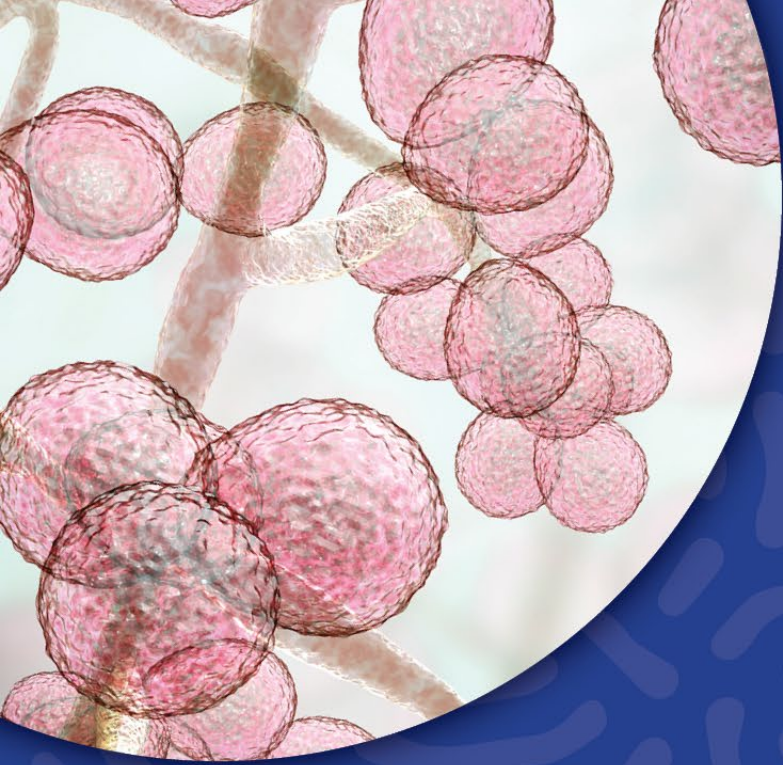
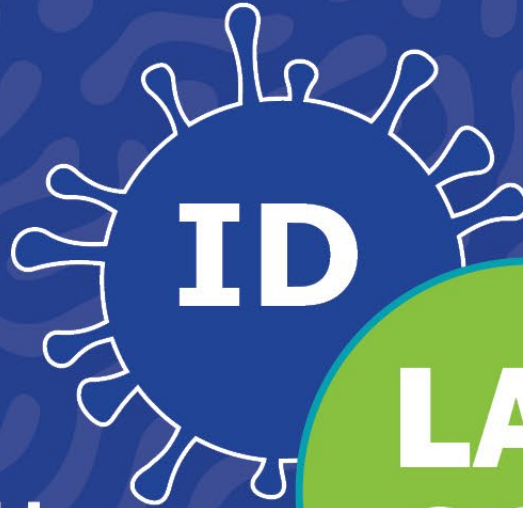


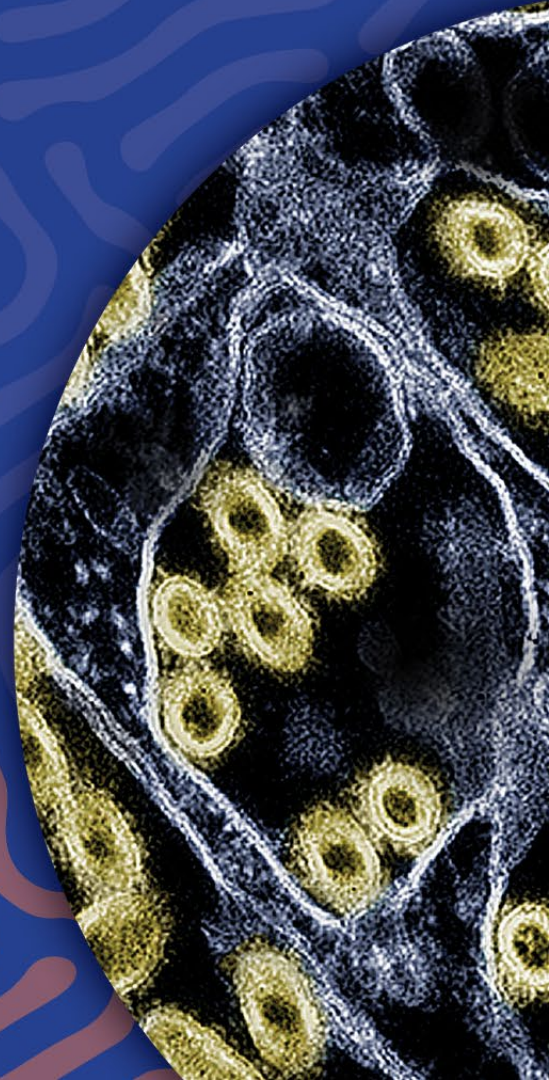


2025



**LAB
CON**

#IDLabCon





Workup of clinical samples for NTM and the role of whole genome sequencing in a public health laboratory

#IDLabCon

Alana Sterkel, PhD, D(ABMM), SM(ASCP)CM

Associate Director, Communicable Disease Division

Wisconsin State Laboratory of Hygiene

Assistant Professor, DPLM, SMPH, UW-Madison



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No Conflicts of Interest

Issues Facing Clinical Mycobacteriology Labs

- Discontinuation of AFB testing products
 - Despite global significance, mycobacteria testing is not financially attractive in the US
- Discontinuation of Hologic Mycobacteria AccuProbes in late 2022

HOLOGIC

AccuProbe®

**MYCOBACTERIUM TUBERCULOSIS
COMPLEX CULTURE IDENTIFICATION TEST**

INTENDED USE

The ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of *Mycobacterium tuberculosis* (TB Complex) isolated from culture. The TB Complex consists of the following species: *M. tuberculosis*, *M. bovis*, *M. bovis BCG*, *M. africanum*, *M. microti* (8), and *M. canetti* (11).

<https://www.hologic.com/package-inserts/diagnostic-products/accuprobe-culture-identification-tests>

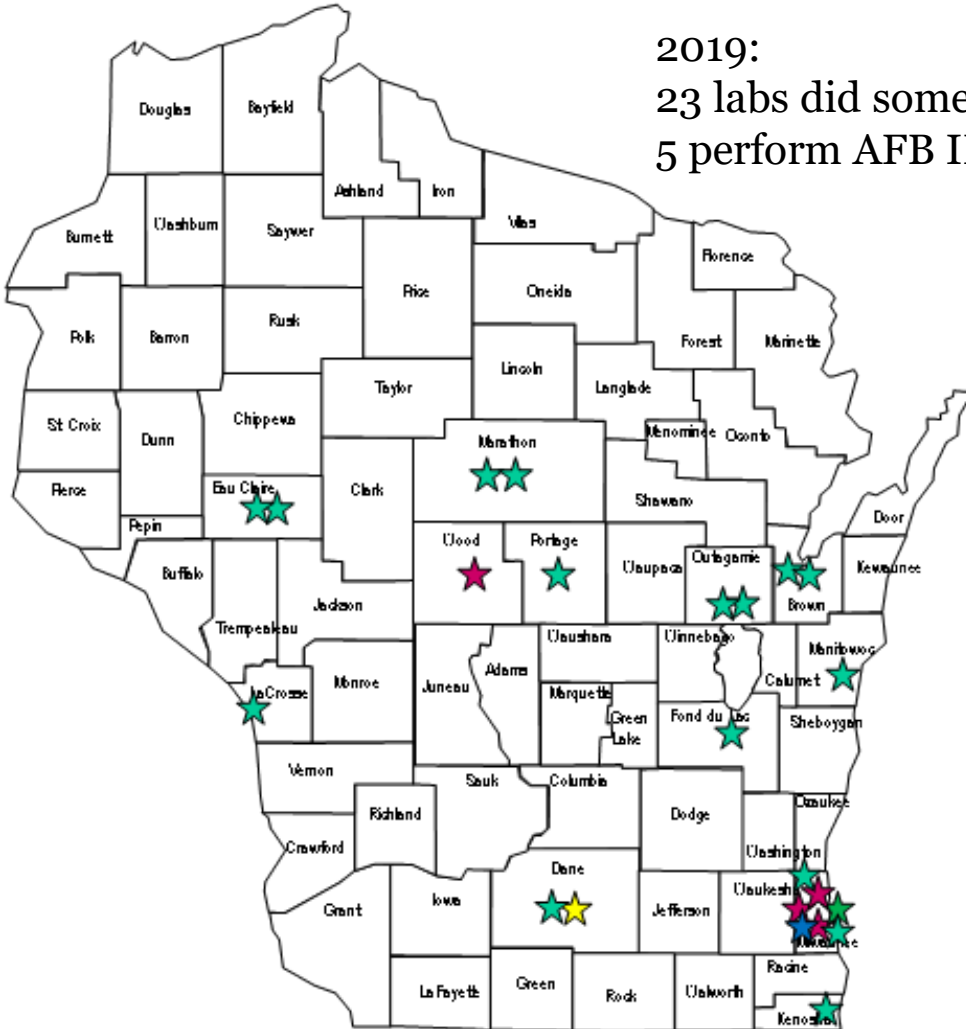
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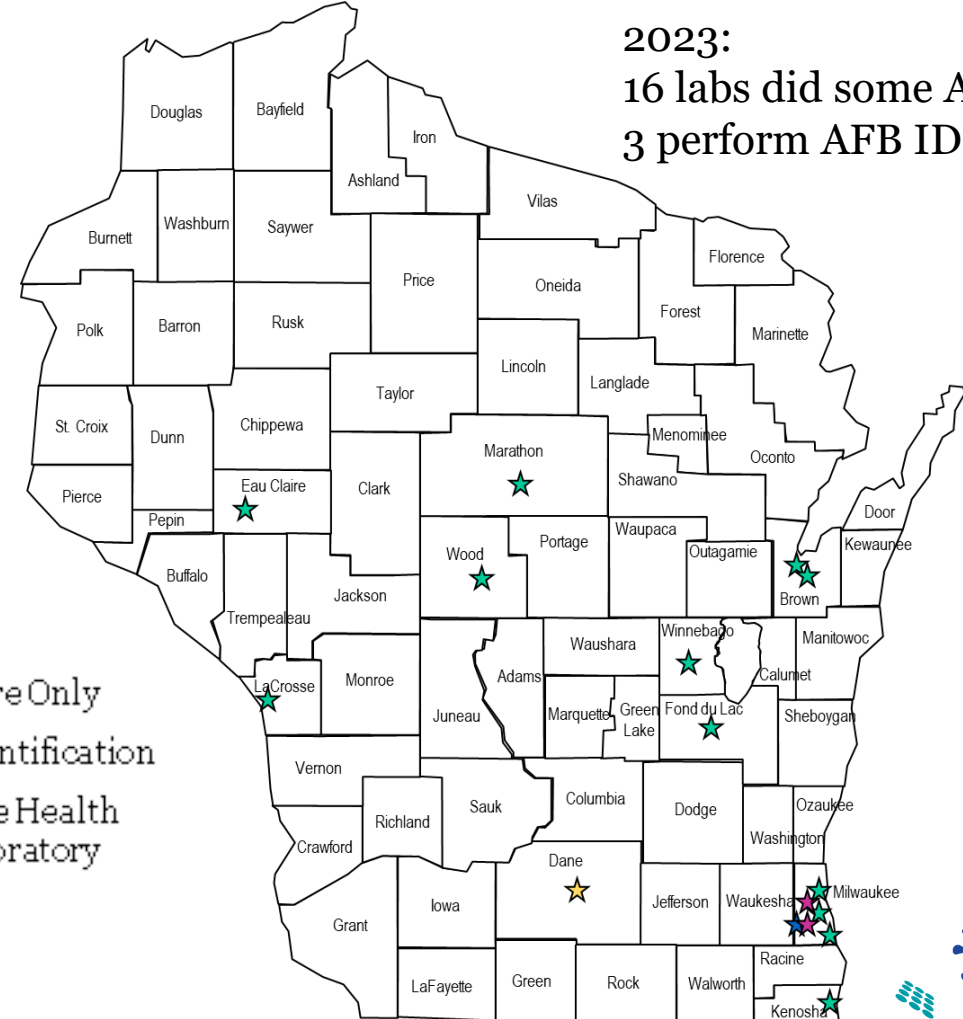
Consolidation of testing

