14th National Conference on
Laboratory Aspects of Tuberculosis

March 10–12, 2026 | Atlanta, Georgia

Which Way to Go?
Diagnostic Decision Making for TB and NTM

Session: Which way to go?

Diagnostic Decision Making for TB and NTM

Max Salfinger, March 11 , 2026 – Atlanta, GA

USF Health
UNIVERSITY of SOUTH FLORIDA

Topics

- Epidemiology of Tuberculosis
- Epidemiology of nontuberculous mycobacterial pulmonary disease
- Ideal algorithm for the laboratory diagnosis of mycobacterial disease
- Ad hoc laboratory survey results
- Implementing change
- Conclusion

U.S. Centers for Disease Control and Prevention

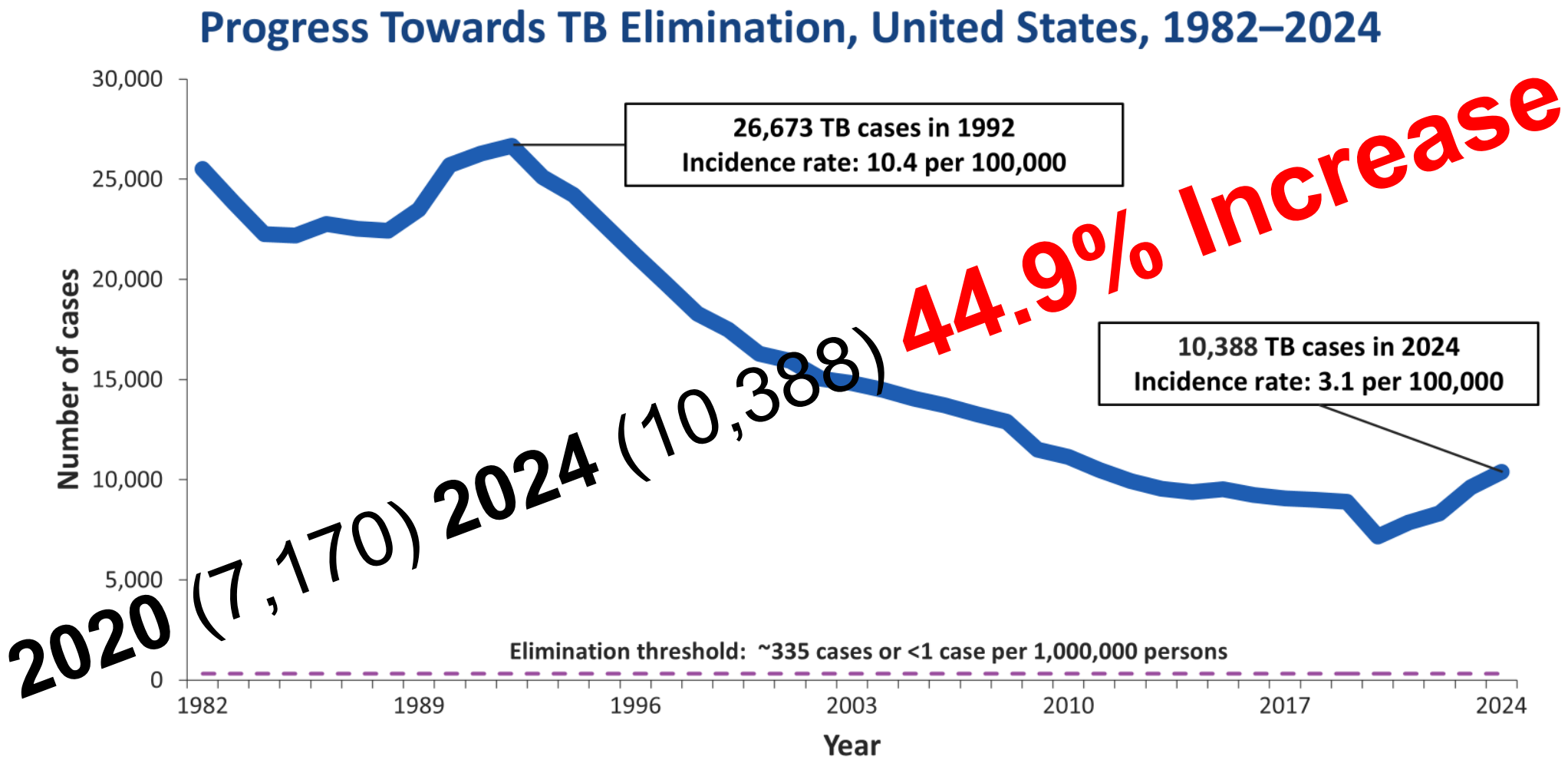
Tuberculosis (TB) Disease in the United States 1993–2024*

