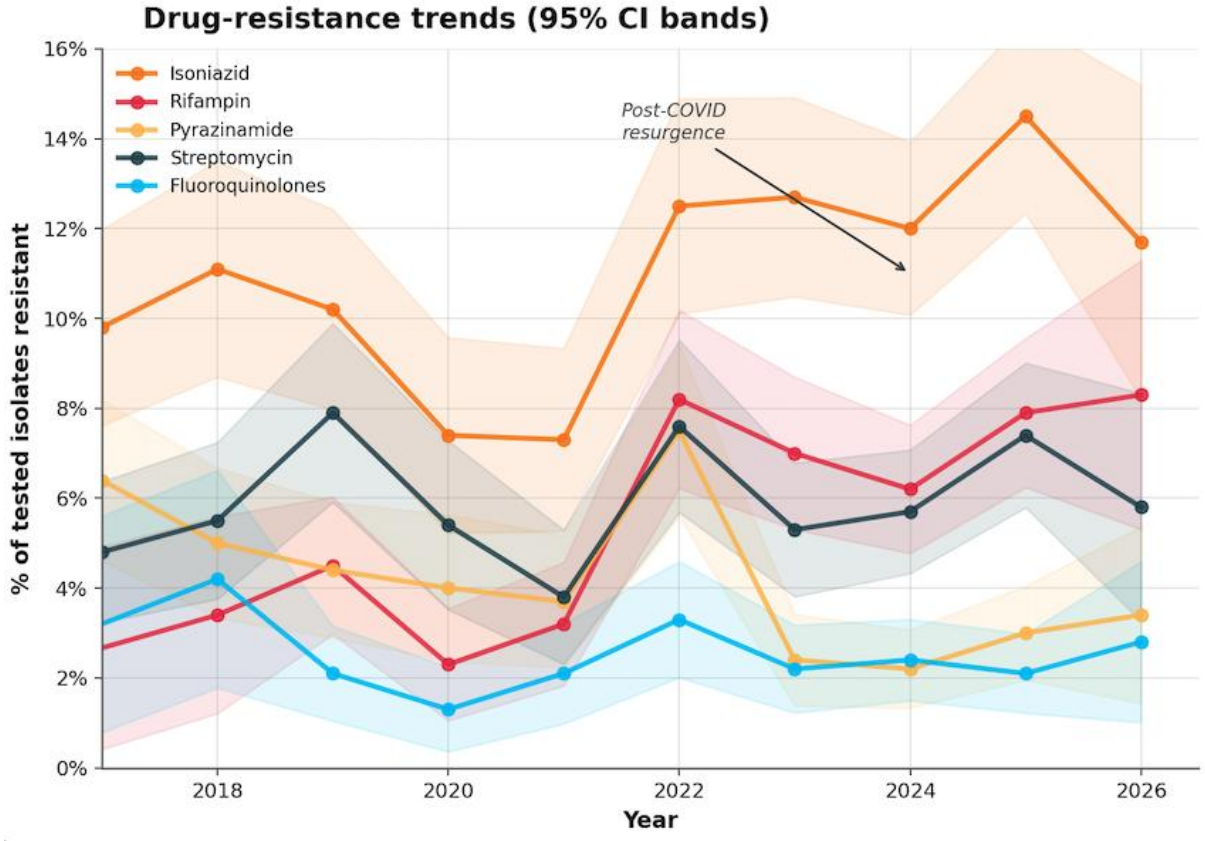
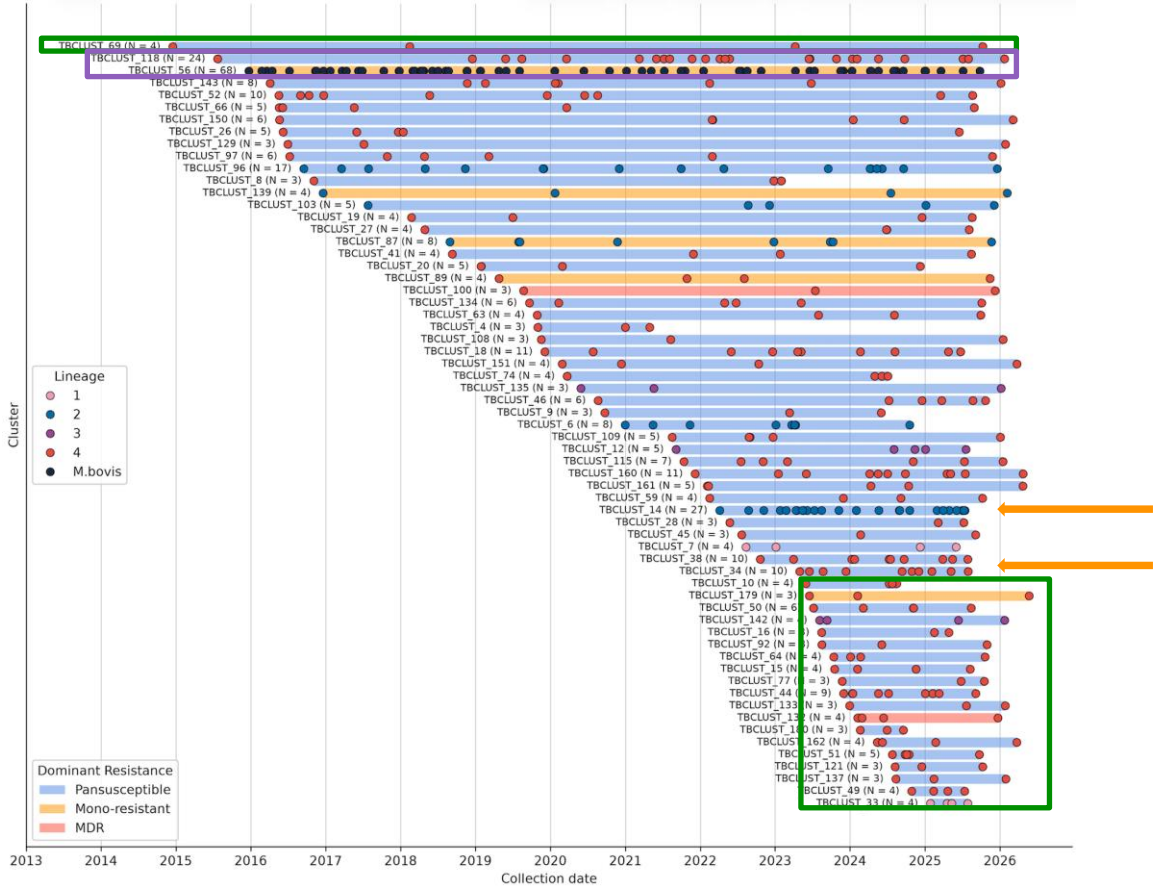
SNP= single nucleotide polymorphism