Wisconsin Mycobacteria Laboratory Network

2019:
23 labs did some AFB testing
5 perform AFB ID



2023:
16 labs did some AFB testing
3 perform AFB ID



- ★ Smear and Culture Only
- ★ Some level of Identification
- ★ City of Milwaukee Health Department Laboratory
- ★ WSLH

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Mycobacteriology at the WI State Lab of Hygiene (WSLH)

Full-service mycobacteriology laboratory

- Primary diagnostic clinical testing
 - From WI clinical labs
 - Public Health Departments state-wide
- Reference laboratory
 - Mycobacteria ID and NAAT testing for clinical labs in WI
- Public health laboratory
 - TB Surveillance
 - Outbreak investigations



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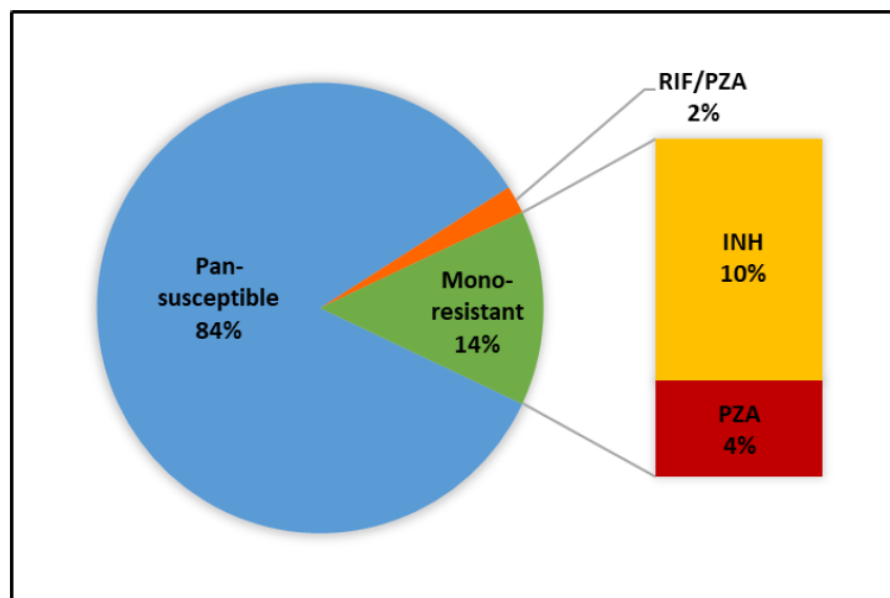
WI is a Low Incidence TB State

Number of Wisconsin Patients with New Isolations of *Mycobacterium tuberculosis* complex

County of Residence	Brown	Dane	Eau Claire	Kenosha	La Crosse	Lafayette	Manitowoc	Marathon	Milwaukee	Pierce	Racine	Sauk	Sheboygan	Waukesha	Waupaca	TOTALS
Pulmonary	2	8	2	1	1	1	1	3	12	1	4	1	1	1	1	40
Extra-pulmonary*	1	1				1		1	4				1	1		10
Totals	3	9	2	1	1	2	1	4	16	1	4	1	2	2	1	50

(‡) 5 cases were clinically diagnosed with no culture growth

(*)Extra-Pulmonary sources of isolation: 3 lymph node, 2 neck, 1 abscess, 1 peritoneal fluid, 1 pleural fluid, 1 lung aspirate, 1 gastric aspirate



WSLH Clinical Specimen Volume

Figure 1. Distribution of Public Health Laboratory Testing Volumes Measured by Total Number of Clinical Specimens Received, 2015 and 2017.



*These jurisdictions referred all TB testing to another laboratory.

Note: DC=Washington, D.C., PHI=Philadelphia, SAN=San Diego, SFO=San Francisco, HOU=Houston, NYC=New York City, LAX=Los Angeles. All others are U.S. Postal Service abbreviations.

Source: Centers for Disease Control and Prevention

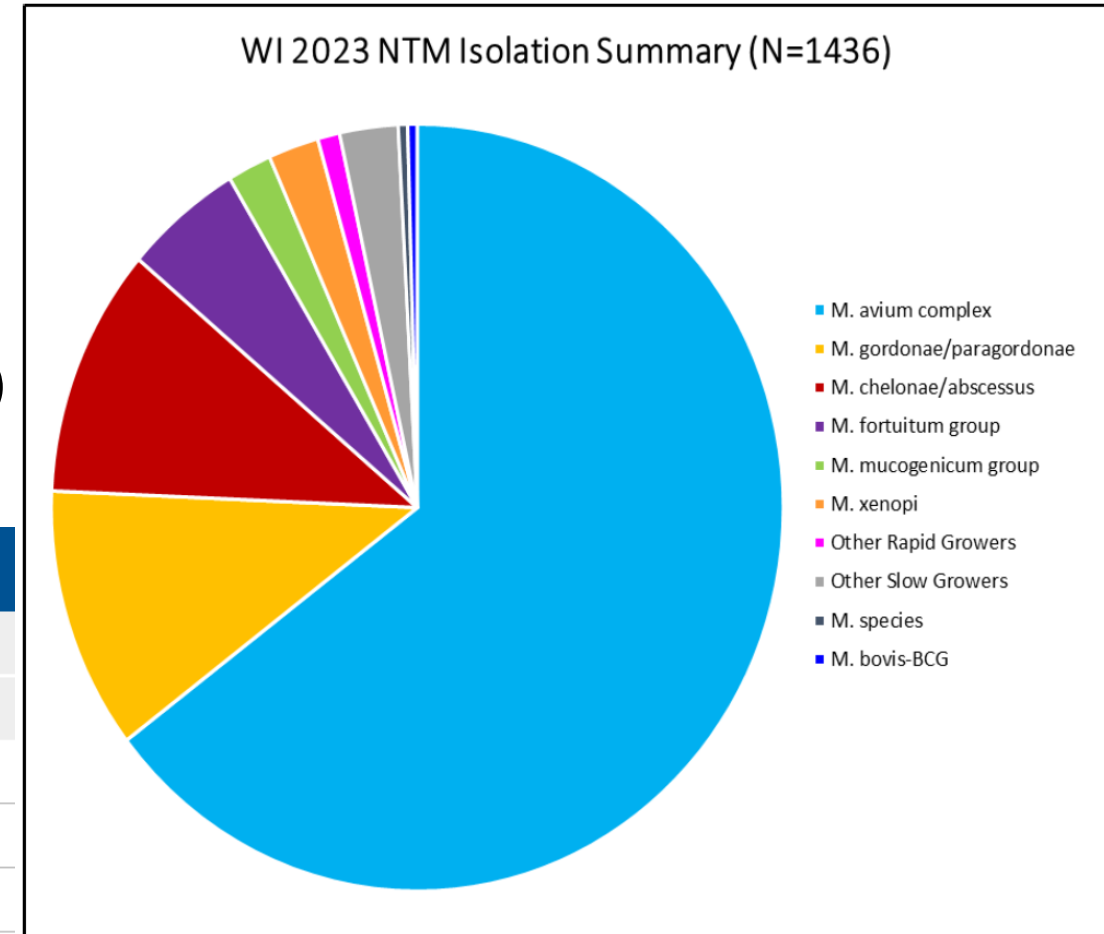


NTMs in Wisconsin

- NTMs are reportable in Wisconsin
- Physician did a 7 year review (2011-2018)

Table 2. Frequency of total, respiratory, and nonrespiratory isolates by nontuberculosis mycobacteria species in Wisconsin adults, 2011-2018

NTM Species	Number (Column %) of Isolates		
	Total (N = 8,135)	Respiratory (n = 7,337)	Nonrespiratory (n = 798)
<i>M. avium</i> complex	5,872 (72.2)	5,608 (76.4)	264 (33.1)
<i>M. chelonae-abscessus</i> grp	779 (9.6)	534 (7.3)	245 (30.7)
<i>M. fortuitum</i> grp	483 (5.9)	365 (5.0)	118 (14.8)
<i>M. mucogenicum</i>	172 (2.1)	132 (1.8)	40 (5.0)
<i>M. xenopi</i>	165 (2.0)	163 (2.2)	2 (0.3)
<i>M. peregrinum</i>	116 (1.4)	111 (1.5)	5 (0.6)
<i>M. kansasii</i>	70 (0.9)	64 (0.9)	6 (0.8)
<i>M. terrae</i> complex	39 (0.5)	35 (0.5)	4 (0.5)



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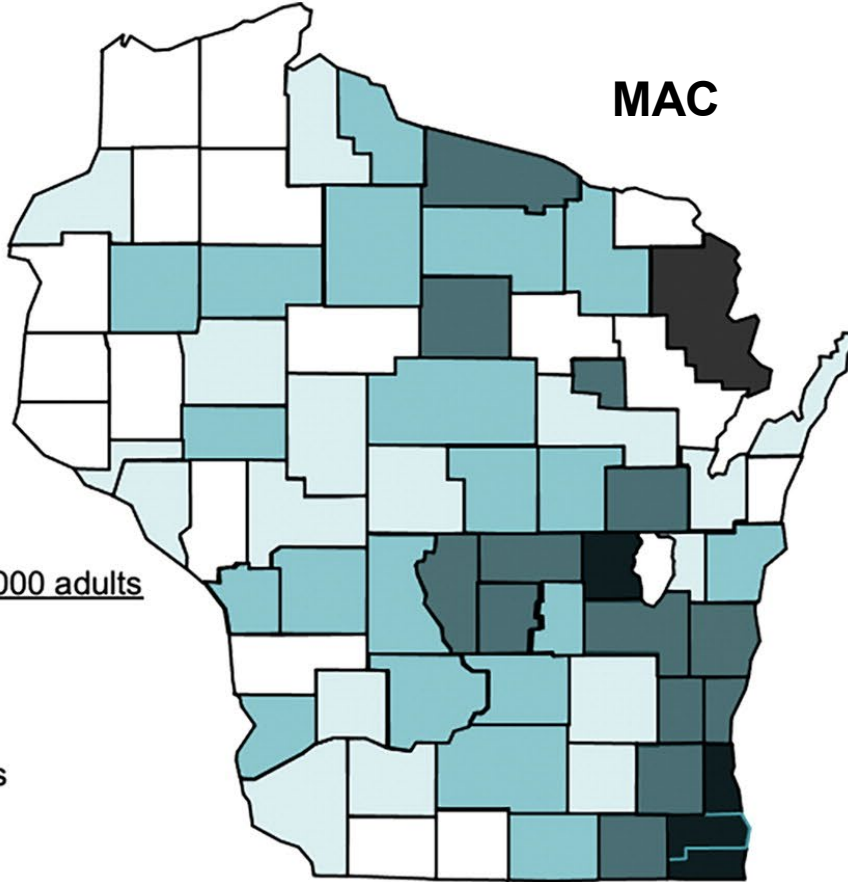
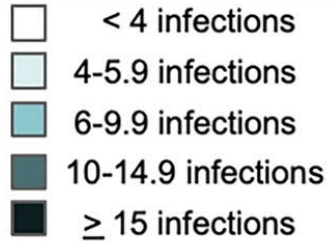