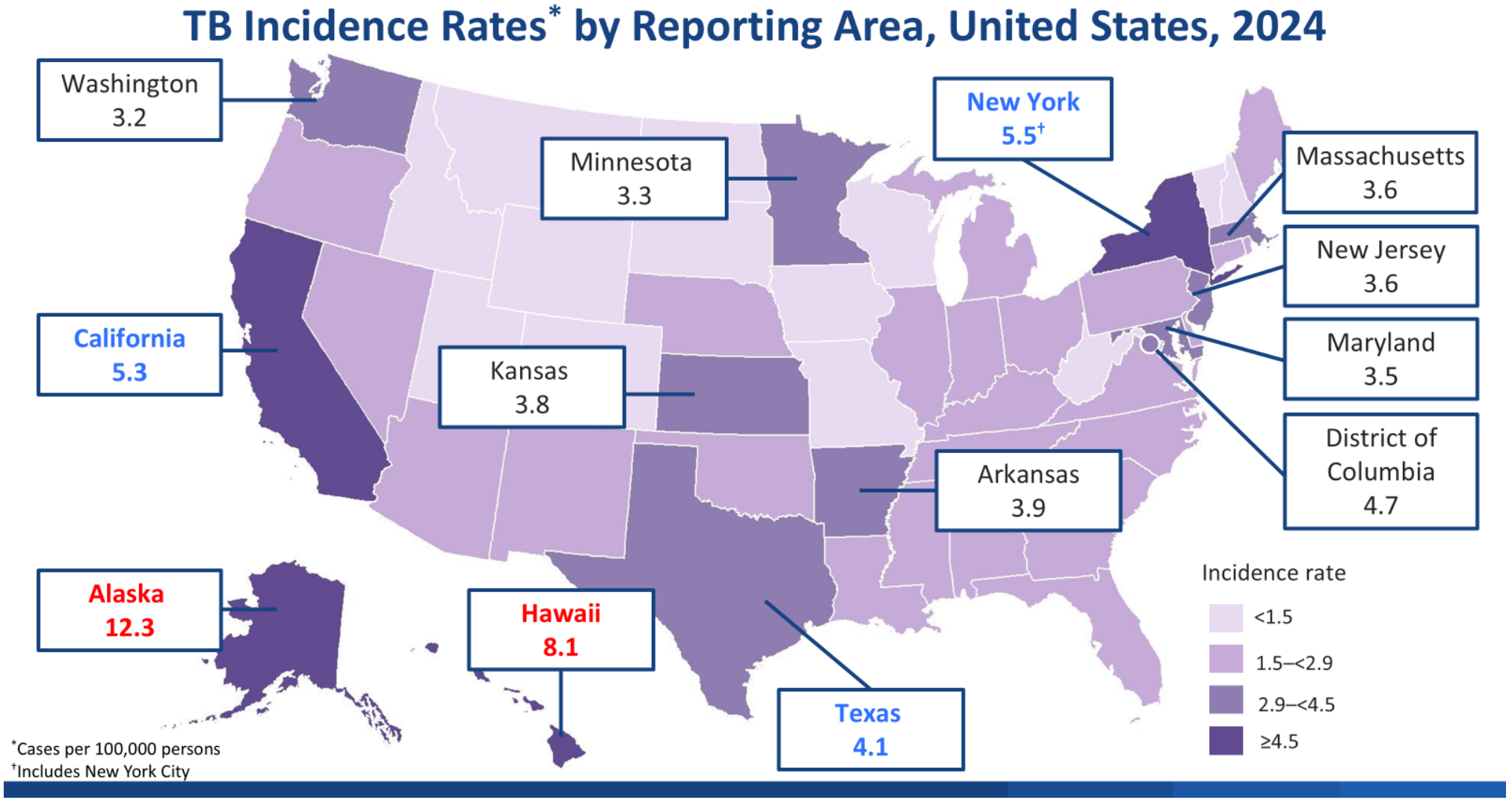
Division of Tuberculosis Elimination

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