5 SNP clusters time difference to most recent case
160
140
120
100
80
60
40
20
0
2017
2018
2019
2020
2021
2022
2023
2024
19
28
24
25
17
20
23
17
25
17
23
20
37
36
39
41
<1 year
1-2 years
2-3 yr
3-4 yr
4-5 yr
5-6 yr
6-7 yr
7-8 yr
8-9 yr


MDR/Pre-XDR/XDR prevalence by year & month
2011 0 0 0 0 0 0 0 0 0 0 0 11
2012 0 0 3 0 2 11 3 3 0 0 0 5
2013 0 0 0 0 0 0 0 5 0 6 0 0
2014 7 0 0 3 3 7 3 0 3 4 0 0
2015 0 0 0 0 0 0 0 0 0 0 0 0
2016 0 0 0 0 0 0 0 0 0 0 0 0
2017 0 0 0 0 0 0 0 0 0 0 0 0
2018 0 0 0 0 0 0 2 0 4 5 5 2
2019 2 6 3 3 1 6 3 2 2 2 5 6
2020 0 2 0 6 5 4 0 2 2 0 0 3
2021 0 0 2 0 0 0 8 0 4 3 2 2
2022 4 2 8 6 4 6 4 14 13 2 5 5
2023 7 8 8 6 5 3 7 4 6 3 4 4
2024 3 6 2 4 4 5 5 7 10 2 11 3
2025 5 10 6 7 1 5 7 3 7 7 6 4
2026 9 13 5 3 0 0 0 0 0 0 0 0
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
% MDR/Pre-XDR/XDR
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0


Drug-resistance trends (95% CI bands)
Isoniazid
Rifampin
Pyrazinamide
Streptomycin
Fluoroquinolones
Post-COVID resurgence
% of tested isolates resistant
16%
14%
12%
10%
8%
6%
4%
2%
0%
2018
2020
2022
2024
2026
Year

TB Clusters Through Time in NYS (≤10 SNP)



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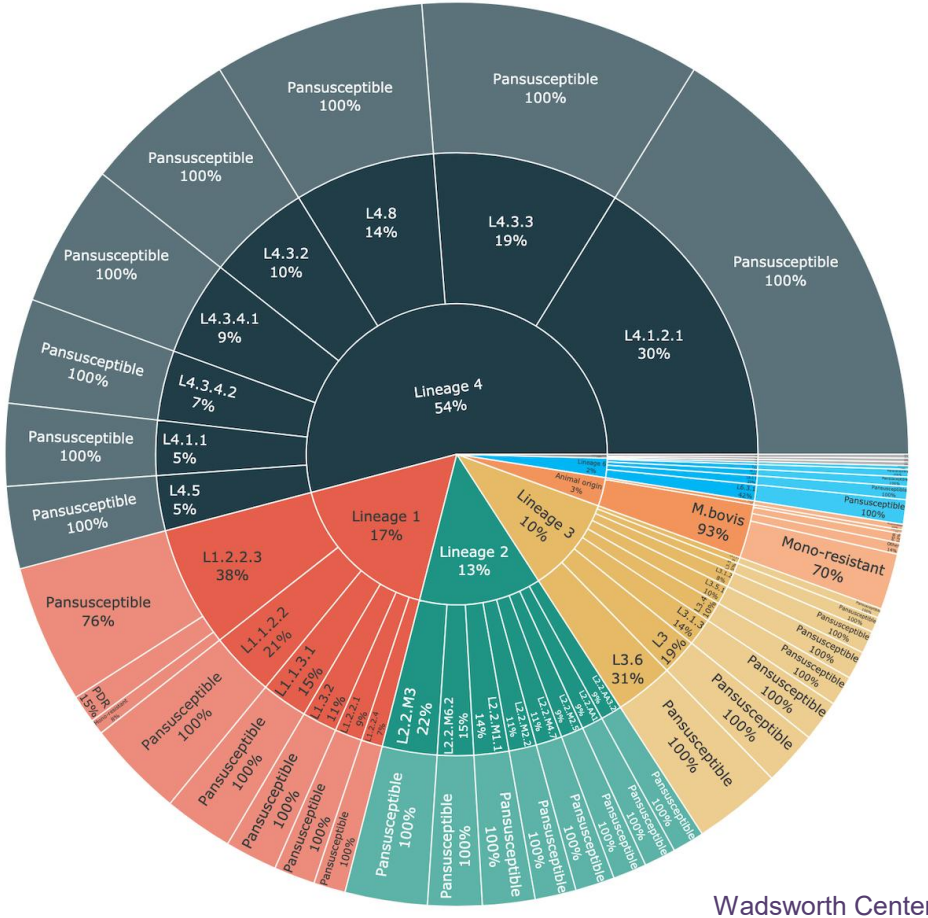
TB Sample Hierarchy: Lineage → Sub-lineage → Resistance