NTM Distribution

A

MAC

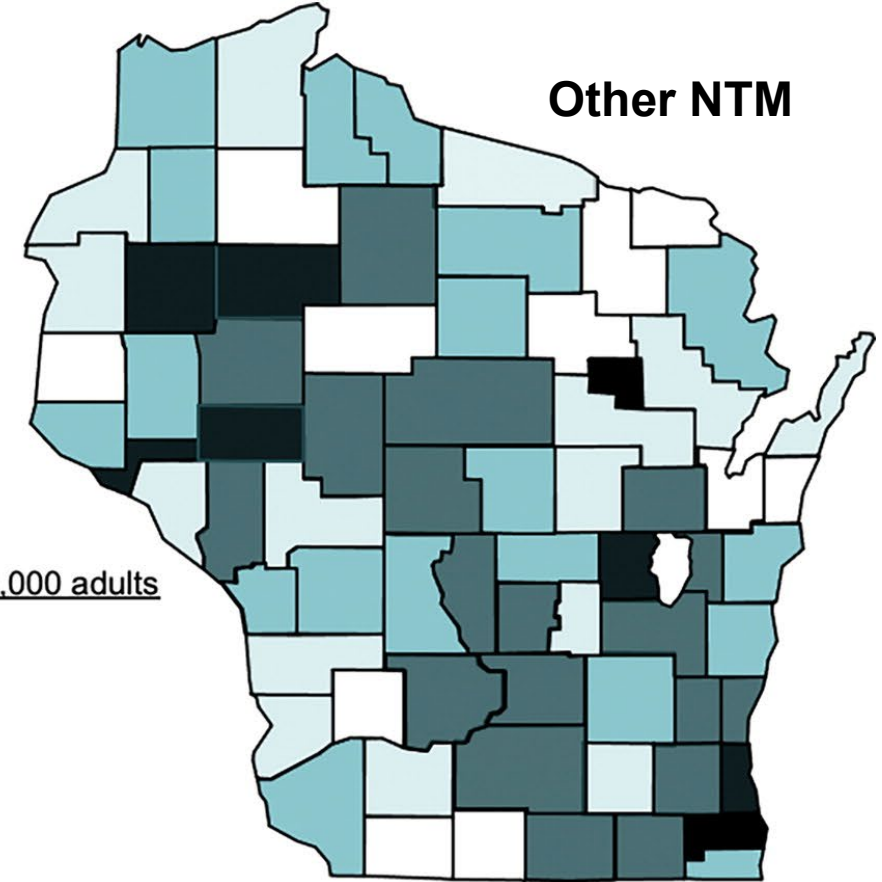
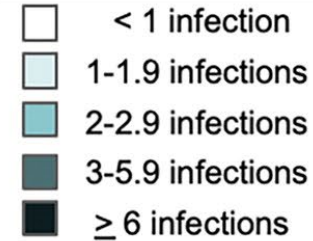
Annual Incidence/100,000 adults



B

Other NTM

Annual Incidence/100,000 adults



*Rates are stable



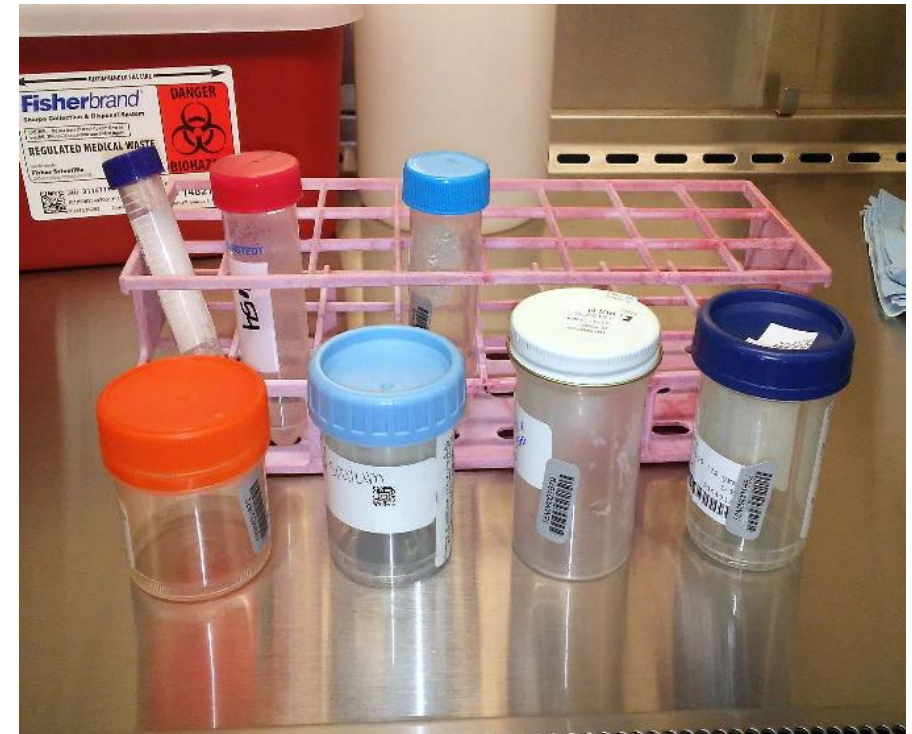
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WSLH Mycobacteriology Testing

Specimen Types

Almost any source is acceptable for AFB culture and smear

- Sputum
 - Induced
 - Expecterated
- Bronchial washing/BAL
- Gastric aspirate
- Fresh tissue
- Bone
- Blood
- Bone Marrow
- CSF
- Body fluids
- Abscess
- Stool
- Urine
- Skin



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Mycobacteriology Testing at WSLH- Processing

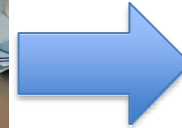


Patient Specimen

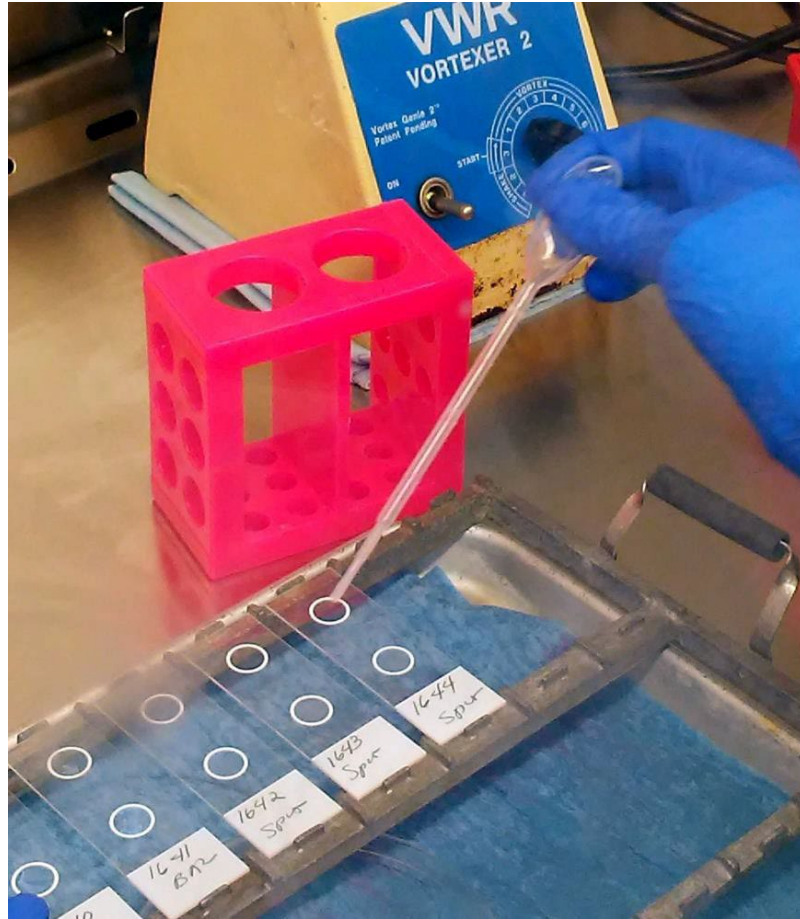


Decontamination and
Concentration
(NALC/NaOH)

- **Digestion:** 0.5% NALC; agent used to liquefy sputum by breaking down mucus and freeing bacilli from cells or tissue
- **Decontamination:** 2% NaOH; agent used to kill normal respiratory flora that grow faster than MTBC and would otherwise overgrow cultures



Mycobacteriology Testing at WSLH- Staining



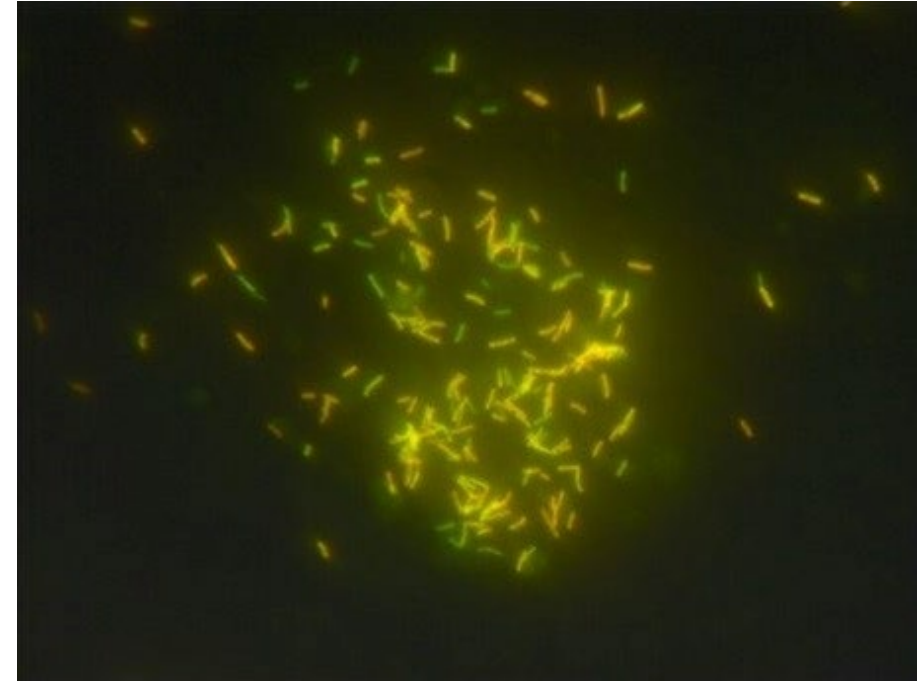
Patient Specimen



Decontamination and
Concentration
(NALC/NaOH)



Smear Microscopy
(Auramine O)