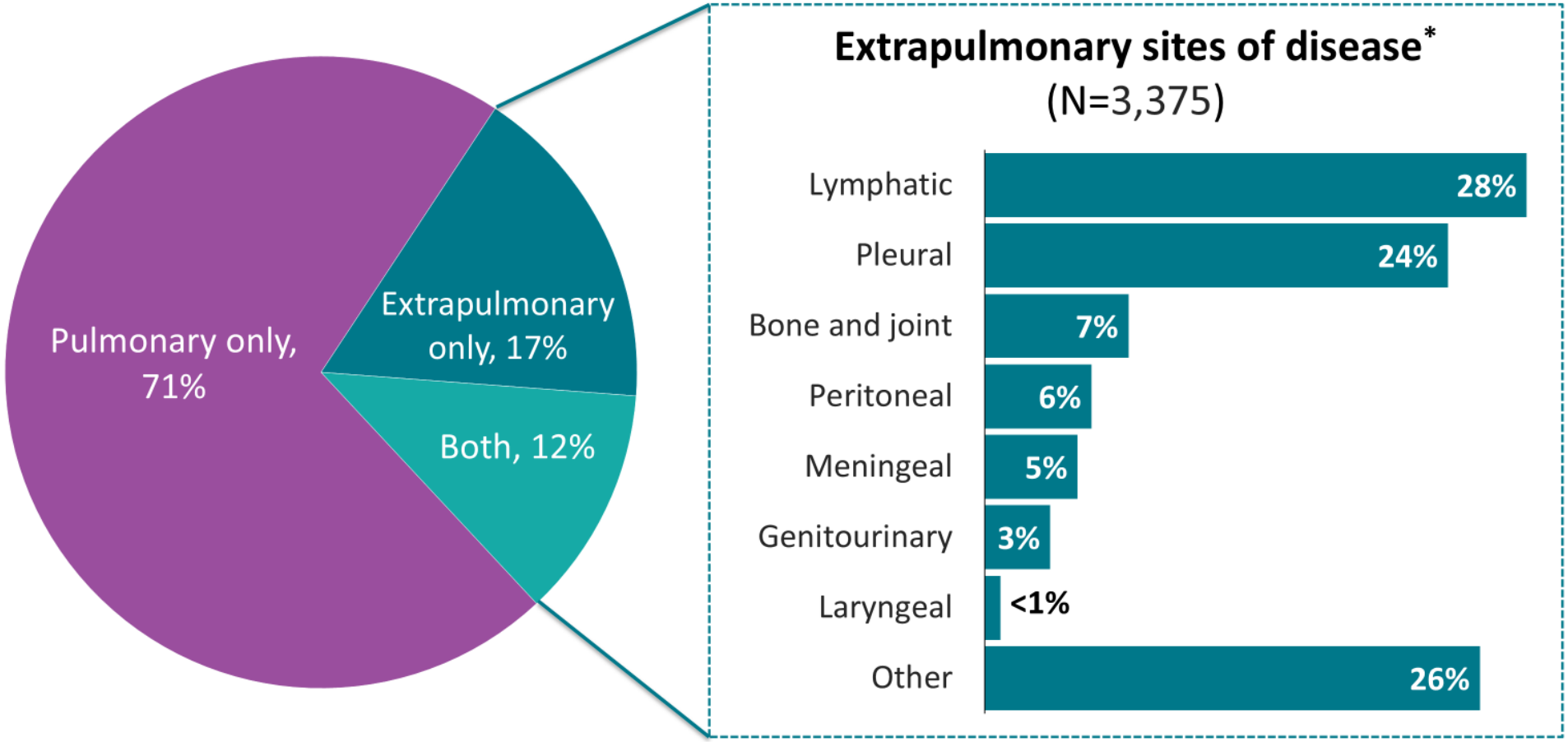
National Tuberculosis Surveillance System

