Should I Stay or Should I Go: Navigating the Landscape of tNGS for TB

NEW YORK STATE | Department of Health | Wadsworth Center

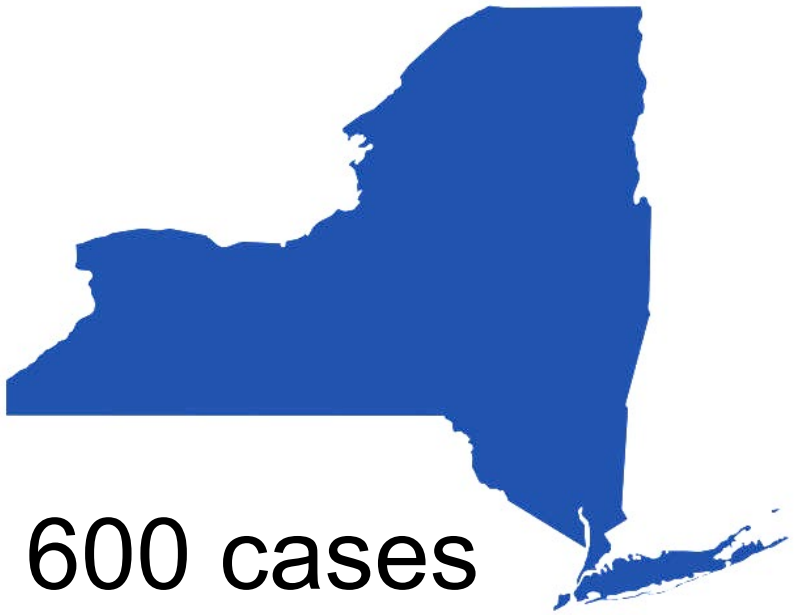
Implementation of a targeted Next Generation sequencing assay for early detection of drug-resistant *Mycobacterium tuberculosis*

Shannon Murphy, PhD

Association of Public Health Laboratories Fellow

July 25, 2023

12.8% NY cases showed clinically relevant resistance



> 600 cases

Susceptibility Profile	Frequency
Pan-susceptible	87.3%
Drug-resistant	10.3%
Multi-drug resistant	2.4%
Extensively drug-resistant	< 0.1%

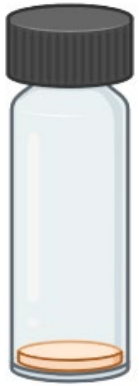
NY Data Compiled from 2022

NEW YORK STATE | Department of Health | Wadsworth Center

Molecular Testing Algorithm for *M. tuberculosis* complex (MTBC)

Processed Primary → MTBC detection → Pyrosequencing (*rpoB*, *katG*, *inhA*, *gyrA*, *gyrB*)

- Limited scope
- Instrument at end of life



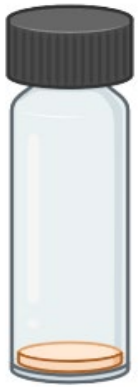
Isolate or Culture → MTBC detection → Whole-Genome Sequencing (WGS) → Phenotypic DST

All sequencing assays are batched and run weekly

NEW YORK STATE | Department of Health | Wadsworth Center

Molecular Testing Algorithm for *M. tuberculosis* complex (MTBC)

Processed Primary → MTBC detection → Targeted Next Generation Sequencing (tNGS) → Pyrosequencing

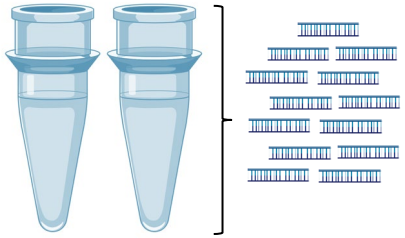


Isolate or Culture → MTBC detection → Whole-Genome Sequencing (WGS) → Phenotypic DST

All sequencing assays are batched and run weekly

NEW YORK STATE Department of Health | Wadsworth Center

Development and validation of a targeted next generation sequencing (tNGS) assay

DNA Extraction	Targeted, Multiplex PCR	Library Preparation	Nanopore Sequencing	Bioinformatic Analysis
				
EZ1 (Qiagen)	13 full length targets 9 antimicrobials	Manual Prep	< 2 hr seq time Flow cell re-use	In-house pipeline Real-time analysis

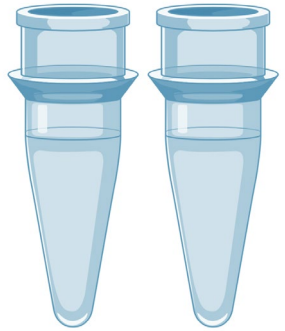
Batched weekly, performed over 2 to 2.5 days
Cost ~ $80/sample

NEW YORK STATE | Department of Health | Wadsworth Center

Targets cover mutations conferring resistance to first- and second-line antimicrobials

Antimicrobial	Loci
Isoniazid	*katG*, *furA-katG* promoter, *mabA*, *inhA*, *mabA-inhA* promoter, *oxyR-ahpC* promoter
Rifampin	*rpoB*
Pyrazinamide	*pncA* promoter
Ethambutol	*embB*, *embC-A* promoter
Fluoroquinolones	*gyrA*, *gyrB*
Streptomycin	*rrs, rpsL*
Kanamycin	*eis* promoter, *rrs*
Amikacin	*rrs*
Ethionamide	*ethA*

* Additional panel in development for BPaL drugs



Full-length genes and/or promoter regions (total = 30 kb)

NEW YORK STATE | Department of Health | Wadsworth Center

Real-time Nanopore sequencing gives faster results

Advantages:

- Flexible run times
- Real-time analysis

Challenges:

- Frequent updates

Nanopore Sequencing

Image: Nanopore

R9 flow cell GridION

Bioinformatic Analysis

In-house pipeline

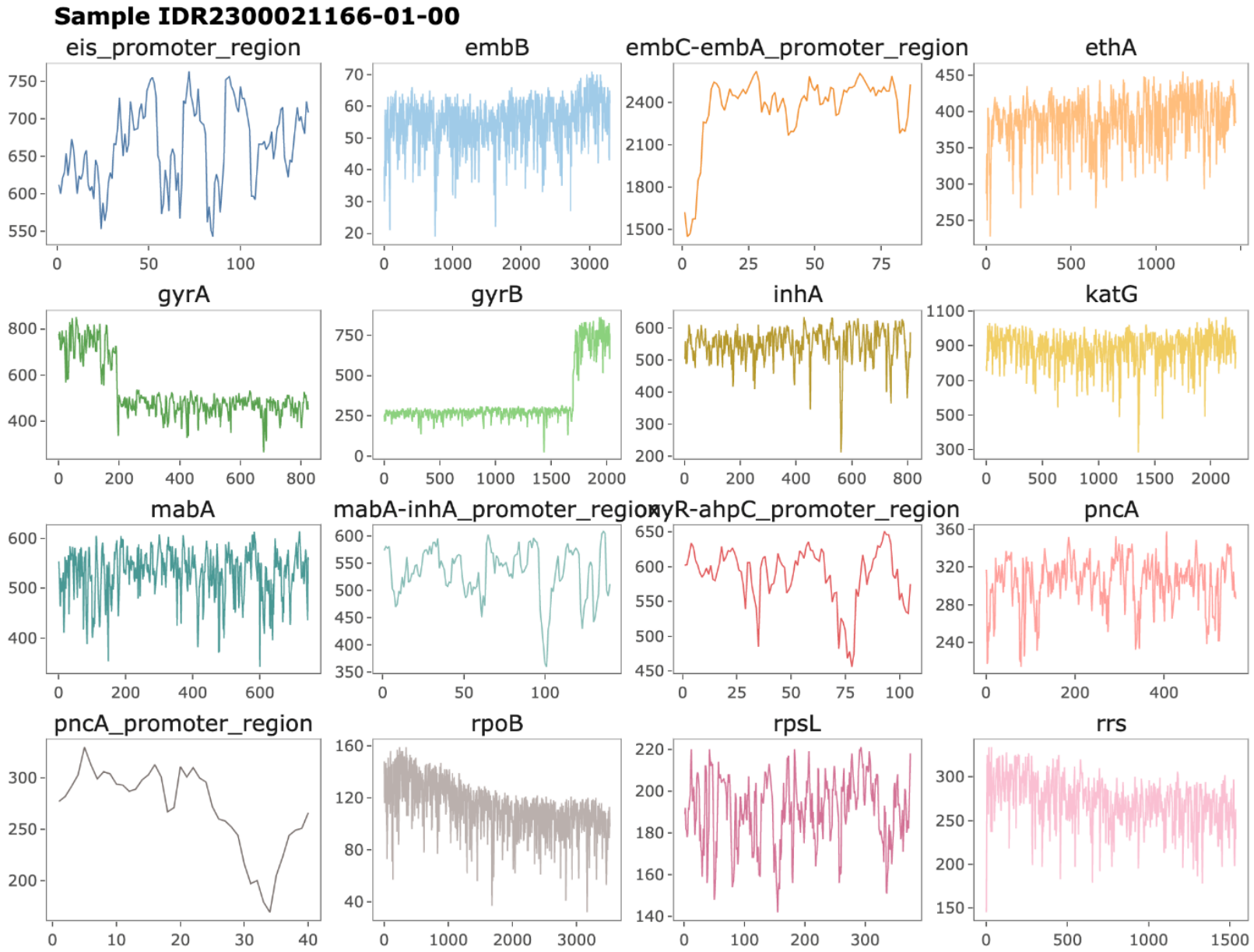
NEW YORK STATE | Department of Health | Wadsworth Center