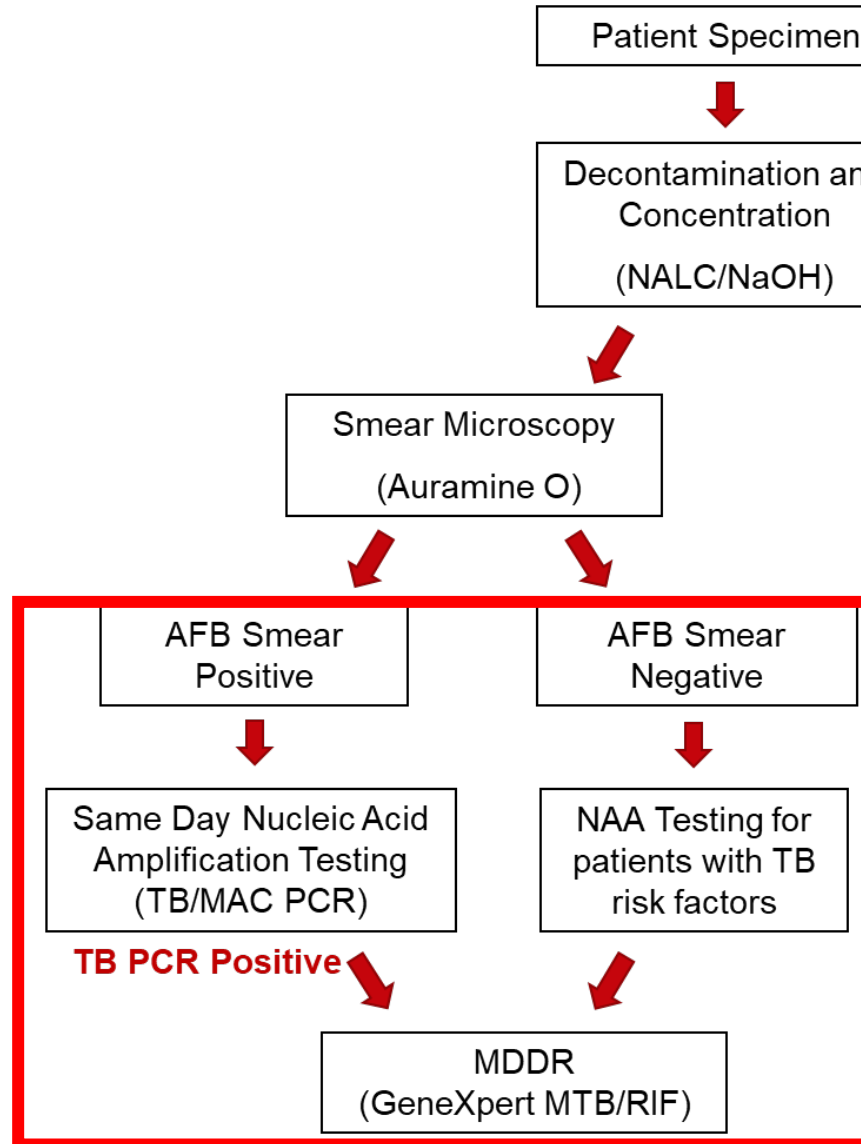
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Mycobacteriology Testing at WSLH- PCR



Xpert MTB/RIF



WSLH has been running a laboratory developed real-time TB PCR (Wadsworth) assay since 2011

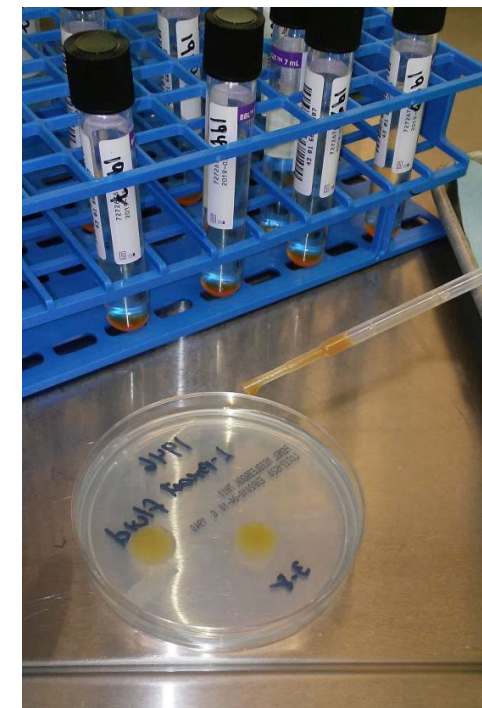
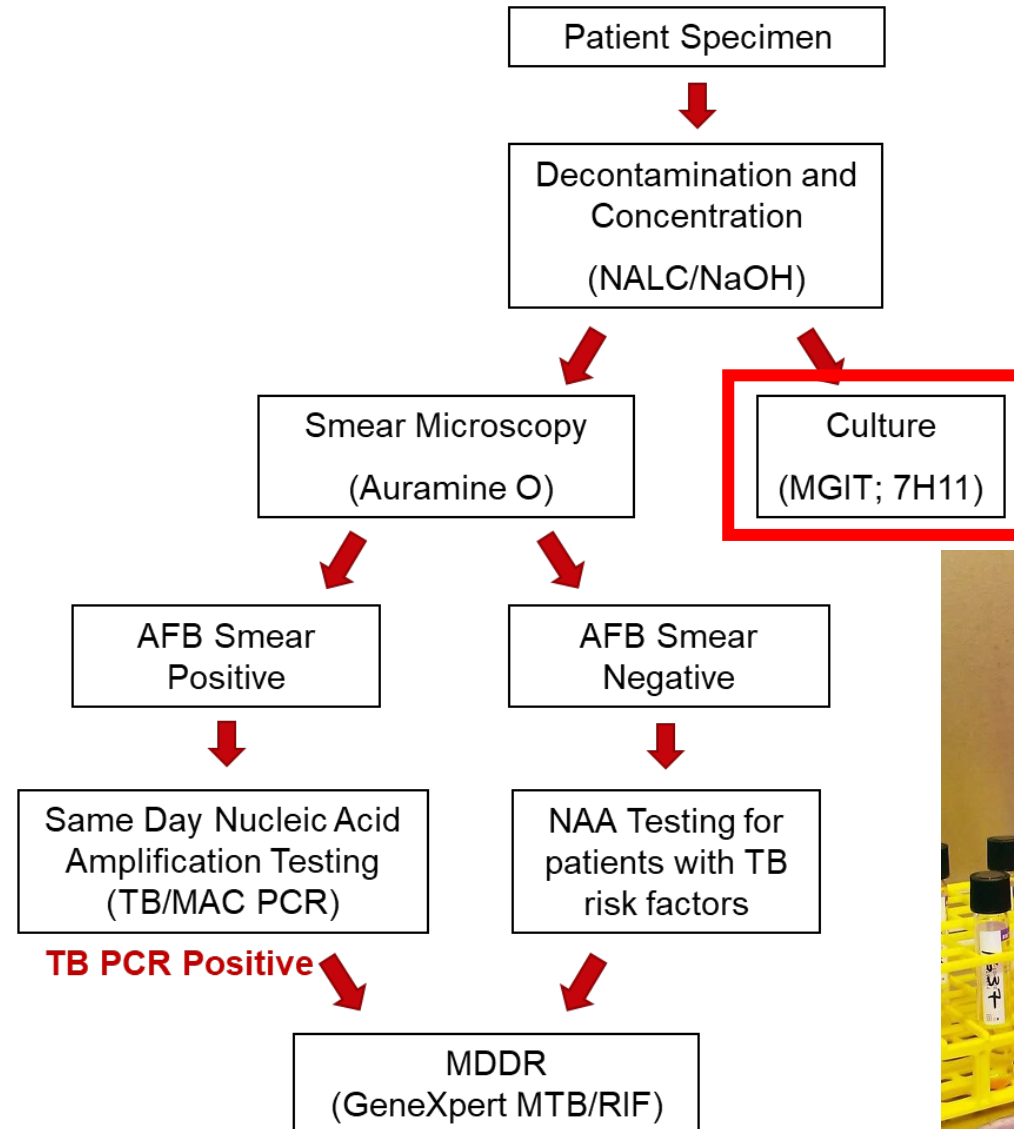
Added MAC in 2013

Added *M. gordonae* in 2024

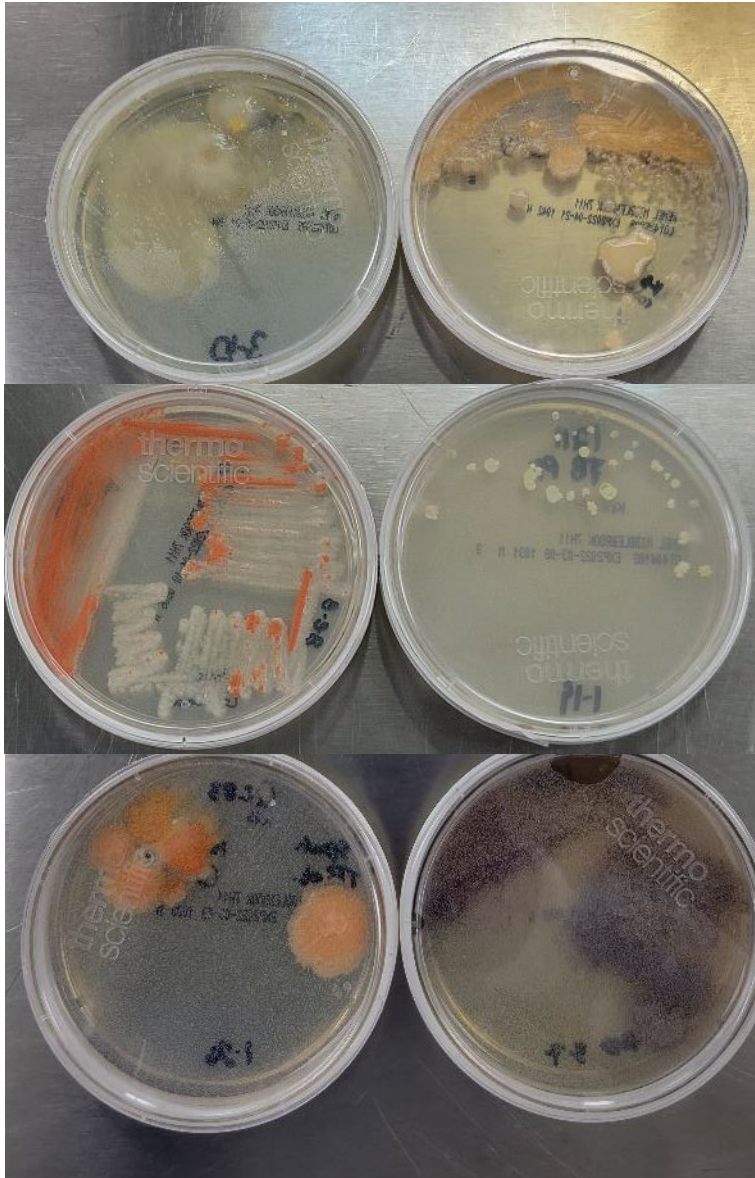
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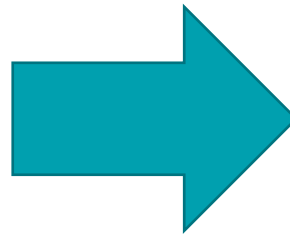
Mycobacteriology Testing at WSLH- Culture