*Data updated as of July 10, 2025


Progress Towards TB Elimination, United States, 1982–2024
30,000
25,000
20,000
15,000
10,000
5,000
0
Number of cases
26,673 TB cases in 1992
Incidence rate: 10.4 per 100,000
10,388 TB cases in 2024
Incidence rate: 3.1 per 100,000
2020 (7 ,170) 2024 (10,388) 44.9% Increase
Elimination threshold: ~335 cases or <1 case per 1,000,000 persons
1982
1989
1996
2003
2010
2017
2024
Year

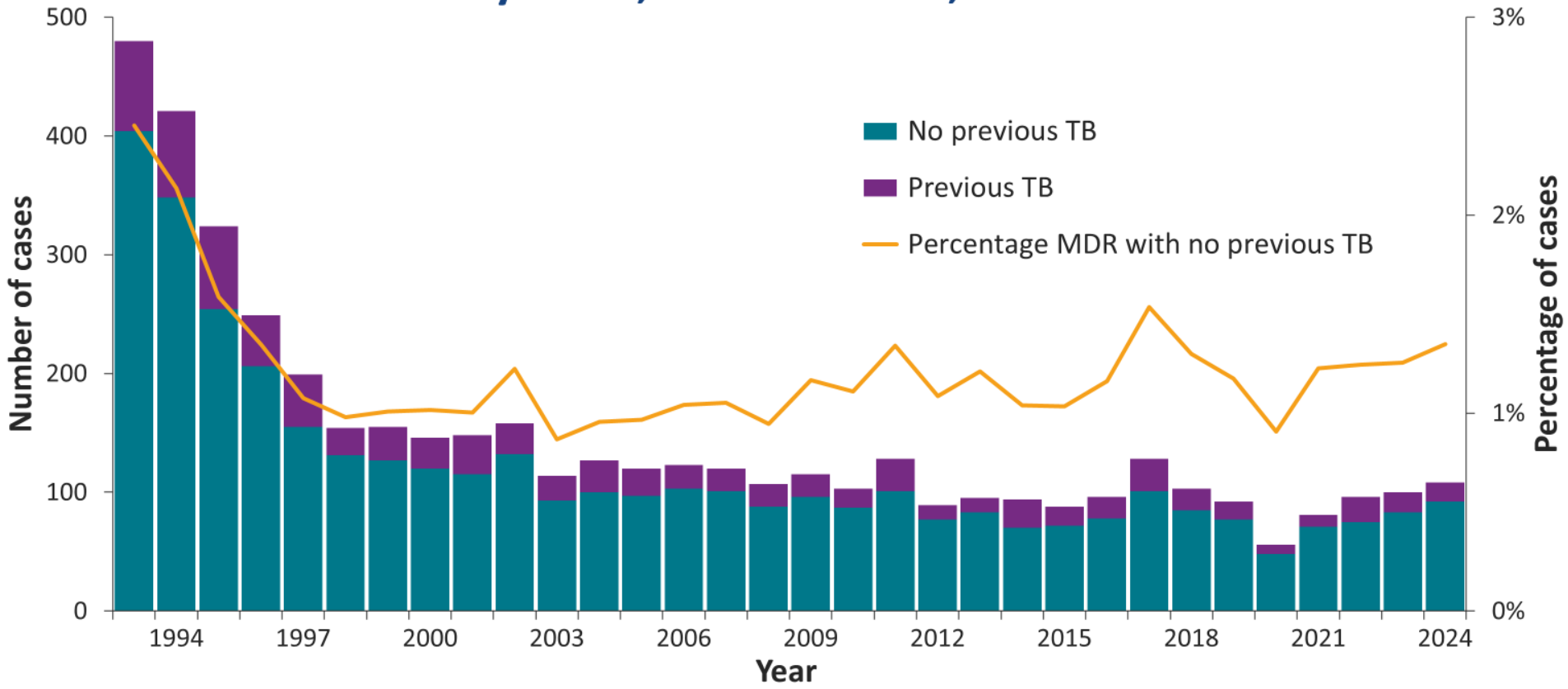

TB Incidence Rates* by Reporting Area, United States, 2024
Washington 3.2
Minnesota 3.3
New York 5.5†
Massachusetts 3.6
New Jersey 3.6
California 5.3
Kansas 3.8
Maryland 3.5
District of Columbia 4.7
Arkansas 3.9
Alaska 12.3
Hawaii 8.1
Texas 4.1
Incidence rate
<1.5
1.5–<2.9
2.9–<4.5
≥4.5
*Cases per 100,000 persons
†Includes New York City

Percentage of TB Cases by Site of Disease, United States, 2024



* Persons might have more than one extrapulmonary site of disease.

Number and Percentage of Multidrug-Resistant (MDR)* TB Cases† by History of TB, United States, 1993–2024