Dashboard

Pascal Lapierre

Loci Status:

Loci	Avg.Depth	Status
rpoB	113	PASS
katG	879	PASS
oxyR-ahpC promoter region	578	PASS
inhA	546	PASS
mabA-inhA promoter region	537	PASS
mabA	526	PASS
pncA	302	PASS
pncA promoter region	296	PASS
embB	55	FAIL
embC-embA promoter region	2292	PASS
rpsL	191	PASS
rrs	268	PASS
eis promoter region	646	PASS
gyrA	517	PASS
gyrB	337	PASS
ethA	385	PASS



Validation plan: compare tNGS results with WGS

Panel	Details
Sensitivity	Serial dilutions of H37rV spiked into processed negative sputa
Specificity	1 McFarland lysates other mycobacteria & other pathogens common in sputum
Reproducibility	Intra-assay, Inter-assay
Accuracy (Cultures/Isolates)	**55 total** 21 dual positive for MTBC and MAC
Accuracy (Primary Specimen)	**72 total** respiratory specimens 35 retrospective, 37 prospective

Direct tNGS is concordant with WGS (performed on cultured isolates)

		WGS (isolates)				
		S	DR	MDR	Pre-XDR	XDR
tNGS (primaries)	S	44	0	0	0	0
	DR	0	5	0	0	0
	MDR	0	0	5	0	0
	Pre-XDR	0	0	0	1	0
	XDR	0	0	0	0	1
	Not Seq	10	1	0	0	0

Limitation: specimens with low TB DNA

April 2023:

tNGS received conditional CLEP approval to test isolates and respiratory specimens

tNGS Reporting

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

Susceptibility Profile*

Rifampin (RIF):	**Susceptible (suggested)**	3/8/2023
Isoniazid (INH):	**RESISTANT (predicted)**	3/8/2023
Pyrazinamide (PZA):	**Susceptible (suggested)**	3/8/2023
Ethambutol (EMB):	**Susceptible (suggested)**	3/8/2023
Streptomycin (SM):	**Susceptible (suggested)**	3/8/2023
Kanamycin/Amikacin (KAN/AMI):	**Susceptible (suggested)**	3/8/2023
Fluoroquinolones (FLQ):	**Susceptible (suggested)**	3/8/2023
Ethionamide (ETH):	**Susceptible (suggested)**	3/8/2023

High-confidence mutations detected*

katG (INH):	**Mutation present: C(-15)T**	3/8/2023

Susceptible (suggested) indicates that no high-confidence or unknown mutations were detected in the targets evaluated. If a positive culture is obtained, Whole Genome Sequencing and culture-based testing (if appropriate) will be performed for final susceptibility results.

tNGS Reporting

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

Susceptibility Profile*

Rifampin (RIF):	**Unknown**	3/10/2023
Isoniazid (INH):	**Susceptible (suggested)**	3/10/2023
Pyrazinamide (PZA):	**Susceptible (suggested)**	3/10/2023
Ethambutol (EMB):	**Susceptible (suggested)**	3/10/2023
Streptomycin (SM):	**Susceptible (suggested)**	3/10/2023
Kanamycin/Amikacin (KAN/AMI):	**Susceptible (suggested)**	3/10/2023
Fluoroquinolones (FLQ):	**Susceptible (suggested)**	3/10/2023
Ethionamide (ETH):	**Susceptible (suggested)**	3/10/2023

High-confidence mutations detected*

rpoB (RIF):	**No high-confidence mutation**	3/10/2023
[Note]	A Glu333Lys mutation is present but its association with rifampin resistance is unknown. This mutation is not considered a high-confidence mutation. If a positive culture is obtained, culture-based testing for select drug(s) will be performed for final susceptibility results.	

Case #1:
Rifampin resistance detected by GeneXpert

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

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

Susceptibility Profile*

Rifampin (RIF):	**Susceptible**
[Note]	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 (ETH):	**Susceptible**

Phe433Phe mutation confirmed via pyrosequencing

NEW YORK STATE | Department of Health | Wadsworth Center

Ocheretina *et al*. 2016. Diagn Microbiol Infect Dis.

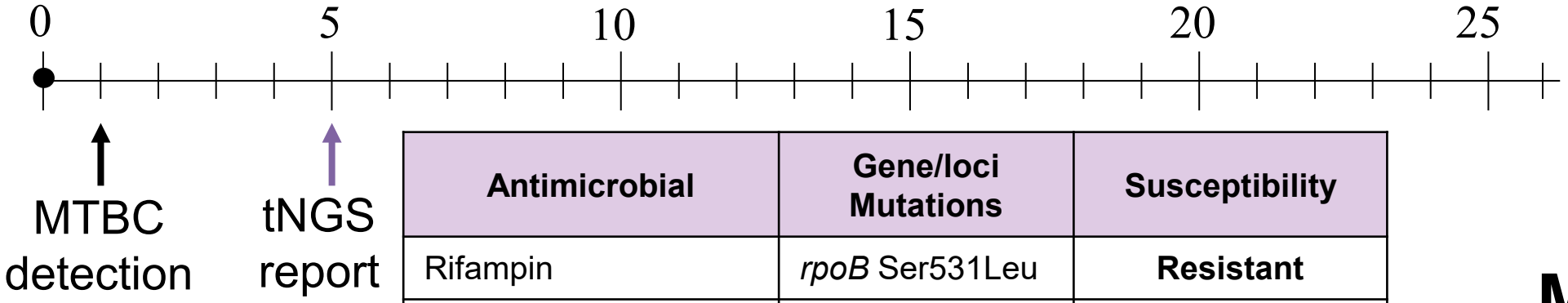
Case #2: Direct detection of extensively drug-resistant (XDR) TB

Patient:
32 weeks pregnant

Rifampin-resistant
MTBC detected in sputa

Sent to Wadsworth Center for testing

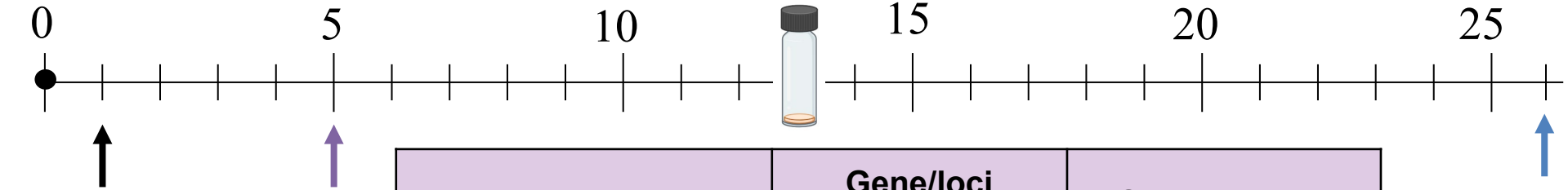
Direct detection of extensively drug-resistant (XDR) TB



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

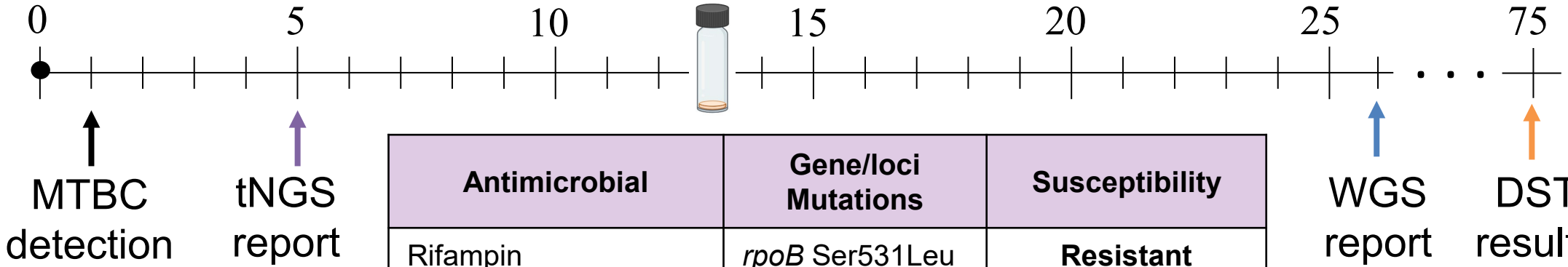
Direct detection of extensively drug-resistant (XDR) TB



Antimicrobial	Gene/loci Mutations	Susceptibility
Rifampin	*rpoB* Ser531Leu	**Resistant**
Isoniazid	*katG* Ser315Thr	**Resistant**
Pyrazinamide	Deletion	**Resistant**
Ethambutol	*embB* Met306Ile	**Resistant**
Streptomycin	*rpsL* Lys43Arg	**Resistant**
Kanamycin/Amikacin	rrs A(1400)G	**Resistant**
Fluoroquinolones	*gyrA* Asp94Asn	**Resistant**
Ethionamide	*ethA* Leu478Arg	Unknown