Mycobacterial Culture: Re-decontamination



Re-decontamination



Mycobacteriology Testing at WSLH- Culture



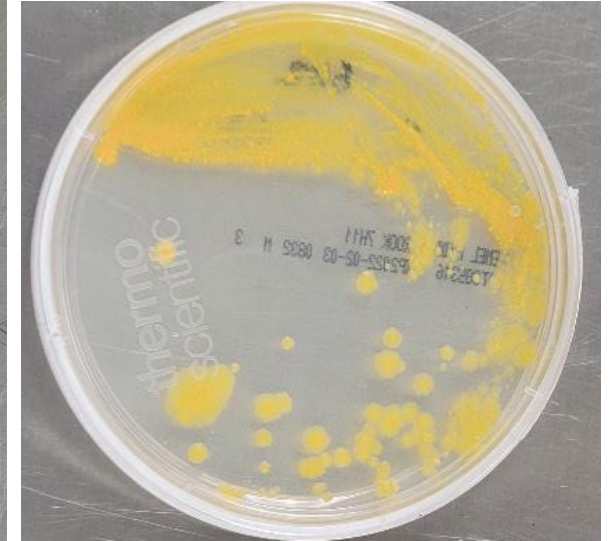
BD BACTEC MGIT 960



7H9 Broth

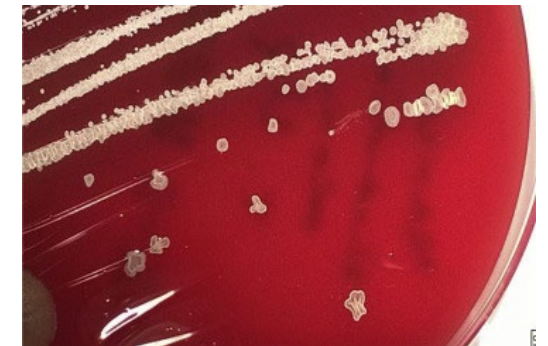


7H11 Media



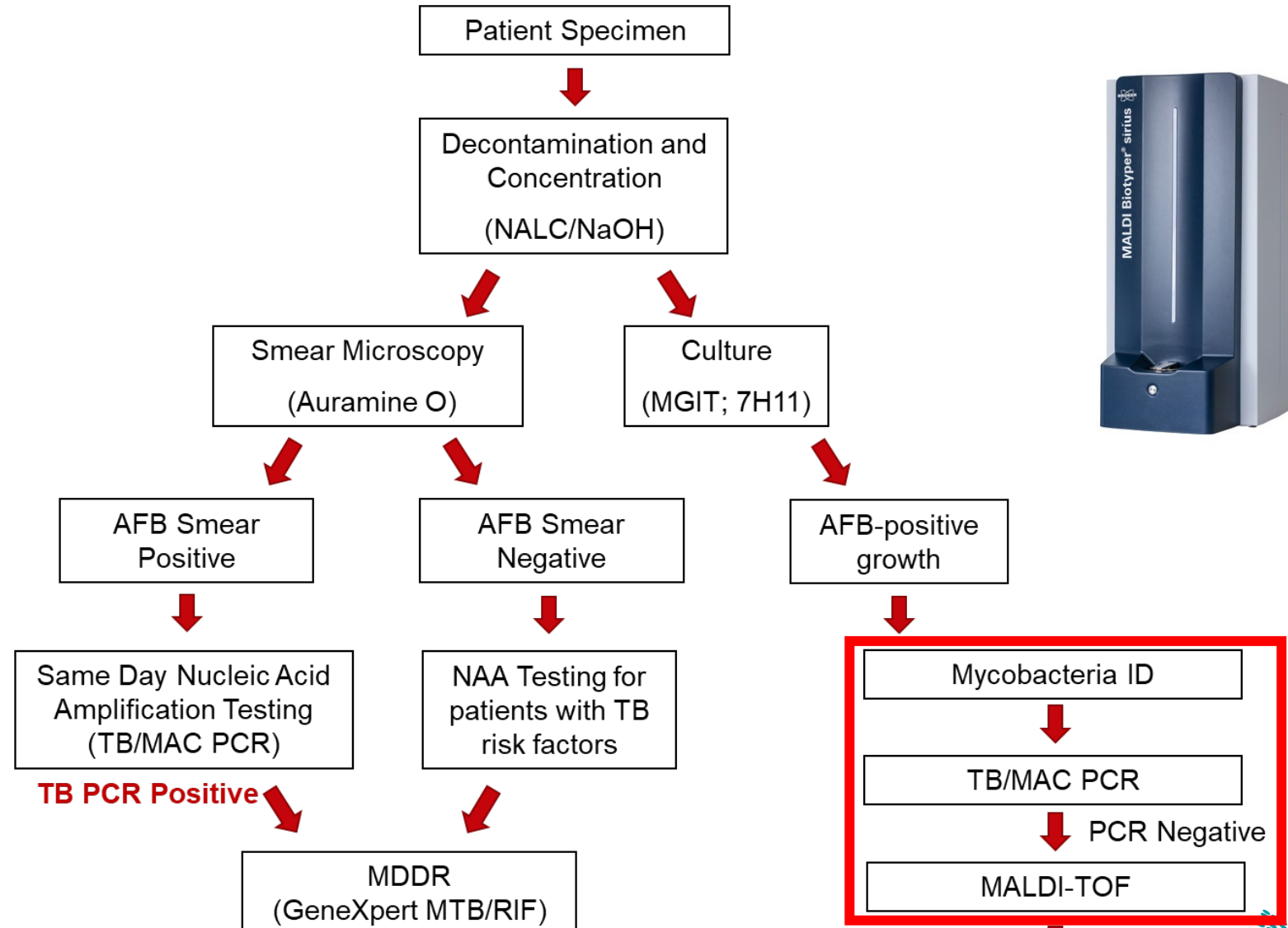
Special Conditions

- Mycobactin J (*paratuberculosis*, *genavense*)
- Ferric ammonium citrate for (*heamophilum*)
- Oxoid X factor (*haemophilum*)
- 31 C (*marinum*) and 42 C (*xenopi*)



Blood agar

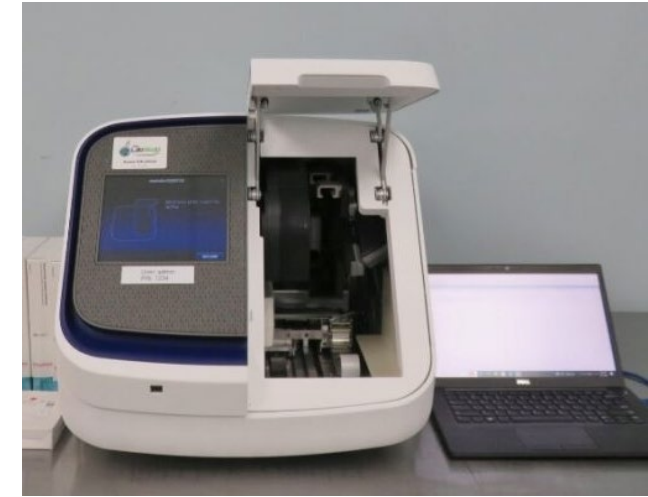
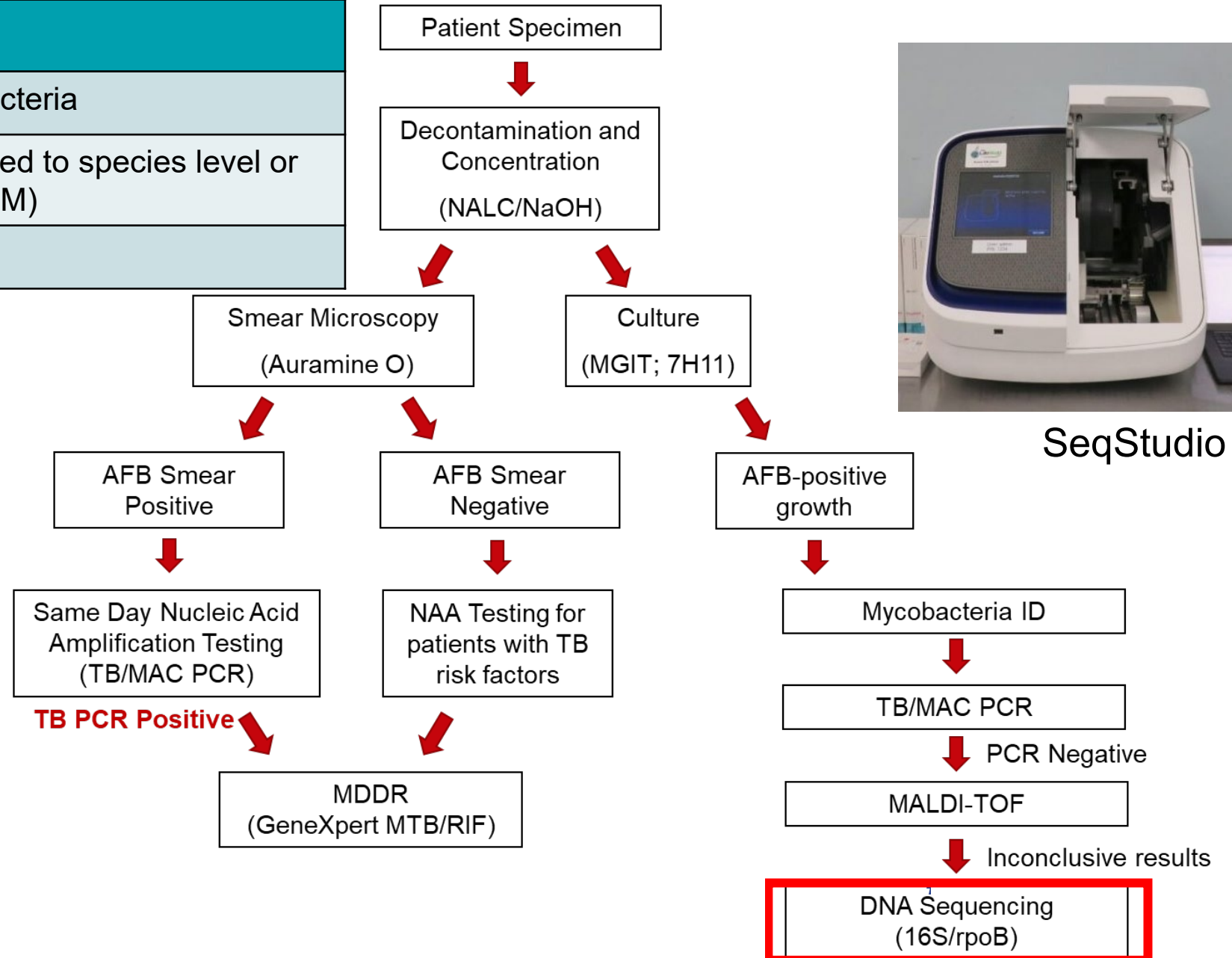
Mycobacteriology Testing at WSLH- Identification