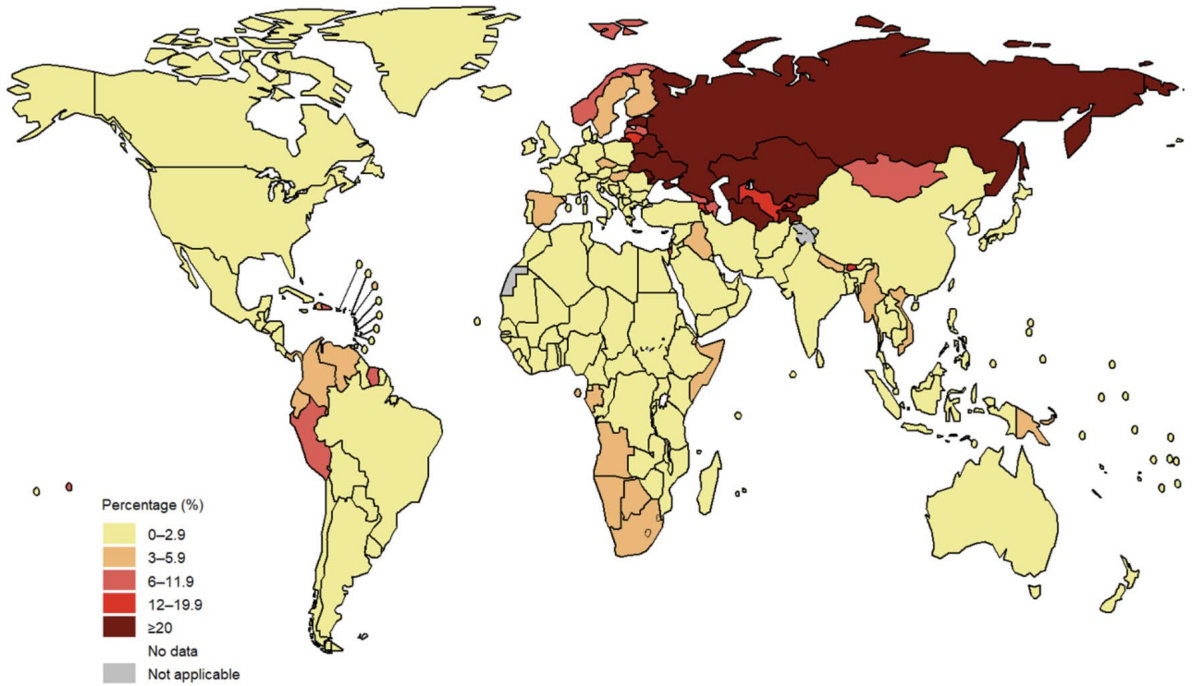


*Starting in 2023, information on drug resistance included results of molecular drug susceptibility testing in addition to growth-based susceptibility testing for isoniazid and rifampin. An isolate is considered resistant to isoniazid or rifampin if either the growth-based test or molecular test detects resistance.

†Excludes persons with unknown origin of birth.

Newly diagnosed TB patients with MDR/RR-TB

Fig. 1.3.6 Percentage of people with TB who had MDR/RR-TB, for those with no previous history of TB treatment, 2023