WGS confirmed mutations and provided additional information

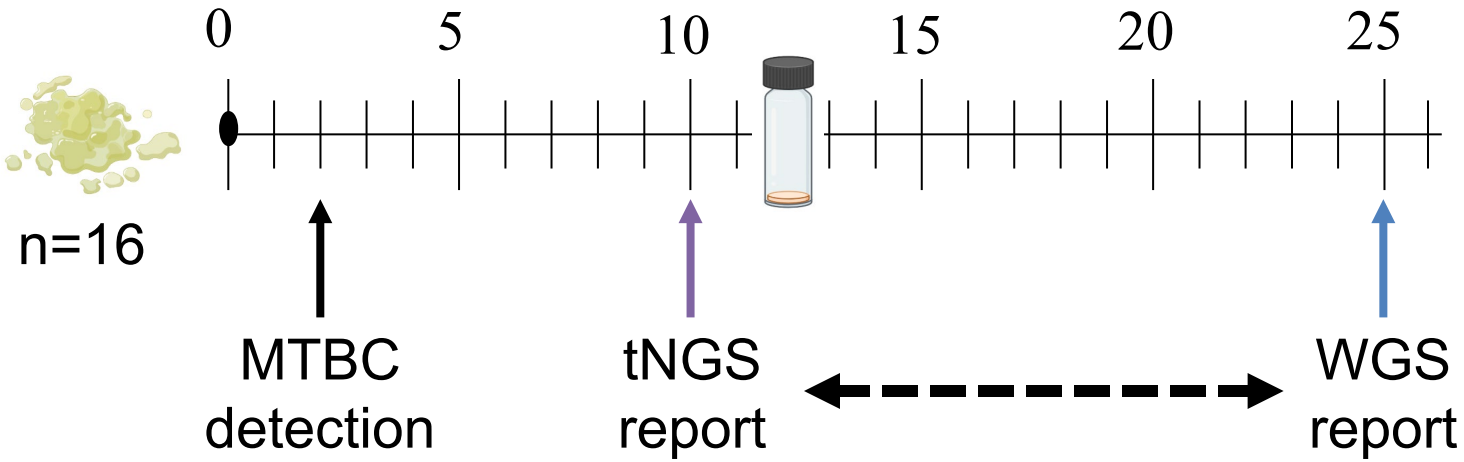
Direct detection of extensively drug-resistant (XDR) TB



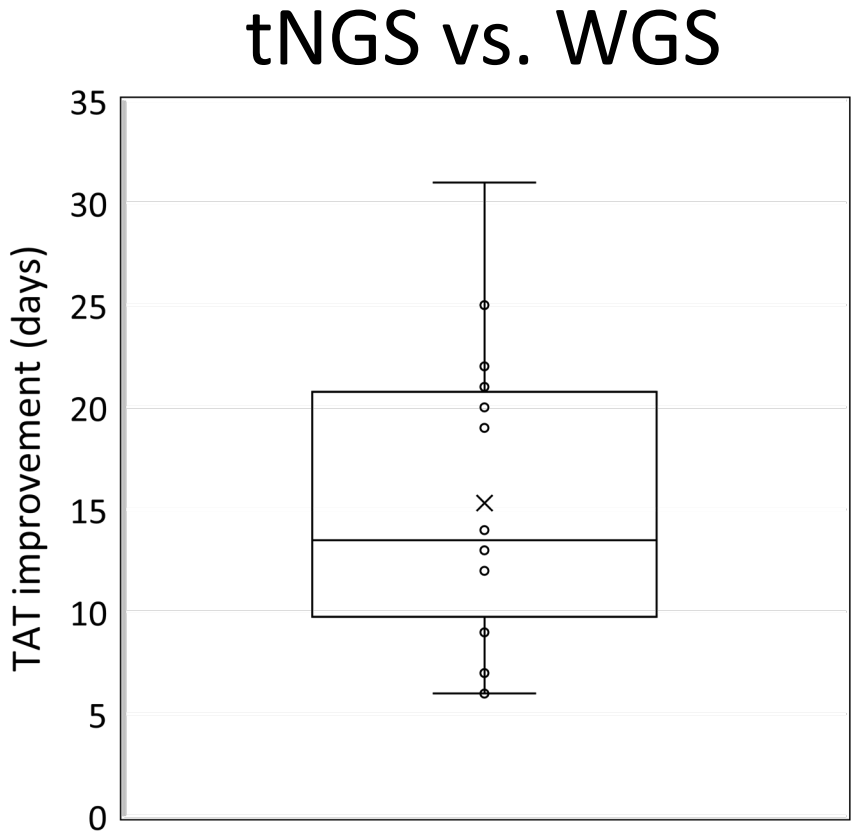
Antimicrobial	Gene/loci Mutations	Susceptibility
Rifampin	*rpoB* Ser531Leu	**Resistant**
Isoniazid	*katG* Ser315Thr	**Resistant**
Pyrazinamide	Deletion	**Resistant**
Ethambutol	*embB* Met306Ile	**Resistant**
Streptomycin	*rpsL* Lys43Arg	**Resistant**
Kanamycin/Amikacin	rrs A(1400)G	**Resistant**
Fluoroquinolones	*gyrA* Asp94Asn	**Resistant**
Ethionamide	*ethA* Leu478Arg	**Resistant**

DST on unknown mutations helps improve our mutation catalog

Direct tNGS improves turnaround times by ~2 weeks compared to WGS



Average improvement of **15.3** days

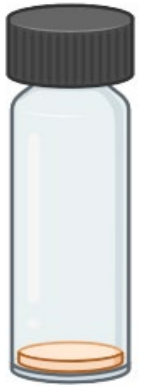


Molecular Testing Algorithm for *M. tuberculosis* complex (MTBC)

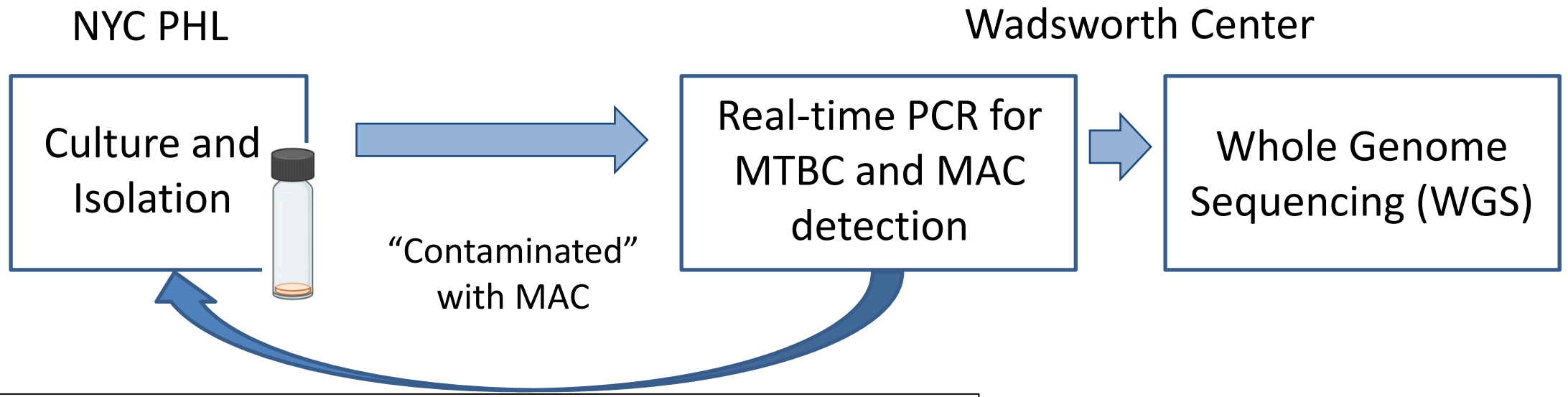
Processed Primary → MTBC detection → Targeted Next Generation Sequencing (tNGS)

Processed Primary → Isolate or Culture → MTBC detection → Whole-Genome Sequencing (WGS) → Phenotypic DST

Contaminated, STAT testing



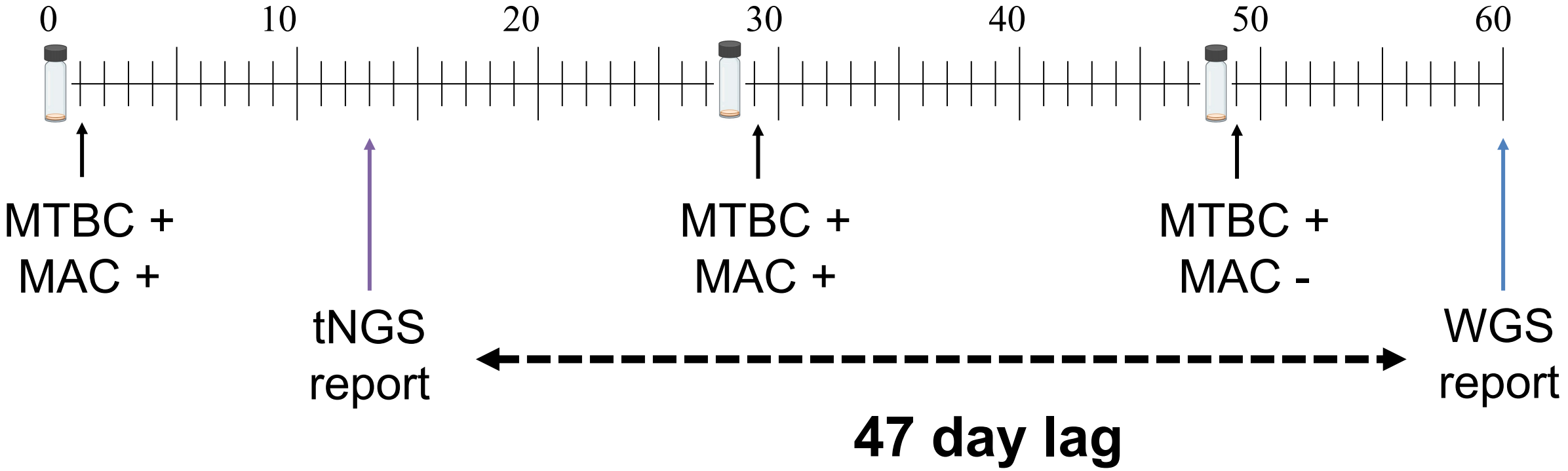
Case #3:
Patients dual positive for MTBC and *M. avium* complex (MAC)



"Whole genome sequencing could not be performed due to a mixed culture. Please resubmit a pure culture of *Mycobacterium tuberculosis*, if warranted."