Mycobacteriology Testing at WSLH- Identification

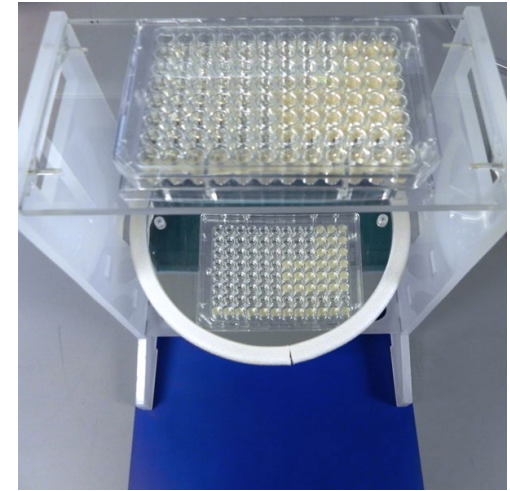
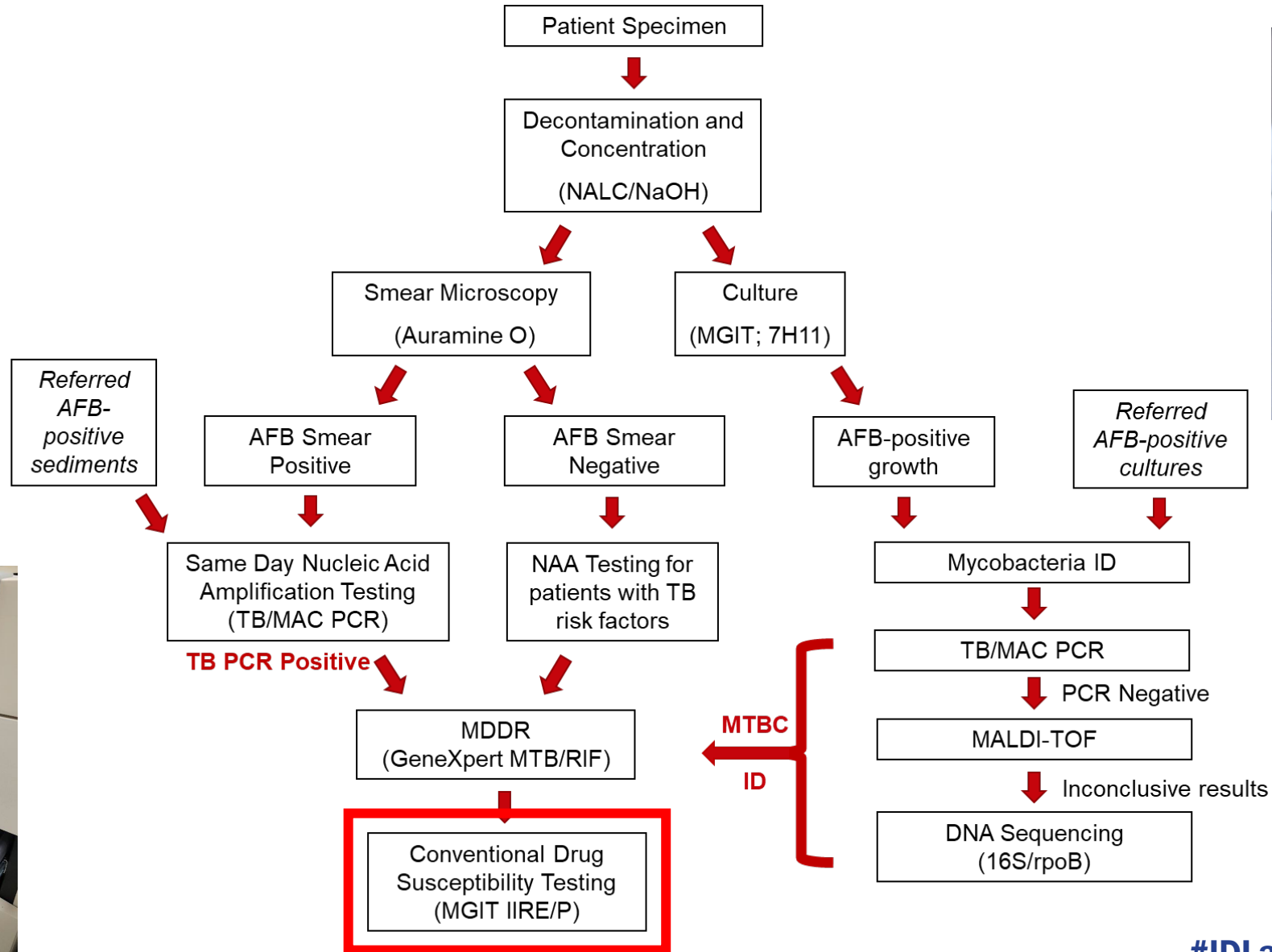
WSLH Results

Negative	Negative for mycobacteria
Positive	Mycobacteria identified to species level or complex (TB and NTM)
	<i>Nocardia</i> sp.

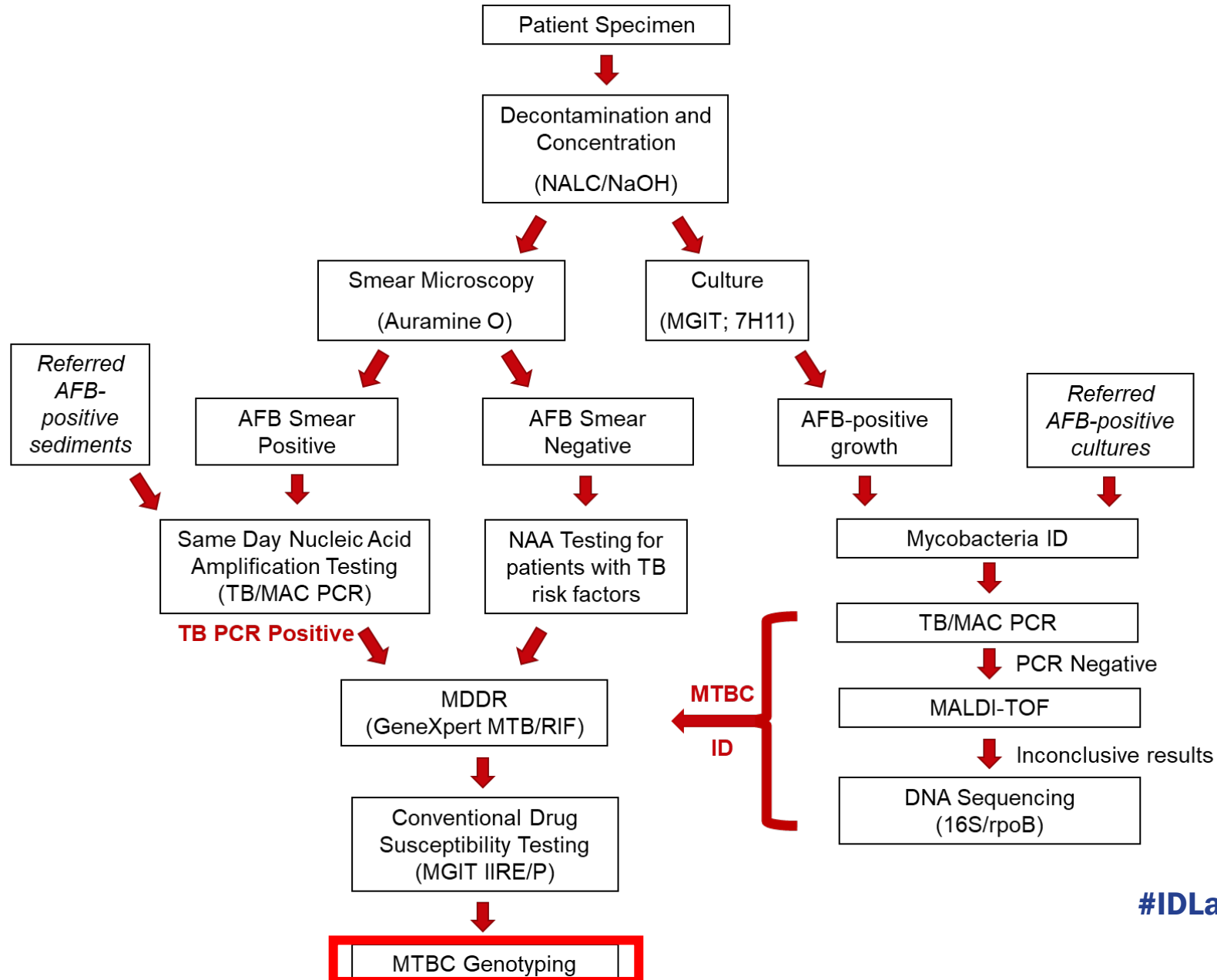


SeqStudio

Mycobacteriology Testing at WSLH



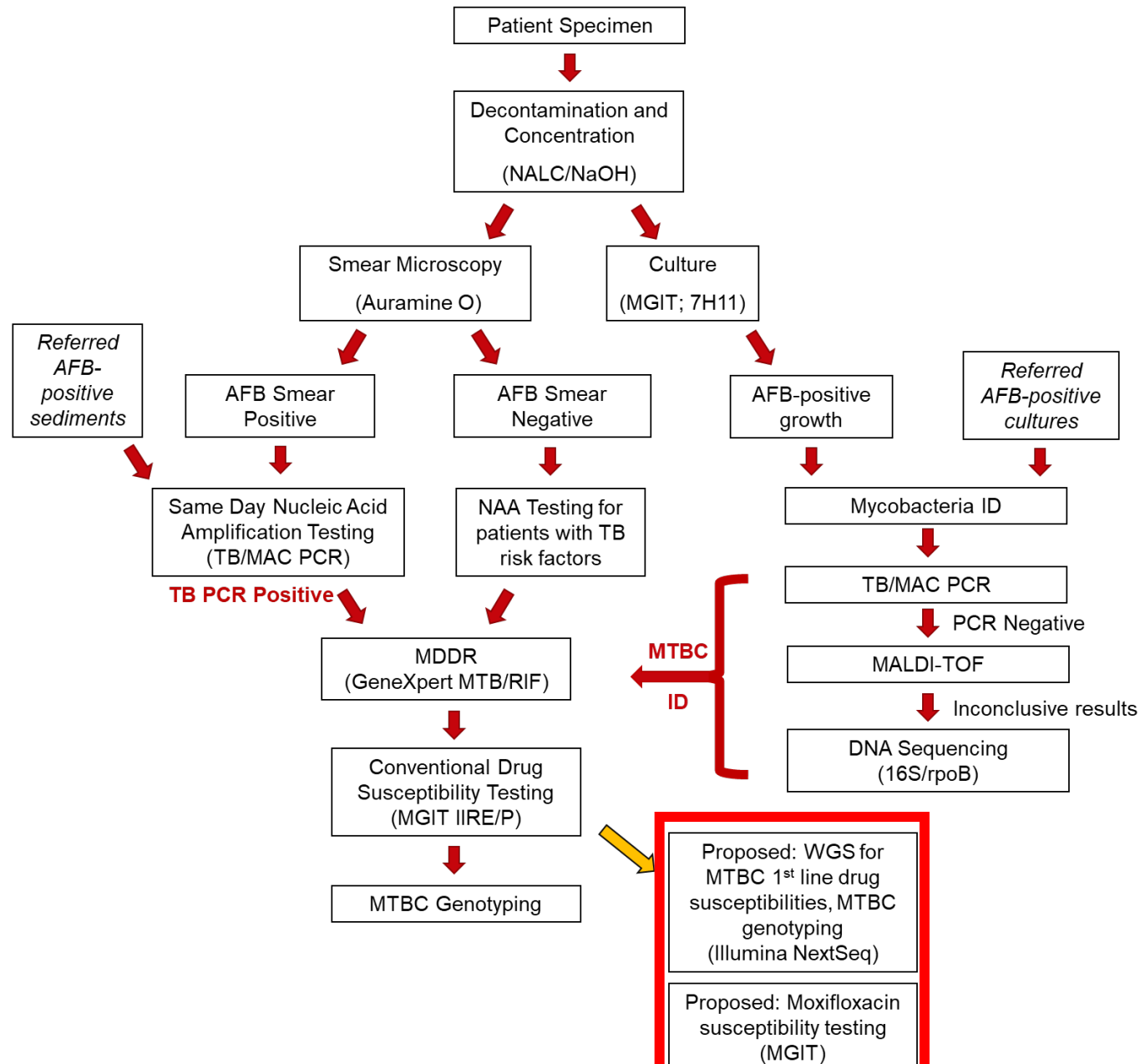
Mycobacteriology Testing at WSLH



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Mycobacteriology Testing at WSLH