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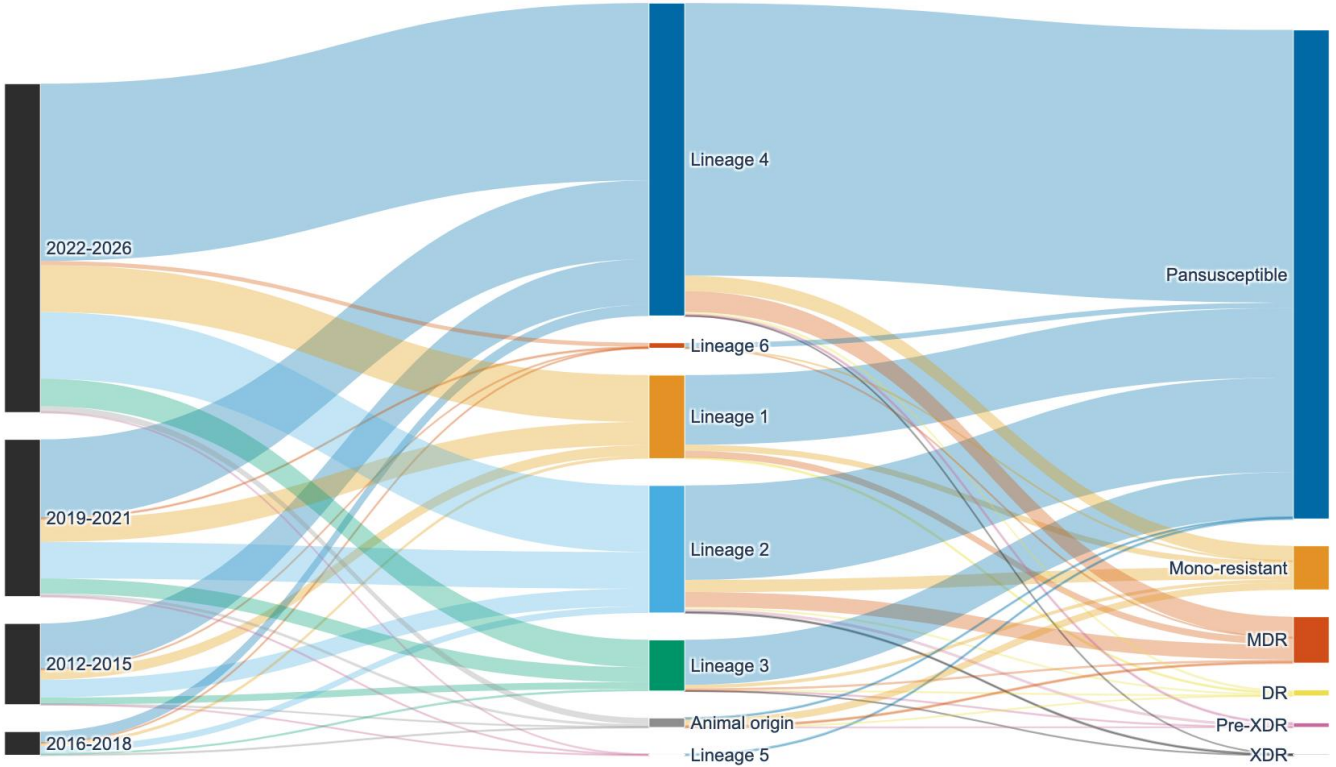
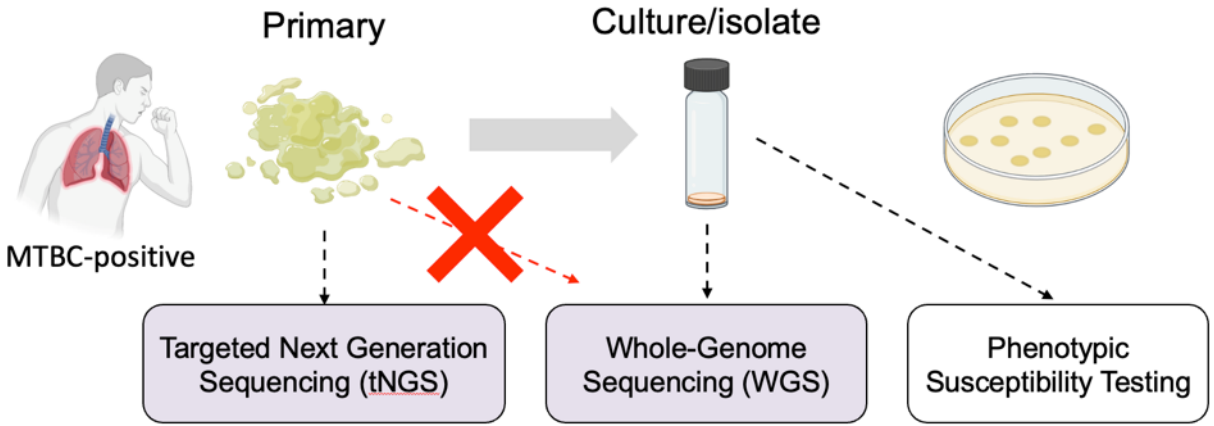
Resistance Trajectory: Flow from Time Period → Lineage → Resistance Category

NYS TB Genomic Surveillance (2012-2026)



Nanopore targeted next generation sequencing (tNGS) assay for *Mycobacterium tuberculosis* complex (MTBC)

NGS direct on primary clinical specimens?