NEW YORK STATE | Department of Health | Wadsworth Center

Patient dual positive for MTBC and *M. avium* complex (MAC)



Average improvement for mixed cultures is 43 days, ranging from 7 to 125 days (n=10).

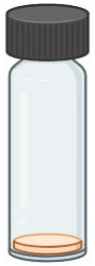
NEW YORK STATE | Department of Health | Wadsworth Center

Case #4:
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

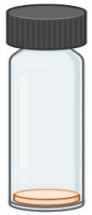
Pan-susceptible
Result in < 24 hours

NEW YORK STATE | Department of Health | Wadsworth Center

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

Targeted Next Generation Sequencing (tNGS) ⟷ Whole-Genome Sequencing (WGS)

Average **15 day** improvement for primary specimens (vs. WGS)



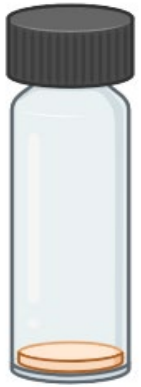
Average **43 day** improvement for contaminated cultures

Stat results available in < 24 hr

NEW YORK STATE | Department of Health | Wadsworth Center

Molecular Testing Algorithm for *M. tuberculosis* complex (MTBC)

Processed Primary → MTBC detection → Targeted Next Generation Sequencing (tNGS)



Isolate or Culture → MTBC detection → Whole-Genome Sequencing (WGS) → Phenotypic DST

Acknowledgements

Mycobacteriology:

- Marie-Claire Rowlinson
- Vincent Escuyer *
- Michelle Dickinson *
- Tanya Halse
- Joe Shea
- Kruthika Patel
- Emily McGrath
- Jim Long
- Justine Edwards
- Donna Kohlerschmidt *
- Susan Wolfe
- Amy Chiefari
- Alexandra Fiero