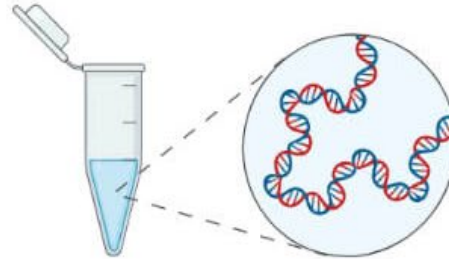
#IDLabCon



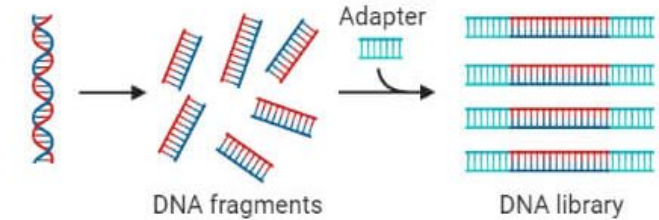
Next-generation sequencing (NGS)

- Millions of short DNA strands ('reads') per specimen
- Whole Genome Sequencing (WGS) vs targeted NGS
- tNGS may be better for primary specimens

Step 1:
DNA extraction

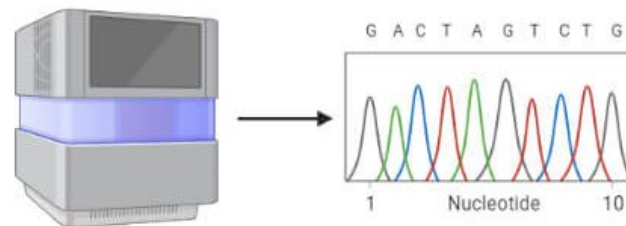


Step 2:
Library preparation

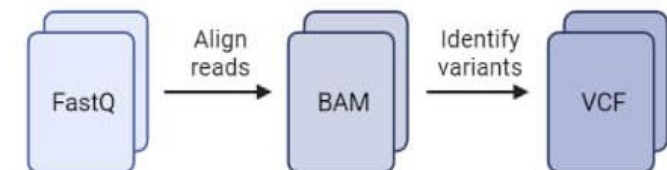


Next Generation Sequencing Workflow

Step 3:
Sequencing

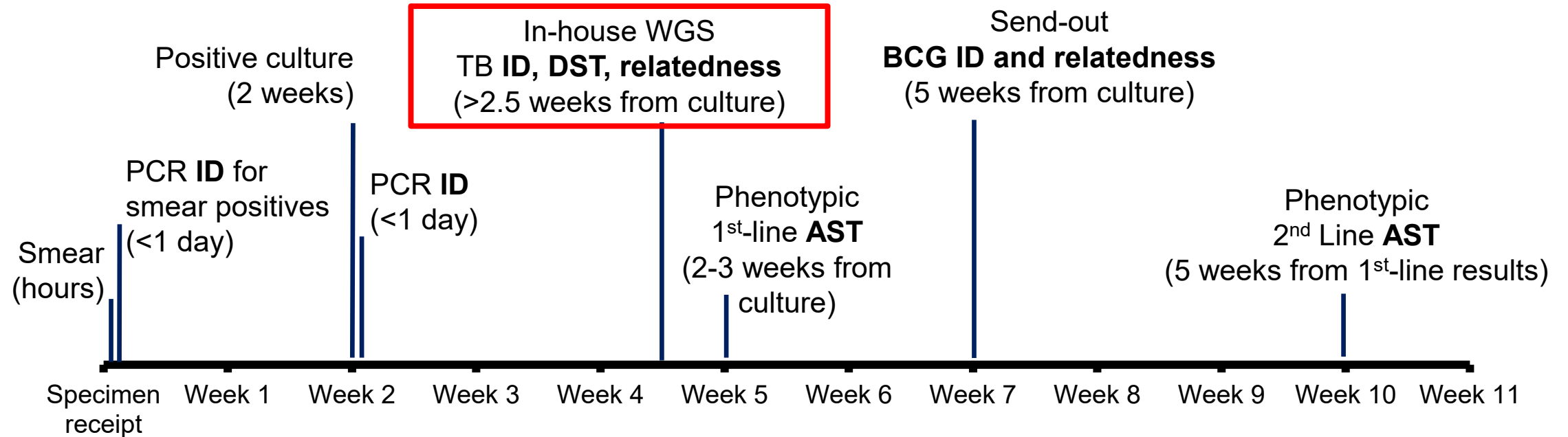


Step 4:
Analysis



<https://www.biorender.com/template/next-generation-sequencing-workflow>

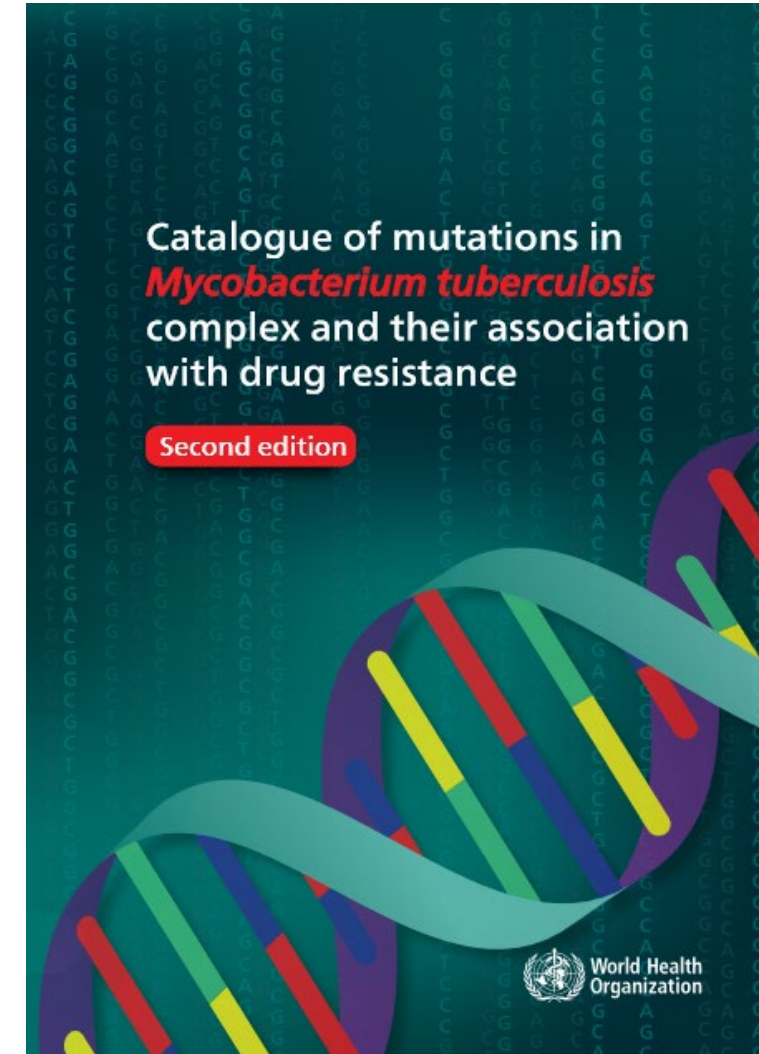
One of the Few Times Sequencing Can Be Faster



TB WGS

TB is an excellent organism for WGS

- TB mutation rate is very slow (0.3-0.5 SNP/genome/year)
- Minimal evidence of recombination or plasmid transfer
- WHO has compiled extensive list of MTBC mutations and their significance
 - 144 pp, >52,000 isolates sequenced



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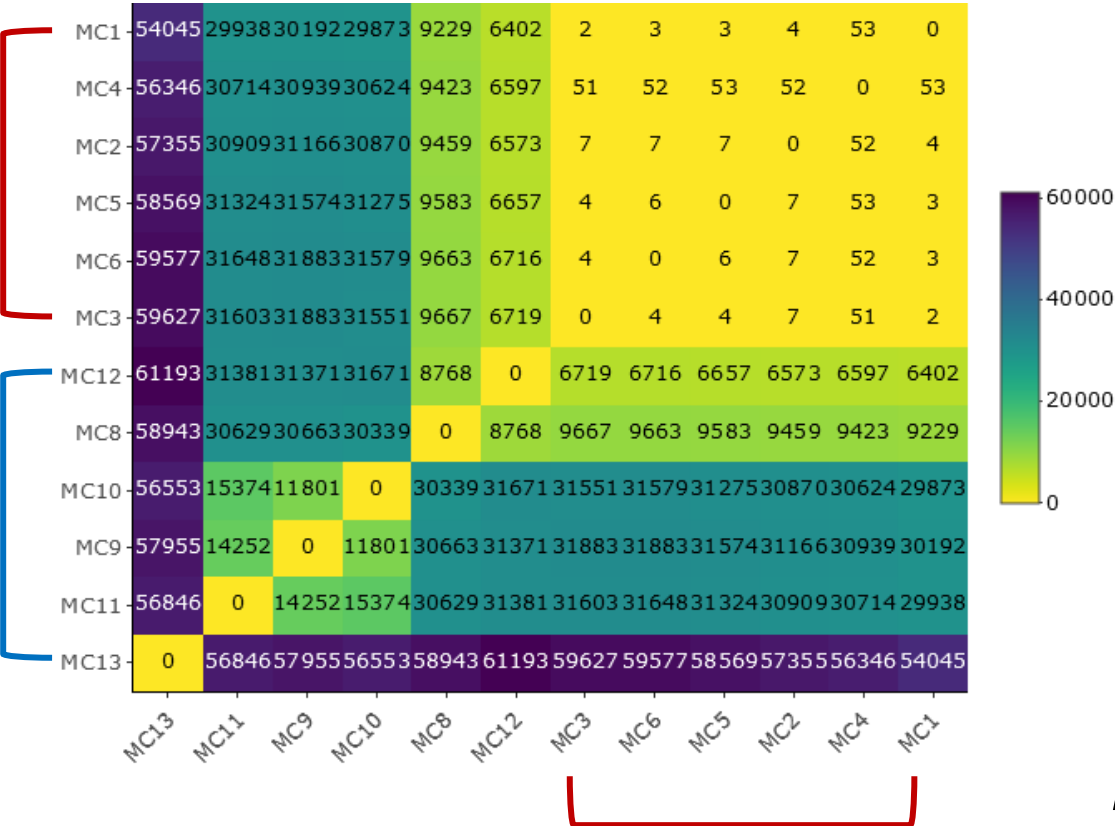


Method Comparison

	PCR	Sanger sequencing	Next-generation sequencing
Cost	\$	\$\$	\$\$\$\$
Time Required	Hours	1-2 days	~ 4-7 days
Percentage of genome targeted	<1%	<1% to 10%	100%
Result	Positive vs. negative, Identify individual mutations	Genotype, Identify multiple mutations in single gene	Genotype, Relatedness, Identify mutations in many different genes, DST