WHO

Topics

- Epidemiology of Tuberculosis
- **Epidemiology of nontuberculous mycobacterial pulmonary disease**
- Ideal algorithm for the laboratory diagnosis of mycobacterial disease
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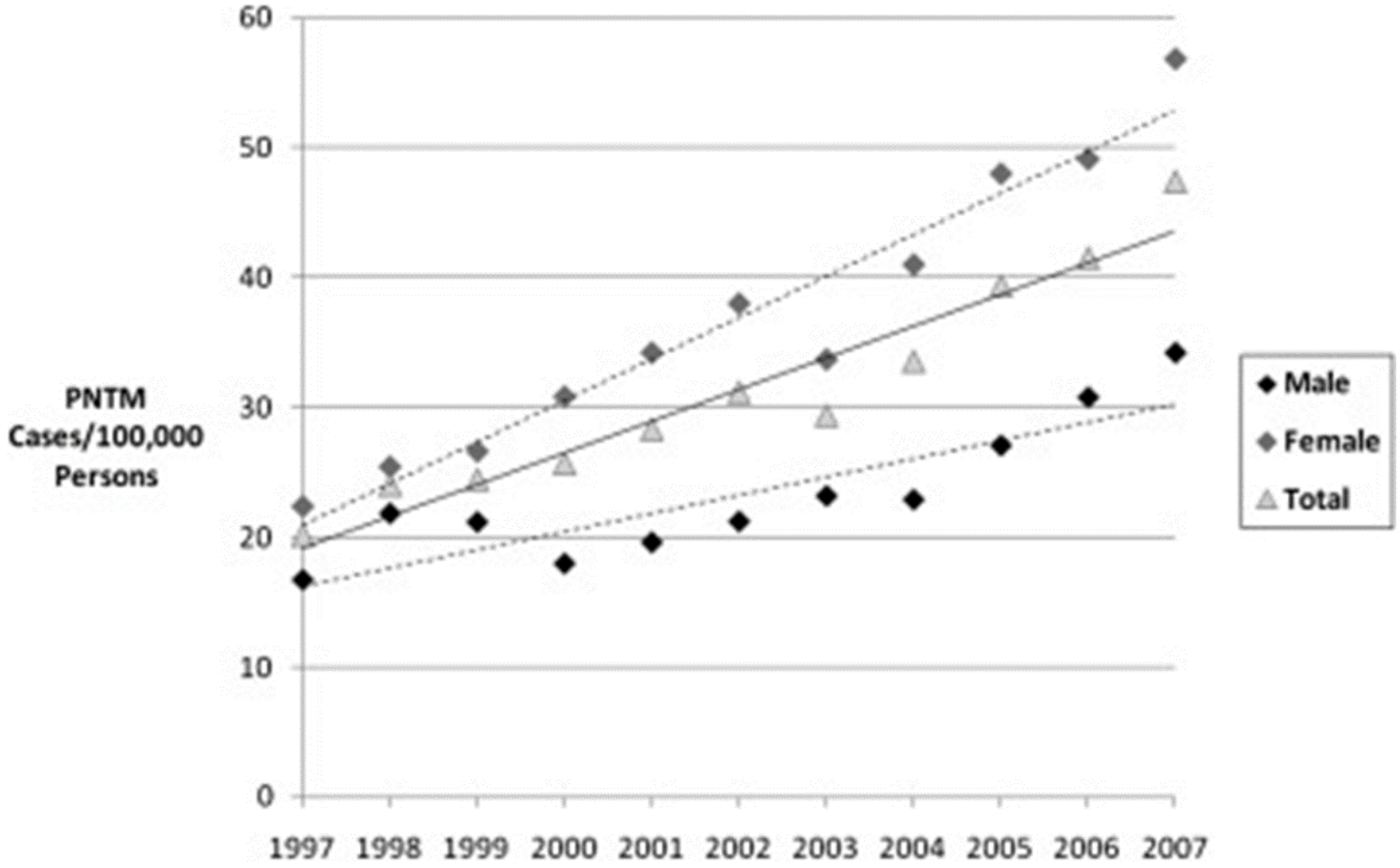
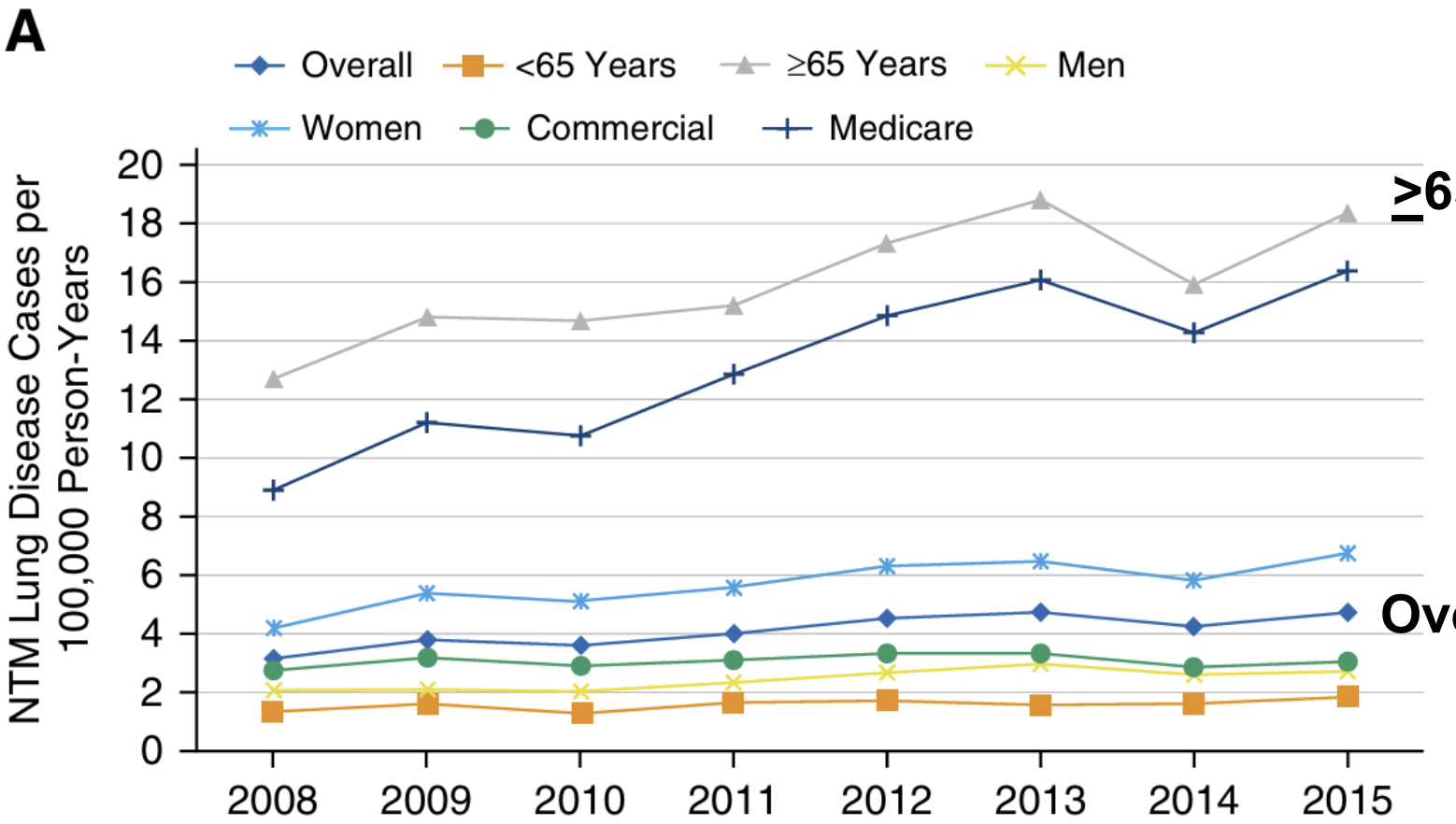
Pulmonary NTM – Medicare Beneficiaries