Carol Smith

Pascal Lapierre*

Kim Musser*

Funding

ELC Grant

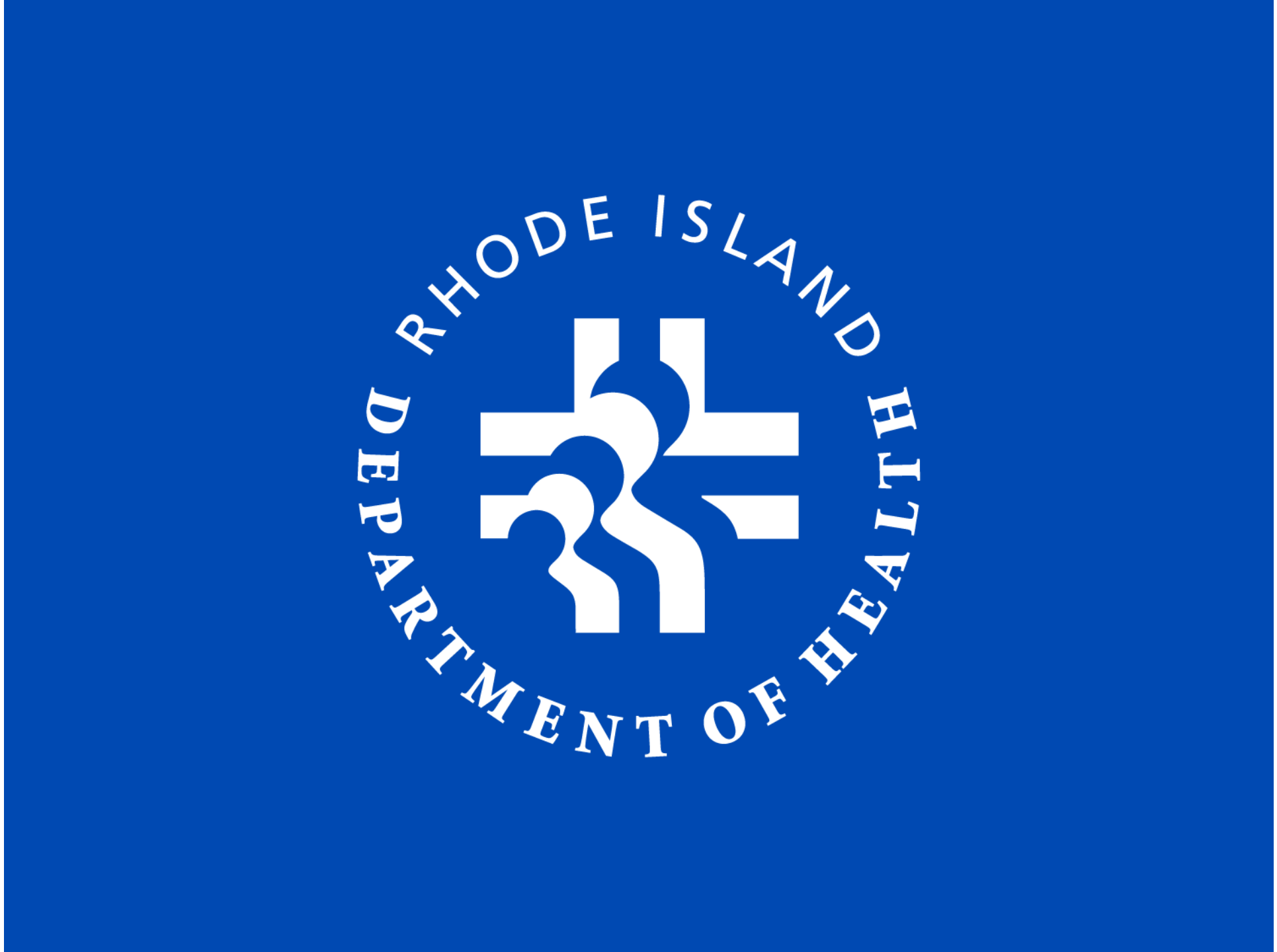
Fellowship

APHL FELLOWSHIP PROGRAMS

Colleagues at the Wadsworth Center

Images from BioRender.com

NEW YORK STATE Department of Health | Wadsworth Center

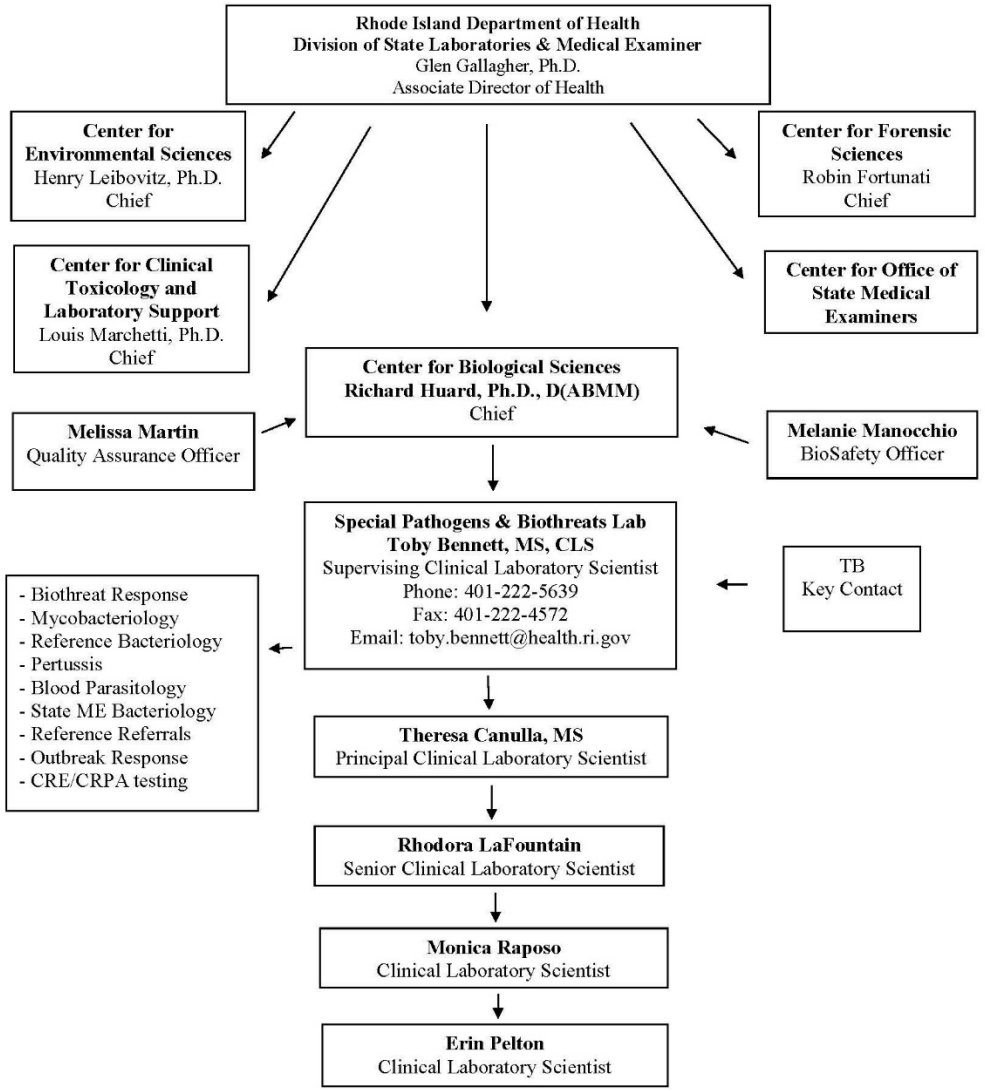

RHODE ISLAND
DEPARTMENT OF HEALTH

Should I Stay or Should I Go: Navigating the Landscape of tNGS for TB

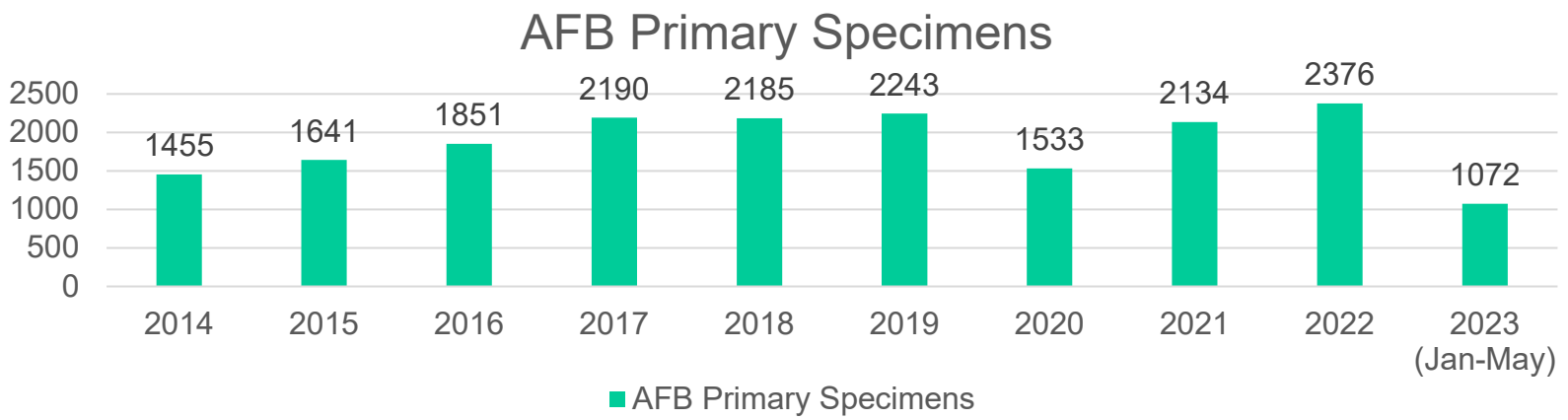
Toby W. Bennett, MS, MLS(ASCP)
Rhode Island State Health Laboratories
Rhode Island Department of Health