Non-tuberculous Mycobacteria: Outbreak Investigation A

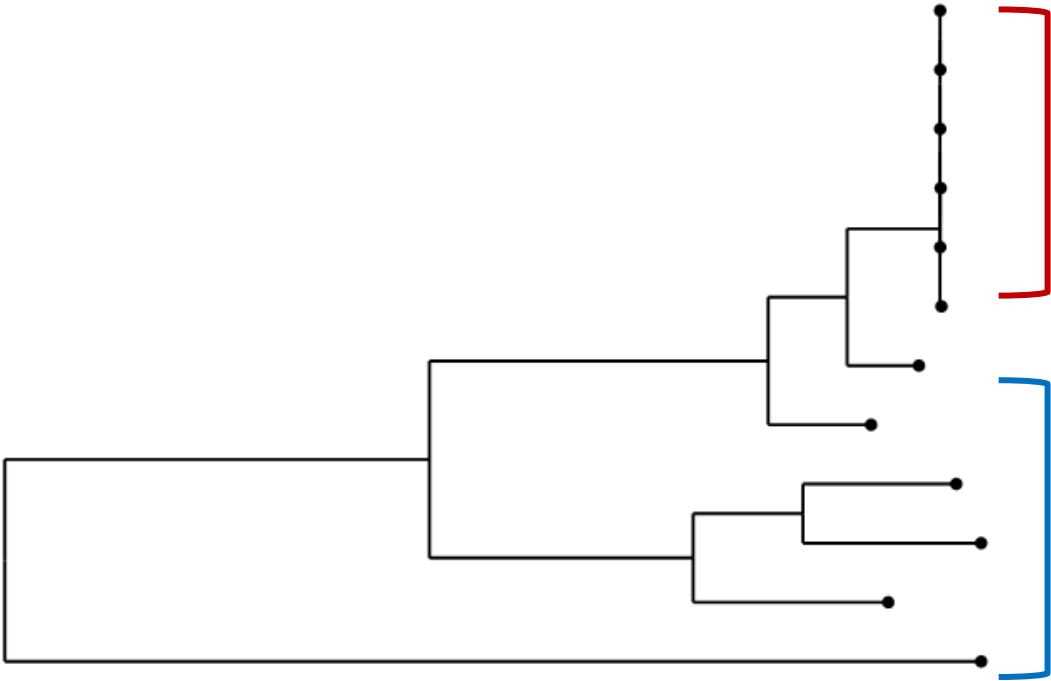
RELATEDNESS HEATMAP
(# SNP)



Suspect patient isolates

Outgroup (species-matched; from other hospitals in WI)

PHYLOGENETIC TREE

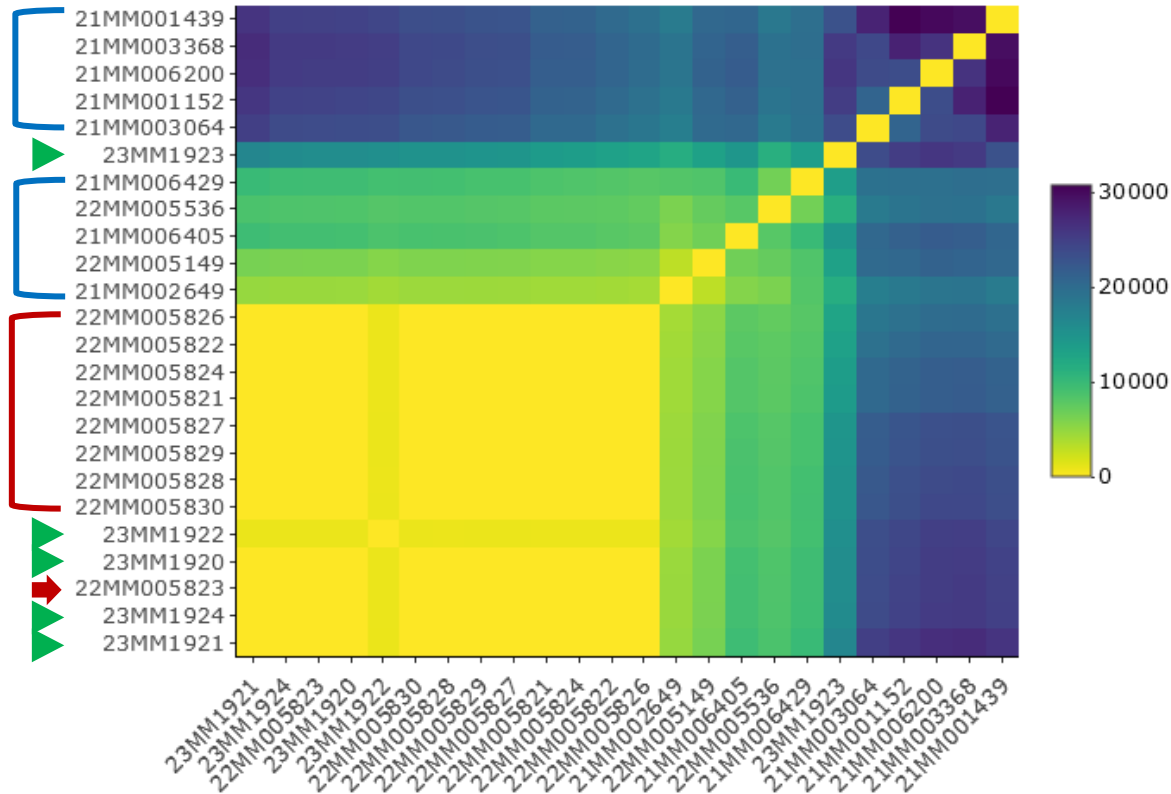


M. mucogenicum

- Rapid grower in fungal cultures
- Patients all had a bronchoscopy
- Environmental testing identified an ice maker

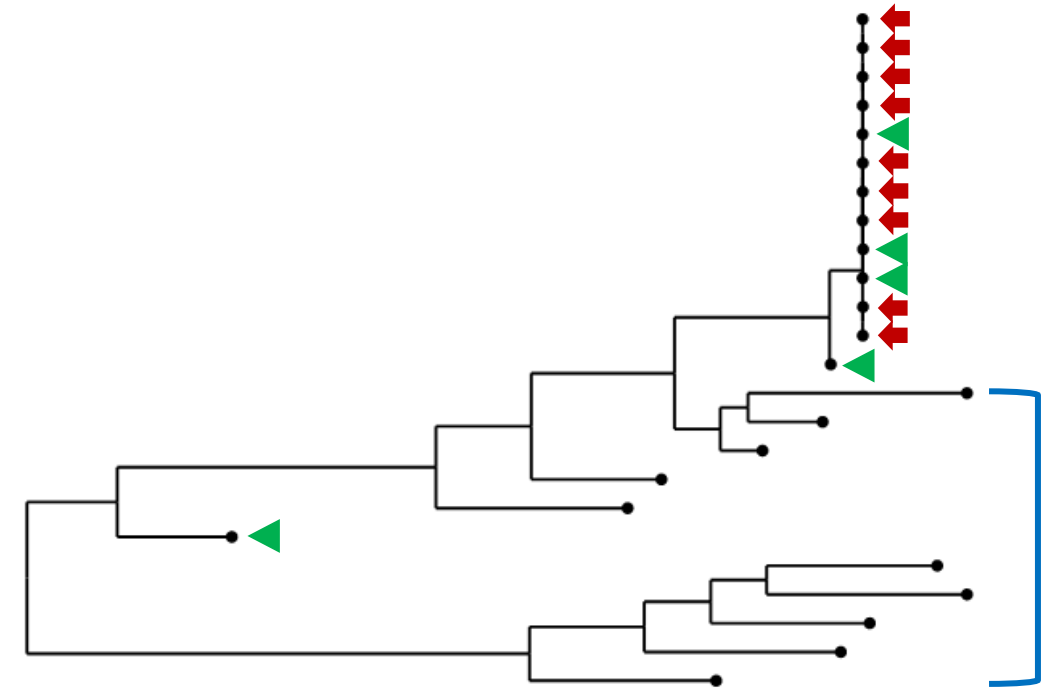
Non-tuberculous Mycobacteria: Outbreak Investigation B

**RELATEDNESS HEATMAP
(# SNP)**



- ➡ **Suspect patient isolates**
- ▶ **Environmental isolates (surgical equipment/suite)**
- Outgroup (species-matched; from other hospitals in WI)**

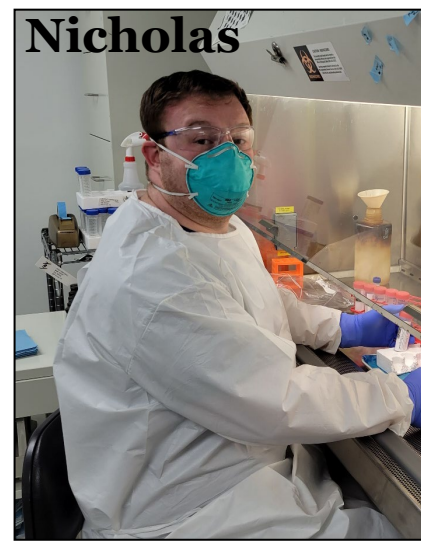
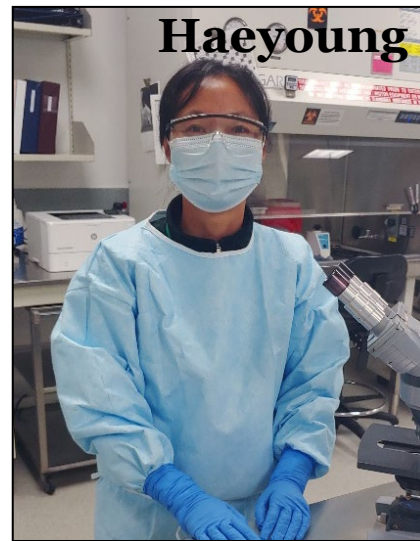
**PHYLOGENETIC
TREE**



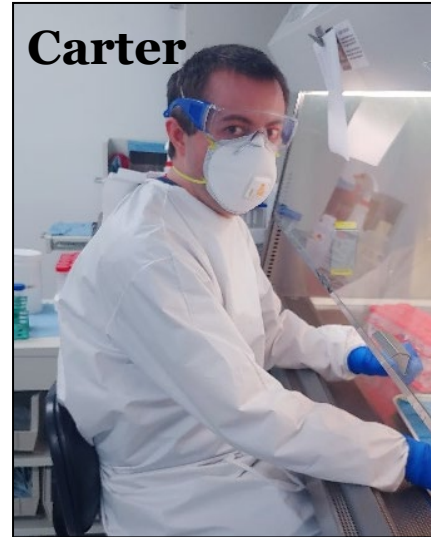
M. chelonae

- Rapid grower in fungal cultures
- Patients all had a bronchoscopy
- Environmental testing identified an ice maker

WSLH Teams



Chrystal



Lab Manager

Laura Louison

Bioinformatics

Kelsey Florek

Abigail Shockey

CJ Jossart

Eva Gunawan

Thomas Bladder

Sequencing

Alicia Mooney

Ava Gehrke-Laundrie

Claire LeFleur

#IDLabCon



Questions

- Should NTM be reportable/notifiable?
- Is sequencing surveillance needed for NTM?
- Should every PHL sequence Mycobacteria?