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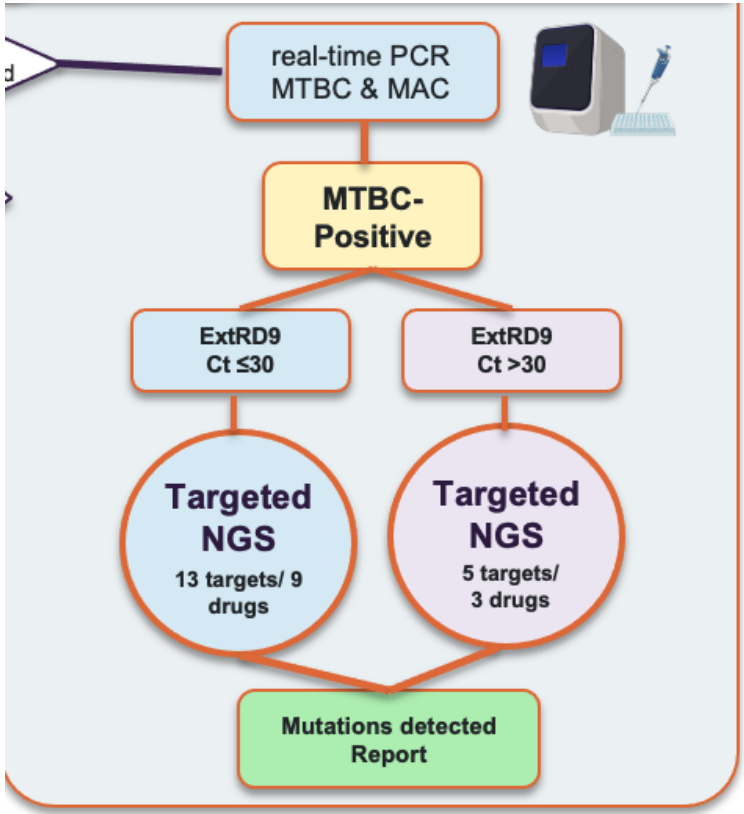
Development and Validation of a Direct Specimen MTBC tNGS assay



- **Offers more information than pyrosequencing: rifampin, isoniazid, fluoroquinolones**
- **Results concordant with WGS**

https://nanoporetech.com/products/gridion
www.Biorender.com

tNGS Loci to Detect DR-TB

Key Improvements:

- Longer amplicons
- Additional targets
- Additional drugs
- Multiplex 2 reactions

- Most tNGS assays run for 1-2 hours/ 50,000 reads, all targets pass
- Positive and Negative controls in every run
- Flow cells reused up to 3 x if Platform QC indicates >800 active pores no repeating barcodes are used
- Revalidation/Validation Plans

Antimicrobials	Loci	tNGS Size (bp)
Isoniazid (INH)	*katG**	2503
	*mabA-inhA**	2902
	oxyR-aphC	1138
Rifampin (RIF)	*rpoB**	3662
Pyrazinamide (PZA)	*pncA*	876
Ethambutol (EMB)	*embB*	3636
	embC-A	1652
Fluoroquinolones (FQ)	*gyrA**	2978
	*gyrB**	2380
Streptomycin (SM)	*rrs*	1927
	rpsL	939
Kanamycin (KAN) /Amikacin (AMI)	*eis* promoter	1329
	rrs	1927
Ethionamide (ETH)	*ethA*	1828
		~30 kb

*pyrosequencing target

Regulatory Approval

Laboratory Developed Test (LDT) -April 2023

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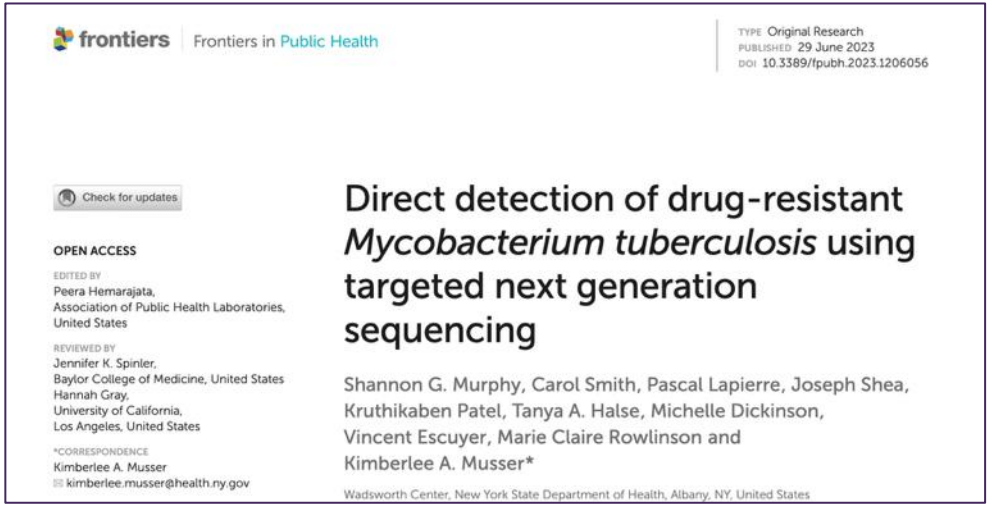
Notification to all NY Microbiology Laboratories-May 2023



frontiers | Frontiers in Public Health

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Direct detection of drug-resistant *Mycobacterium tuberculosis* using targeted next generation sequencing

Shannon G. Murphy, Carol Smith, Pascal Lapierre, Joseph Shea, Kruthikaben Patel, Tanya A. Halse, Michelle Dickinson, Vincent Escuyer, Marie Claire Rowlinson and Kimberlee A. Musser*