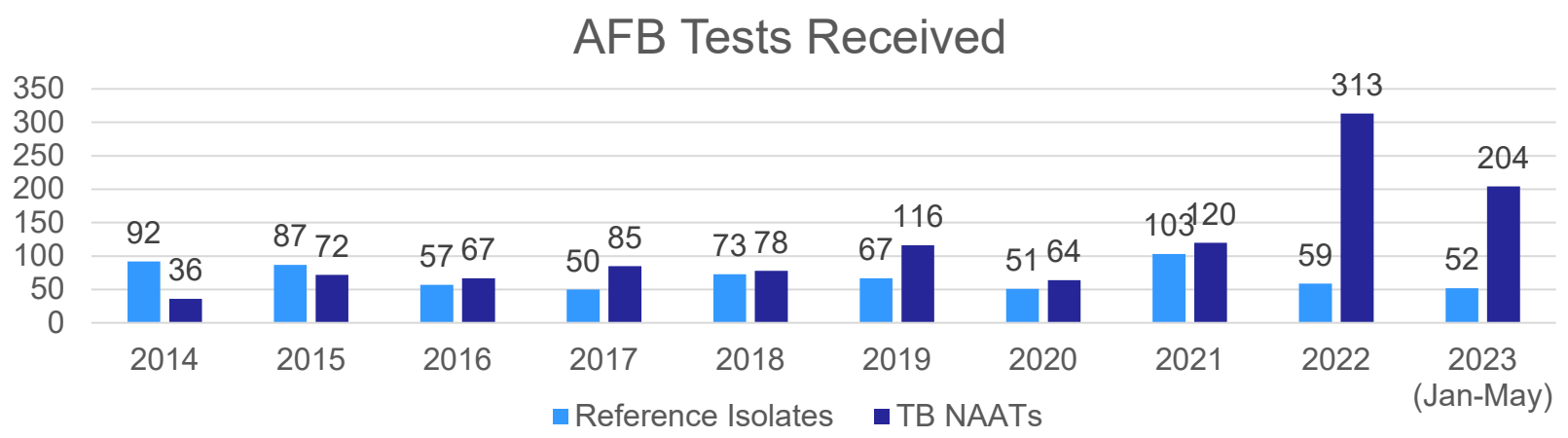
Rhode Island State Health Laboratory

Organizational Chart

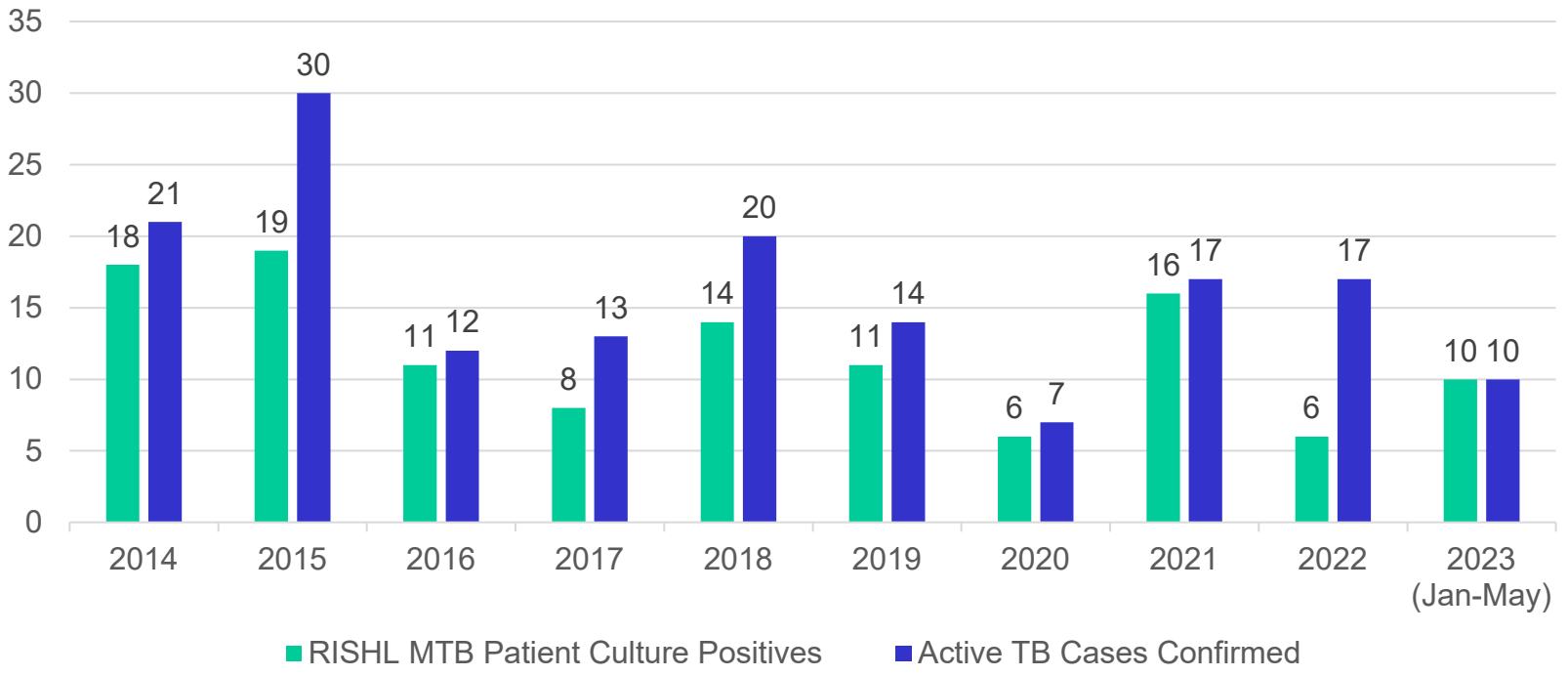


RISHL AFB Stats 2014-2023

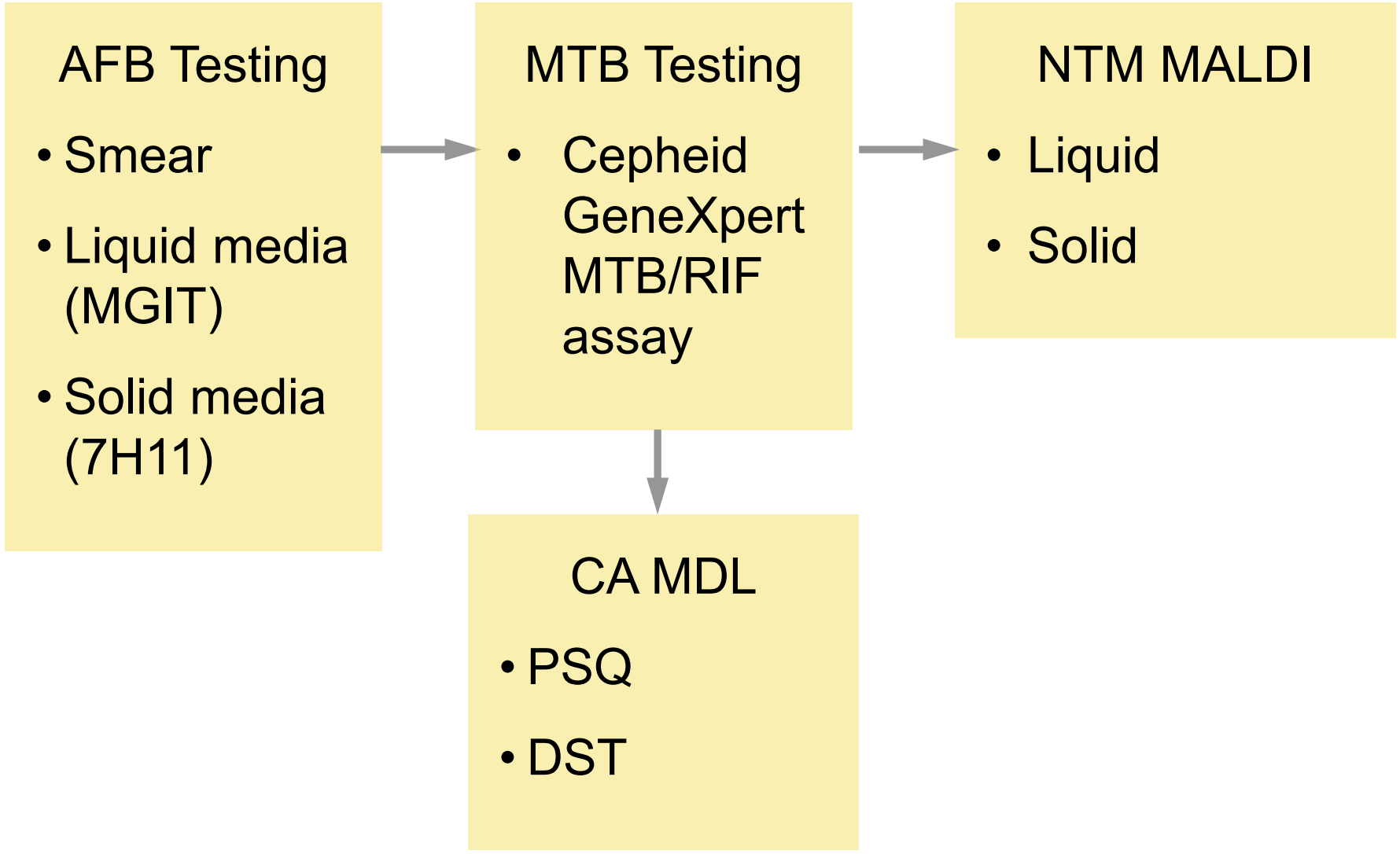




RIDOH MTB Positive Cases 2014-2023



RISHL AFB Workflow



Workflow for Positive MTB Cases

- TB NAAT positive respiratory sediment
  - If smear positive will forward to CA MDL

- MTB positive culture (broth or solid)
  - Send pure isolate to CA MDL
    - if rifampin resistance is not detected on Cepheid
  - Send pure isolate to CDC MDDR
    - If rifampin resistance is detected on Cepheid
  - Will ask provider if they will want to request PSQ and for them to email a clinical history

RISHL "No Go for tNGS"

- Low incidence state
  - 6-19 MTB lab positive patients per year
  - Most cases are 1st line susceptible
- Prioritizing workflow of understaffed Sequencing Lab
  - Pipeline to choose from/CLIA validation
- Results from CA MDL PSQ (INH/RIF)
- Availability of tNGS through CDC MDDR

RISHL – Happy to Help

- tNGS special request test
- Results sent to provider that ordered test as well as RIDOH Epidemiology and TB Clinic
- Anything is possible in future

RISLH – HOPE(ful)

Thank You TB Partners

- No disclosures of conflicts of interest/financial relationships with commercial entities
- Special acknowledgments to:
  - California Department of Health Microbial Diseases Lab
  - Centers for Disease Control and Prevention Division of Tuberculosis Elimination
  - Association of Public Health Laboratories

Toby W. Bennett, MS, MLS(ASCP)
Supervising Clinical Laboratory Scientist
401.222.5639
toby.bennett@health.ri.gov
www.health.ri.gov

Implementing Genoscreen's Deeplex assay in Florida

Matthew D. Schimenti MPH, M(ASCP)
Bureau of Public Health Laboratories – TB Control
Florida Department of Health

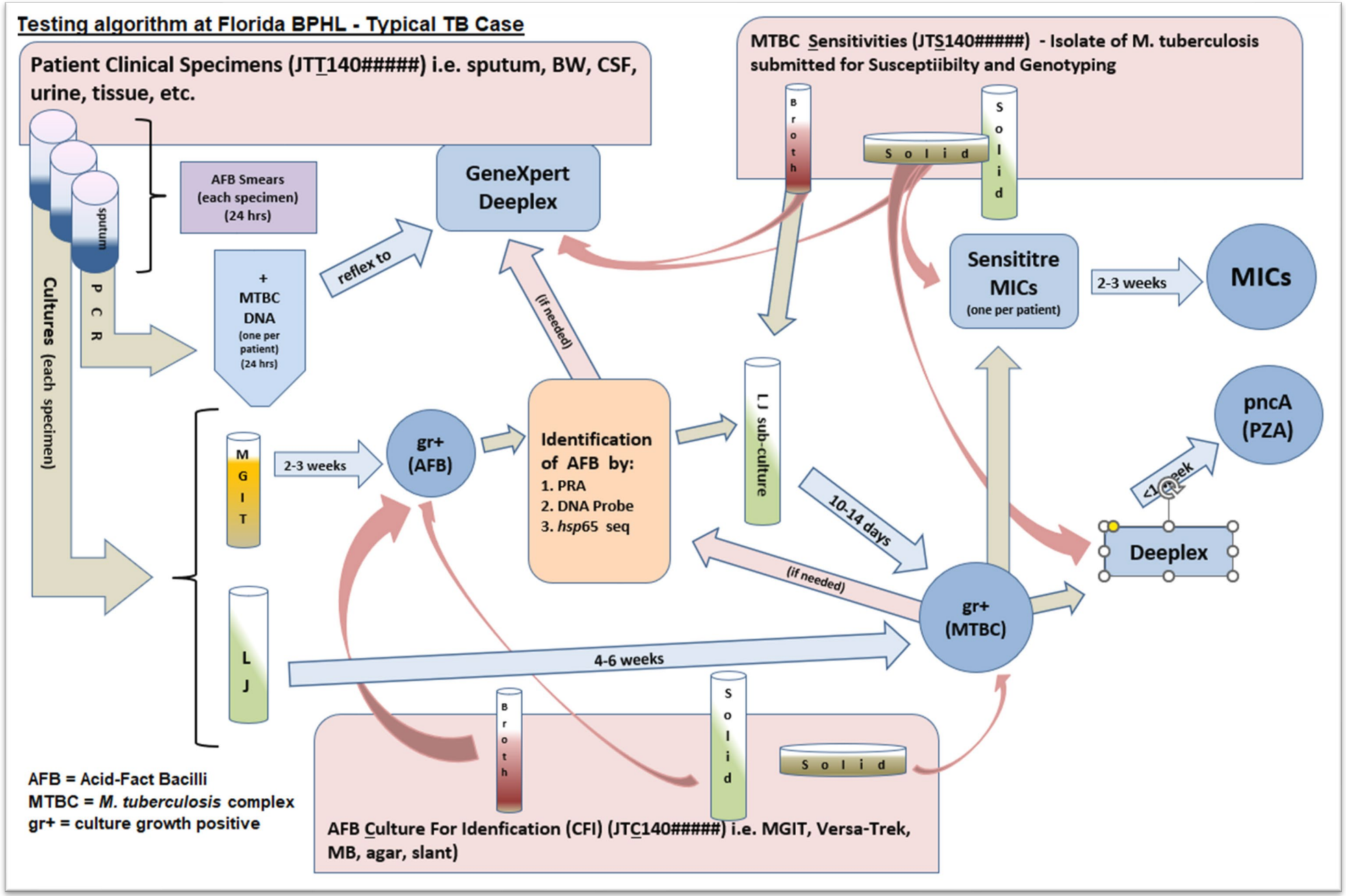


Figure 1. *Jacksonville Mycobacteriology Laboratory Workflow in Summary, 2023, retrieved from TB Staff.*

Florida HEALTH

Deeplex Workflow

Inactivation
- Boil for 30 minutes

Extraction
- Bead-Beating with Zirconium Beads
- Maxwell Promega
  - RSC Genomic DNA Kit (AS1880)
- KingFisher Flex
  - MagMax Ultra Viral/Pathogen (A42356)

Multiplex PCR
- 24-plex
- Internal Control

Deep Sequencing
- Nextera XT
  - UDI indexes
- iSeq 100 i1 v2 – 13 samples/run

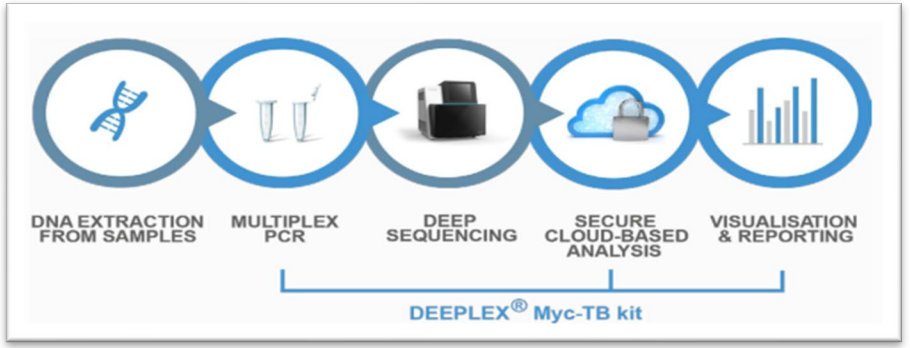


Figure 2. *Genoscreen's Workflow for Deeplex Assay. Adapted from "Deeplex® Myc-TB User Manual, 2020, retrieved from https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/.*

Genomic targets	Drugs
rpoB	rifampicin
inhA	isoniazid, ethionamide
fabG1	isoniazid, ethionamide
katG	isoniazid
ahpC	isoniazid
*pncA**	pyrazinamide
embB	ethambutol
*gidB**	streptomycin
*rpsL**	streptomycin
*rrs**	streptomycin, amikacin, kanamycin, capreomycin
eis	kanamycin
*tlyA**	capreomycin
gyrA	fluoroquinolones
gyrB	fluoroquinolones
*ethA**	ethionamide
rrl	linezolid
rplC	linezolid
*rv0678**	bedaquiline, clofazimine

Figure 3. *Targets Included With the Deeplex Assay, Adapted from "Deeplex® Myc-TB User Manual, 2020, retrieved from https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/.*

Florida HEALTH

Deeplex Turnaround Time

Platforms	Specifications	Run time (2x150pb)	Number of samples	Number of tests (including controls)
Iseq® 100	1,2Gb	19h	13	16
MiniSeq®	Mid Output (2,1Gb)	17h	21	24
	High Output (6,6Gb)	24h	66/69	72
MiSeq®	Nano kit (300Mb)	17h	1	4
	Micro kit (1,2Gb)	19h	13	16
	Full kit (4,5Gb)	24h	45	48
NextSeq®	Mid Output (32,5Gb)	26h	372	384
	High Output (100Gb)	29h	Not enough indices available	1333 (theorical)

Figure 4. *Supported Sequencing Platforms for the Deeplex Assay, Adapted from "Deeplex® Myc-TB User Manual, 2020, retrieved from https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/.*

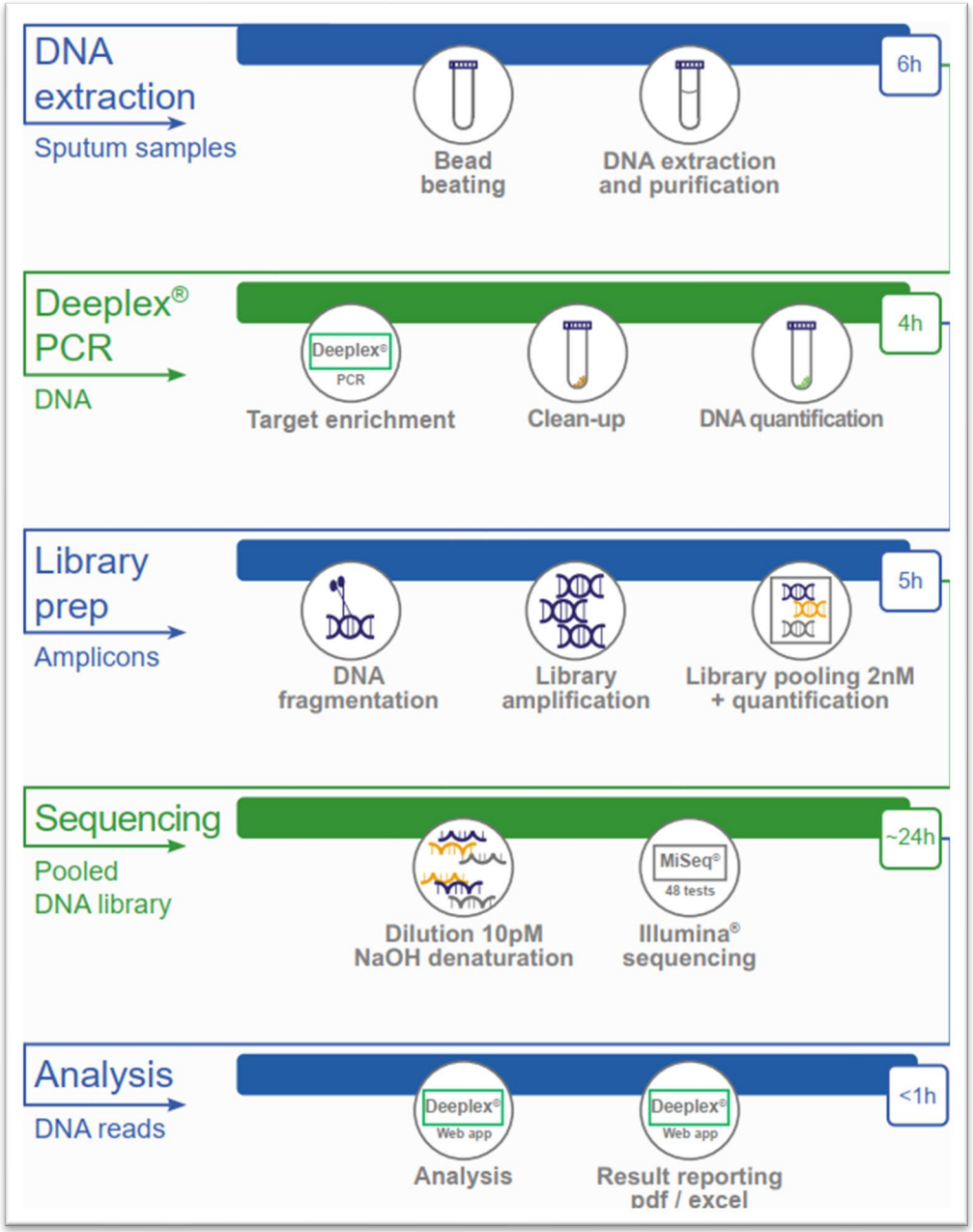


Figure 5. *Deetailed Deeplex Workflow, Adapted from "Deeplex® Myc-TB User Manual, 2020, retrieved from https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/.*

Deeplex Detailed Report

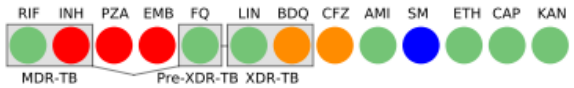
Report date May, 19 2023 08:53:15

GenoScreen Innovative Genomics

DEEPLEX® Myc-TB Report

[redacted]

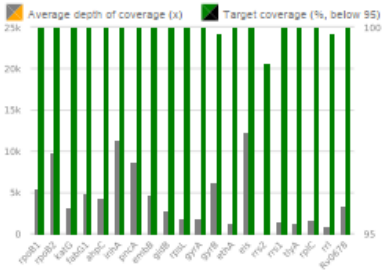
Date of submission	May, 19 2023 07:49:20
Analysis mode	Deeplex Myc-TB V3. 0.1 - Extended catalogue
Quality	⊕⊕⊕
Experiment set	230516_MS



Legend [1]

Sample controls and metrics[2]

Sequencing Run:	
Positive control*	VALID
Negative control*	VALID
Sample:	
Internal control*	VALID
Composite reference coverage	99.992%
Median depth of coverage*	4084x



***hsp65*-based species identification**

Av coverage depth (x)	Consensus length	% Identity	E-value	Best match
1298.8	400.0	100.000	0.0	*Mycobacterium tuberculosis* complex

Drug resistance associated variants[3]

Gene	Genomic position	Codon change	% Variant	Dx-score	AA change	Drug *	Confidence	Resistance level	Reference
embB	4247431	atg306ata	99.860	357.75	M306I	EMB	Associated with resistance	Resistant	WHO 2021
katG	2155168	agc315acc	100.000	142.50	S315T	INH	Associated with resistance	Resistant	WHO 2021
pncA	2288827	gtg139ctg	99.930	1883.25	V139L	PZA	Associated with resistance - Interim	Resistant	WHO 2021
rv0678	779053	cag22tag	5.040	41.50	Q22*	n/a	n/a	Resistant	n/a
rv0678	779265	delT	43.990	0.00	frameshift	n/a	n/a	Resistant	n/a
rv0678	779315	delG	9.010	0.00	frameshift	n/a	n/a	Resistant	n/a
rv0678	779447	delC	16.690	0.00	frameshift	n/a	n/a	Resistant	n/a
rv0678	779181	insG	6.920	0.00	frameshift	n/a	n/a	Resistant	n/a

Uncharacterized and uncertain significance variants[3]

Uncharacterized variants designate sequence variants of as yet unknown association with drug resistance or drug susceptibility. Uncertain significance variants designate variants that could not be characterized yet as either drug resistant or drug susceptible according to the current WHO confidence grading classification.