Wadsworth Center, New York State Department of Health, Albany, NY, United States

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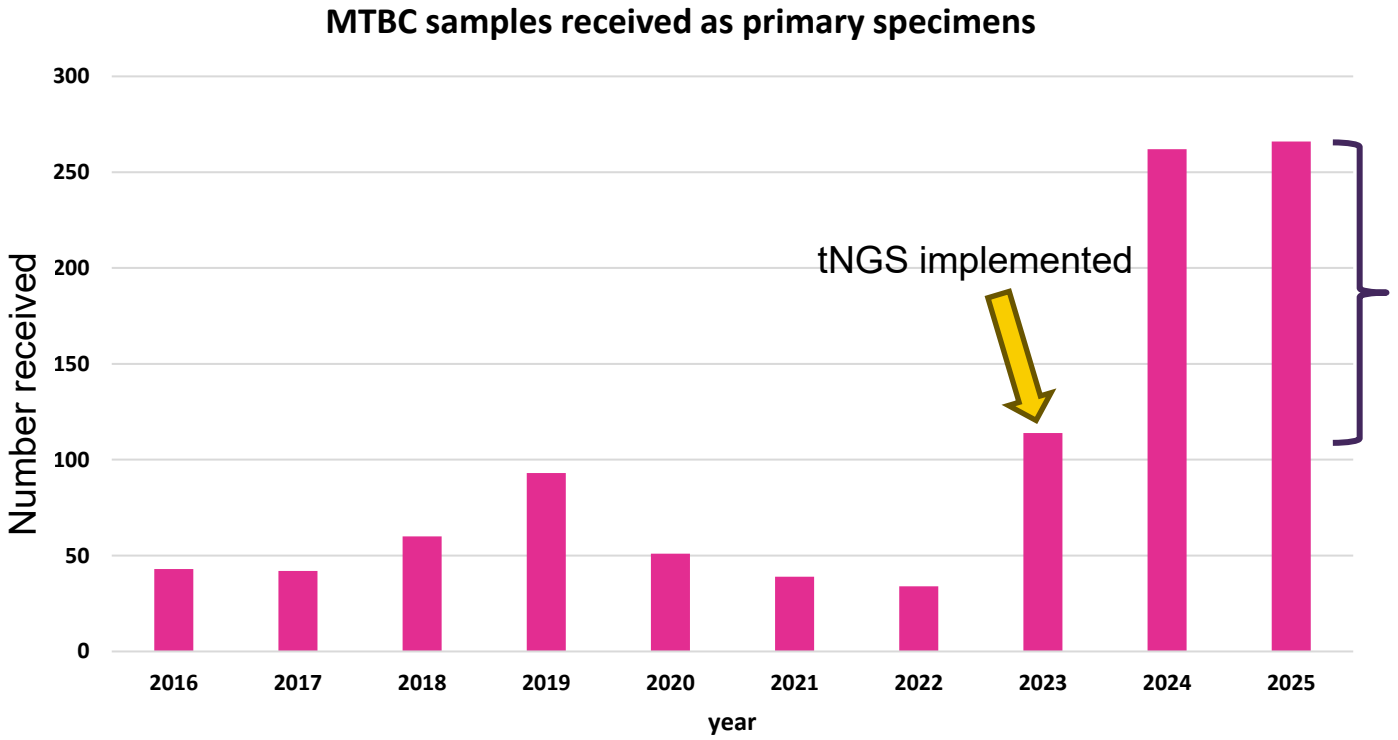
tNGS MTBC Testing Increase



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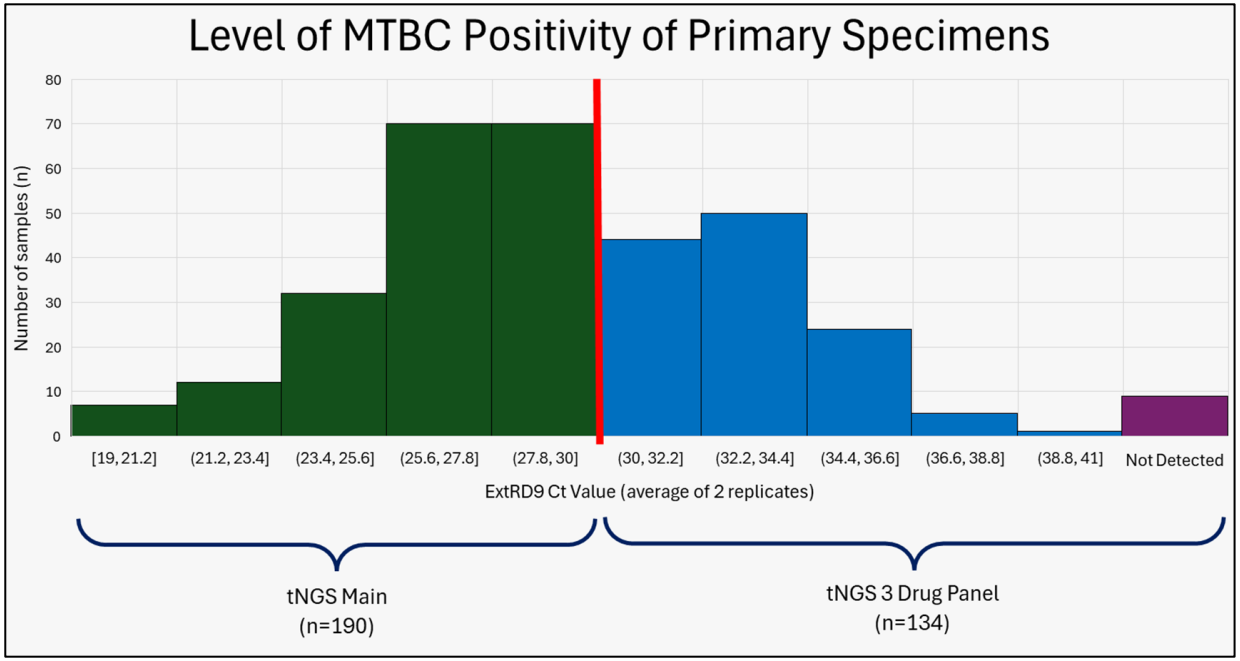
tNGS Assay PCR levels