Gene	Genomic position	Codon change	% Variant	Dx-score	AA change	Drug*	Category	Reference
gidB	4407917	cgc98tgc	99.770	860.50	R98C	STM	Uncertain significance	WHO 2021

Spoligotype

Octal code/ Binary code	Av. coverage depth (x)	SIT	SITVIT occurence	Clade
700076777760671	2776.5	70	100	X3
11100000000011111011111111111111100001101111				

SNP-based phylogenetic lineage

Other than 4.9

Potential mixed infection

Mixed infection is signaled by a phylogenetic variant detected at less than 97%, indicating the simultaneous presence of 1 strain harboring this variant present at this percentage and another strain sharing the same sequence as the reference at this position, present at approx. 100% minus this percentage.

Not detected.

Figure 6,7. *Sample Deeplex Report, Adapted from "Deeplex® Myc-TB User Manual, 2020, retrieved from https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/.*

Florida HEALTH

Results Visualization

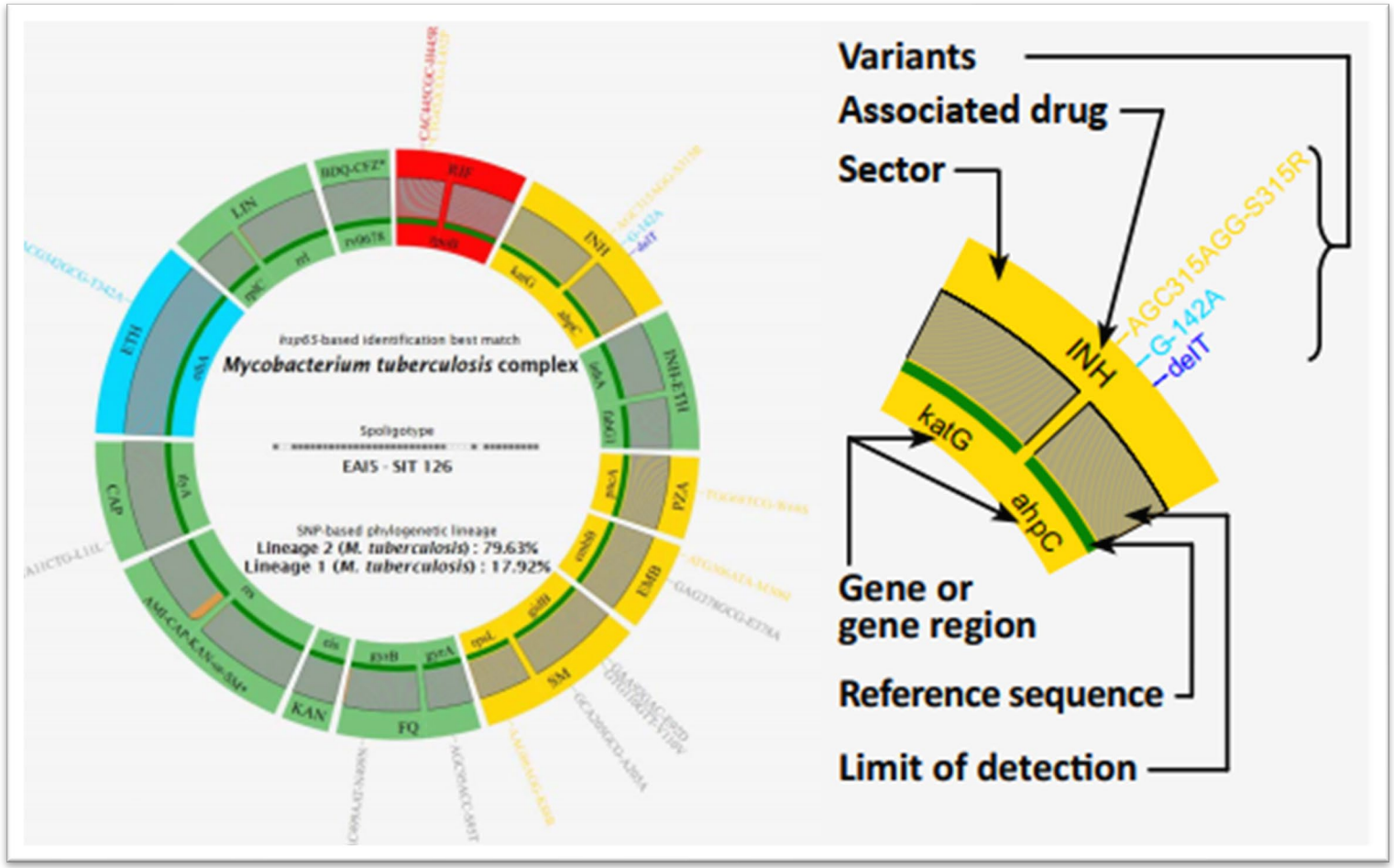
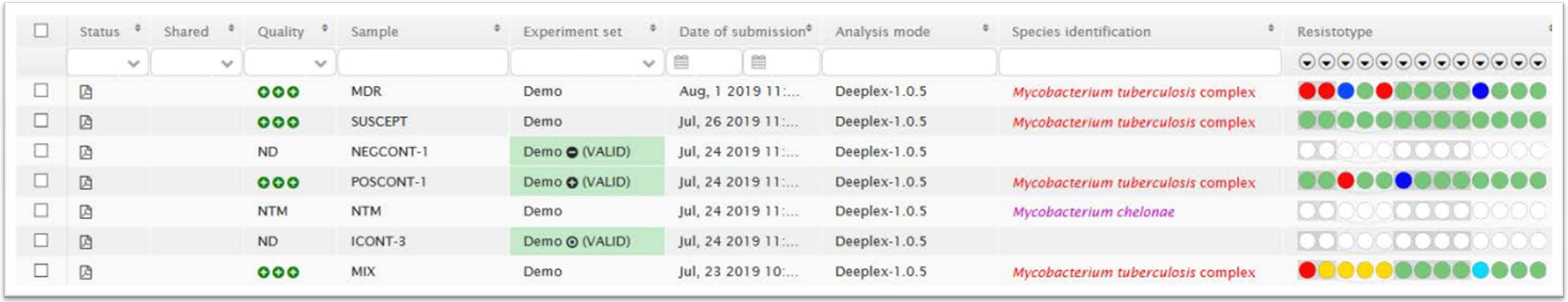


Figure 8. *Detailed Description of the deeplex map, Adapted from "Deeplex® Myc-TB User Manual, 2020, retrieved from https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/.*

Florida HEALTH

Results Visualization



Status	Shared	Quality	Sample	Experiment set	Date of submission	Analysis mode	Species identification	Resistotype
		●●●	MDR	Demo	Aug, 1 2019 11:...	Deeplex-1.0.5	*Mycobacterium tuberculosis* complex	
		●●●	SUSCEPT	Demo	Jul, 26 2019 11:...	Deeplex-1.0.5	*Mycobacterium tuberculosis* complex	
		ND	NEGCONT-1	Demo ⊖ (VALID)	Jul, 24 2019 11:...	Deeplex-1.0.5		
		●●●	POSCONT-1	Demo ⊕ (VALID)	Jul, 24 2019 11:...	Deeplex-1.0.5	*Mycobacterium tuberculosis* complex	
		NTM	NTM	Demo	Jul, 24 2019 11:...	Deeplex-1.0.5	*Mycobacterium chelonae*	
		ND	ICONT-3	Demo ⊙ (VALID)	Jul, 24 2019 11:...	Deeplex-1.0.5		
		●●●	MIX	Demo	Jul, 23 2019 10:...	Deeplex-1.0.5	*Mycobacterium tuberculosis* complex	

Figure 9. *Results Overview on the Deeplex Website, Adapted from "Deeplex® Myc-TB User Manual, 2020, retrieved from https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/.*

Tentative Implementation Plan

CLIA-Certified Assay

LIMS Integration

Broad Sample Source Validation

Single FTE for Deeplex/GeneXpert

Extraction Batching?

- Maxwell vs KingFisher vs Manual

Contact Information

Figure 10. TB Staff by Matthew Schimenti, authorized by TB Staff.

Matthew D. Schimenti MPH, M(ASCP)

Bureau of Public Health Laboratories – TB Control

Florida Department of Health

Matthew.Schimenti@Flhealth.gov

References

- Genoscreen. July 2020 – v3. Deeplex® Myc-TB User Manual
- Illumina. May 2019. Document # 15031942v05. Nextera XT DNA Library Prep
- Promega Corporation. 10/22. TM708. Maxwell® RSC Genomic DNA Kit
- ThermoFisher Scientific. Applied Biosystems. MAN0018075. B.0. MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit

Florida HEALTH

#1: How did you decide whether or not to implement tNGS?

- What factors contributed to the decision?
- Were your TB program and clinicians involved in discussions?
- Labs that did not implement: What factors (if any) would steer you toward implementation?

#2: If you decided to implement tNGS. How did you determine which methodology/platform to utilize?

#3: How many and what type of samples did you use to validate your assay? Where did you obtain them and what was the comparator method?

#4: How did you choose which data analysis pipeline to use? What factors did you take into consideration?

#5: What was the main challenge encountered while bringing on a tNGS assay?

#6A: How would you address your physician and TB program needs in terms of result report content and formatting?

- What considerations affected your selection of reportable targets for tNGS assay?

#6B: What is your process for sending isolates out for tNGS?

- How are results passed on to partners (are results forwarded as is or is some kind of manual entry or reformatting required)?
- Are results sent to submitters, TB programs or both?
- How do you maintain the ability to address any questions related to the results?

#7: How does your decision to implement or not implement tNGS impact laboratory workflows, turn around times, personnel, and costs?

#8: How was tNGS incorporated into your testing algorithm? Do you see this being sustainable?

Questions?