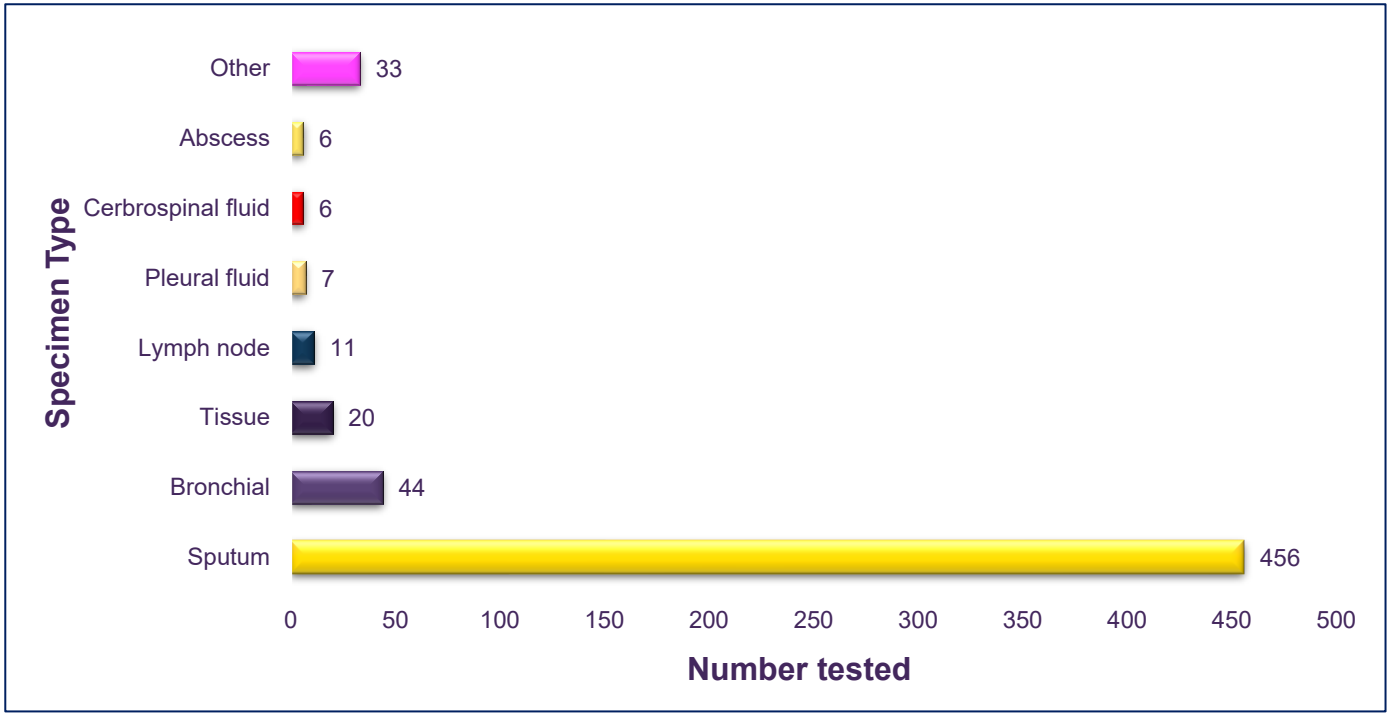
324 PCR positive specimens
- 59% tNGS Main
- 41% tNGS 3 Drug Panel

96.2% respiratory

26 PCR negative specimens not tested by tNGS

'Not Detected' for ExtRD9 PCR target only; these were positive for multi-copy IS6110 target

tNGS Testing by Specimen Type



- **463/580 (80%) Pan susceptible (No High Confidence Mutations)**
- **64/580 (11%) Failed QC for 4 or more targets on**
- **12 multidrug resistant (MDR) MTBC**
- **1 extensively drug resistant (XDR) MTBC**

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Impact of tNGS as an early detection method for drug-resistant MTBC

Targeted Next Generation Sequencing (tNGS)

4–7-day turnaround time

Whole-Genome Sequencing (WGS)

14–21-day turnaround time from specimen receipt

- Average 15-day improvement for primary specimens
- Average 43-day improvement for mixed/ contaminated cultures
- Stat results available in < 24 hr
- Cost <$75, batched 1 x per week

- Comprehensive DR profiling
- Comprehensive genome analysis
  SNP Relatedness
- Continued assessment of mutations

Now $48 with NextSeq 1000

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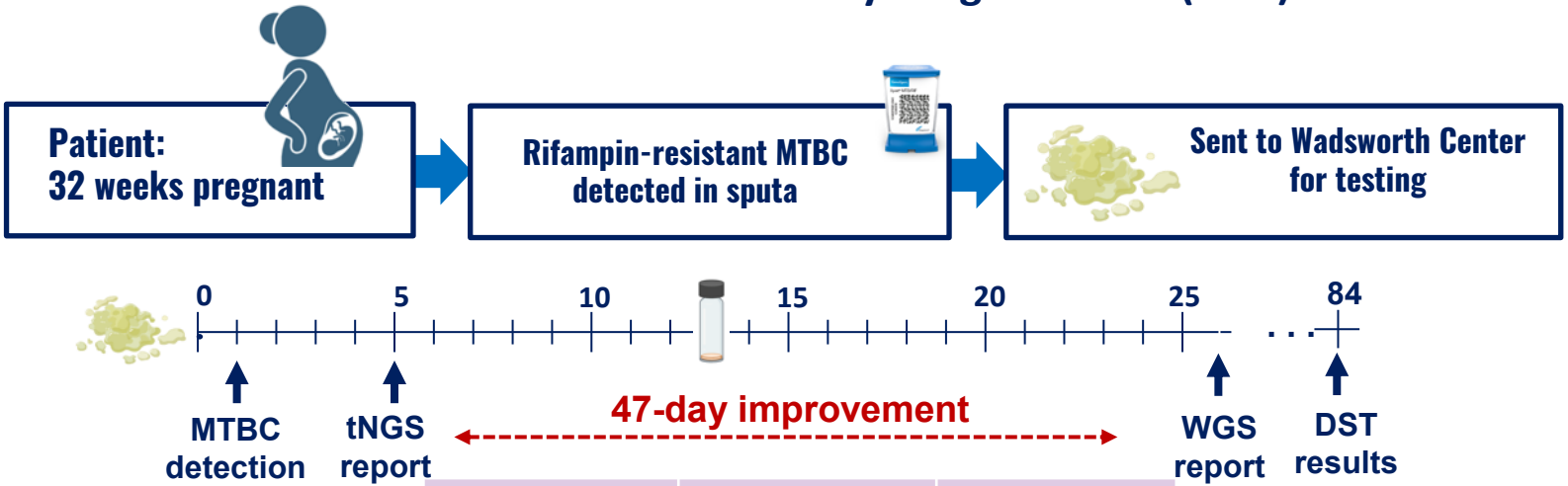
Case #1: Direct detection of extensively drug-resistant (XDR) MTBC



Antimicrobial	Gene/loci Mutations	Susceptibility
Rifampin	*rpoB* Ser531Leu	Resistant
Isoniazid	*katG* Ser315Thr	Resistant
Pyrazinamide	No amplification	Not Determined
Ethambutol	*embB* Met306Ile	Resistant
Streptomycin	*rpsL* Lys43Arg	Resistant
Kanamycin/Amikacin	*rrs* A(1400)G	Resistant
Fluoroquinolones	*gyrA* Asp94Asn	Resistant
Ethionamide	*ethA* Leu478Arg	Unknown

Milestone:
First time NYS has detected an XDR case direct from a patient sample

Case #2: Rifampin resistance detected by GeneXpert

Patient → Rifampin-resistant MTBC detected in sputa → Sent to Wadsworth Center for testing

False rifampin-resistant GeneXpert result caused by silent *rpoB* mutation

Case #3: STAT testing for pediatric TB case

12 months old
Very sick

TB-positive relative

CSF grew an MTBC-positive isolate

Sent to Wadsworth Center for WGS

Pan-susceptible
Result in < 24 hours

Direct Molecular Identification and Drug Susceptibility Prediction- Targeted Next Generation Sequencing

Susceptibility Profile:	
Rifampin (RIF):	Susceptible
	A silent mutation Phe433Phe mutation is present in the gene rpoB but does not confer resistance to rifampin. However, this mutation can generate a false rifampin resistance result with the GeneXpert MTB/RIF assay.
Isoniazid (INH):	Susceptible
Pyrazinamide (PZA):	Susceptible
Ethambutol (EMB):	Susceptible
Streptomycin (SM):	Susceptible
Kanamycin/Amikacin (KAN/AMI):	Susceptible
Fluoroquinolones (FLQ):	Susceptible
Ethionamide:	Susceptible

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BPaL SUSCEPTIBILITY TESTING IN THE US

Together, bedaquiline, pretomanid, and linezolid comprise the BPaL regimen, which has been endorsed by the World Health Organization (WHO) for the treatment of MDR-TB.

MDR-TB is defined by TB strains that are resistant to at least the two first-line drugs: rifampin and isoniazid.

Phenotypic drug-susceptibility testing (DST) for BPaL drugs is limited nationwide, only a small number of laboratories are currently performing this testing. The Wadsworth Center is among the few facilities that currently offer phenotypic testing for BPaL drugs as well as clofazimine and delamanid.

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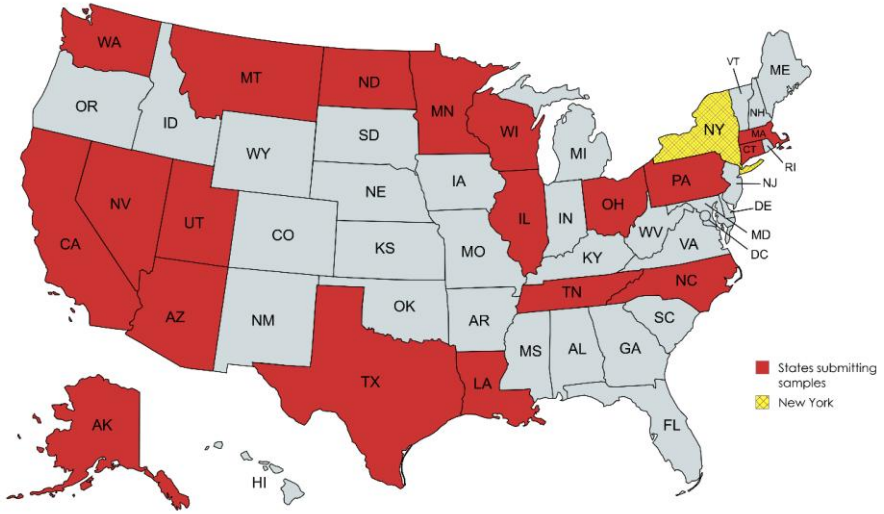
ACCESS TO BPaL SUSCEPTIBILITY TESTING IN THE US

- Currently only public health laboratory in the US offering clinical susceptibility testing for the three BPaL drugs. Offering testing to the rest of the country
- Member of the BPaL Implementation group in the US led by CDC. One of the two reference laboratories for phenotypic testing with Johns Hopkins Medical Microbiology laboratory
- Laboratory expert for the Global TB Institute, one of the National Tuberculosis Centers of Excellence funded by CDC
- Since implementation in 2023, tested over 100 MDR/XDR isolates for ~40 states in addition to the provinces of British Columbia, Ontario and Nova Scotia in Canada



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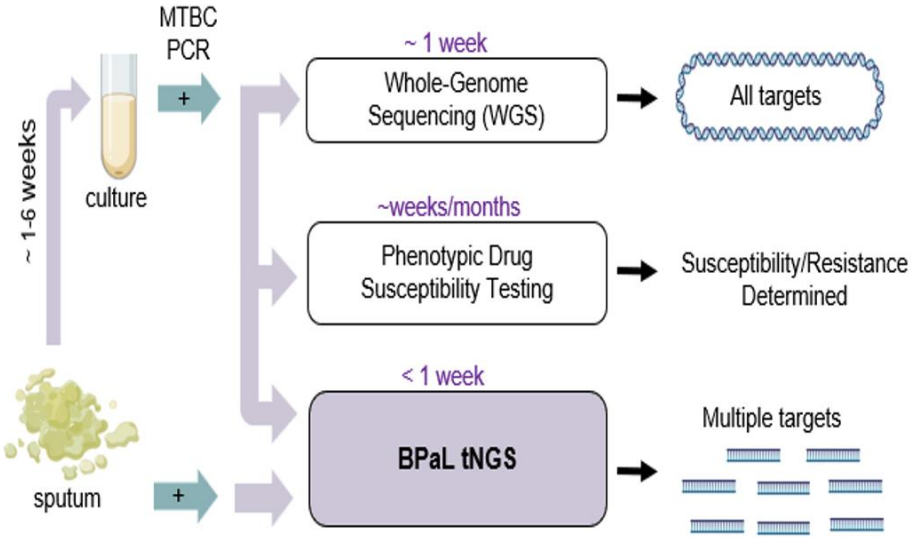
BPaL Drug Susceptibility Testing in NYS



BPaL Targeted NGS (tNGS) Assay Design

- Primers designed to amplify 12 full-length genes/gene regions associated with resistance to the BPaL regimen drugs
- 12 targets amplified within one multiplexed PCR

Antibiotic	Gene Targets
Bedaquiline	*atpE*
Bedaquiline/ Clofazimine	*rv0678*, *rv0678-mmpS5* promoter region, *pepQ*
Pretomanid/ Delamanid	*fbiA*, *fbiB*, *fbiC*, *fbiD*, *fgd1*, *ddn*
Linezolid	*rplC*, *rrl*

- Amplicon size: 246 – 3,138 bp
- One multiplexed pool

Image from BioRender.com

Future Directions

- Maintain and improve tNGS assays
  - Improve sensitivity full tNGS
  - Evaluating commercial assays
- Revalidation plans
- Direct specimen WGS
- Evaluating MTBC in wastewater

Microreact TB Dashboard

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Wadsworth Center Microreact Dashboard

BIGGEST WIN

- Impact on patient treatment and management
- Impact on TB case surveillance
- Turnaround times NGS
- Reduced cost and staff time associated with phenotypic DST
- Sharing with others:
  - SOPs
  - Validation strategies
  - Pipelines

BIGGEST CHALLENGE

- Instrument and test discontinuation
- Validation and revalidations
- Navigating sequencing improvements
- Complex data analysis- LIMS pulls
- **Early adopter**

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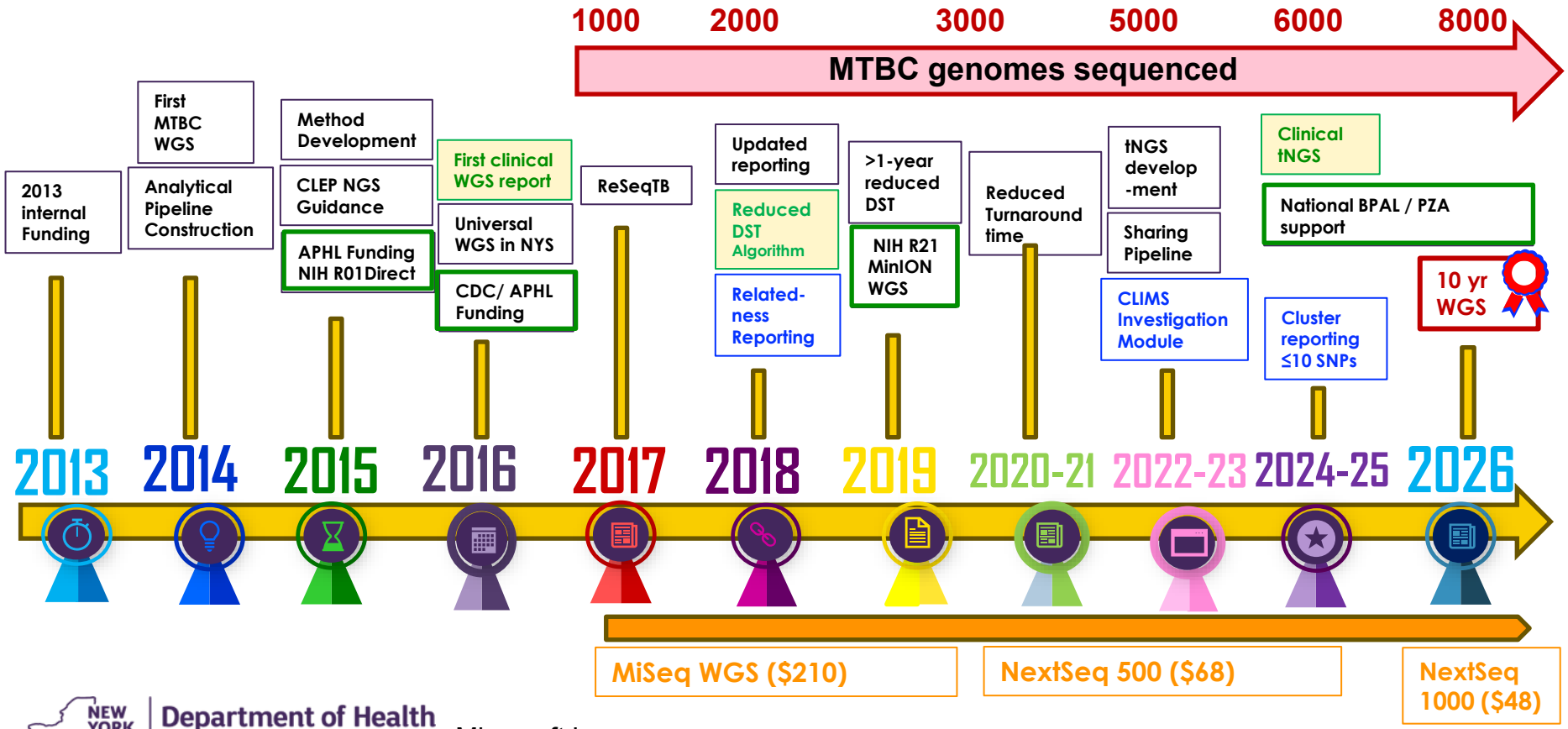
Wadsworth Center MTBC NGS Timeline



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Acknowledgements

Core TB WGS/tNGS Team

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APHL

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Wadsworth Center, NYSDOH
Public Health Genomics Initiative

Establishment of Mycobacterium tuberculosis complex WGS Reference Centers

National Institutes of Health
Office of Extramural Research

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

R03 NIH- Use of whole genome sequencing for tuberculosis diagnostics

APHL FELLOWSHIP PROGRAMS

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