Am J Respir Crit Care Med 2012
Adjemian et al

Yearly incidence of NTM lung disease(2008-2015) in a US national health insurance plan – 27 million people



≥65 yrs: 18/100k

Overall: 5/100k

Winthrop KL et al Ann Am Thorac Soc 2020

Canada: Up and down

We measured annual prevalence of microbiologically defined nontuberculous mycobacterial lung disease in Ontario, Canada. *Mycobacterium avium* prevalence was 13 cases/100,000 persons in 2020, a 2.5-fold increase from 2010, indicating a large increase in true *M. avium* lung disease. During the same period, *M. xenopi* decreased nearly 50%, to 0.84 cases/100,000 persons.

Marras TK. Emerg Infect Dis. 2023

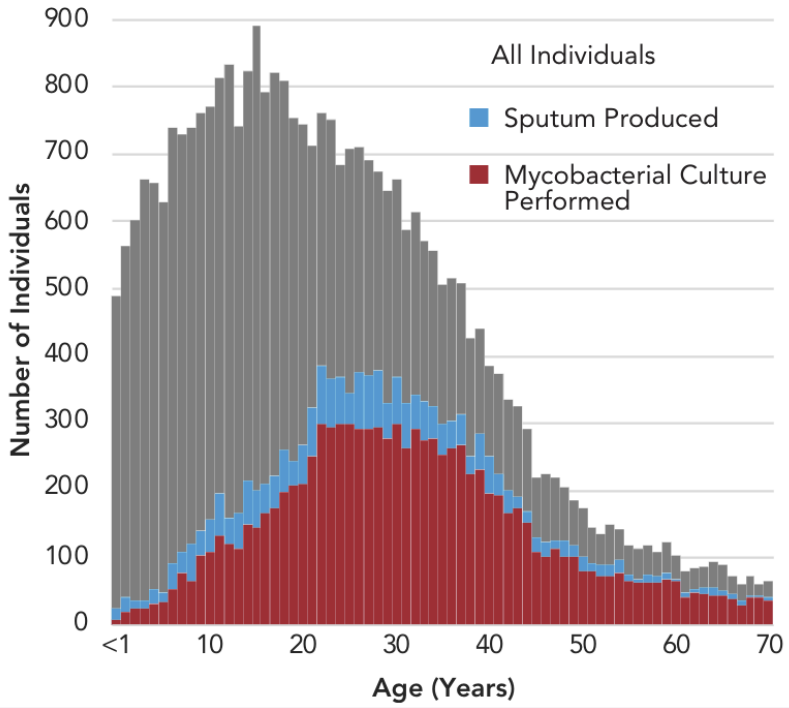
Four integrated health care delivery systems*, 1991-2007

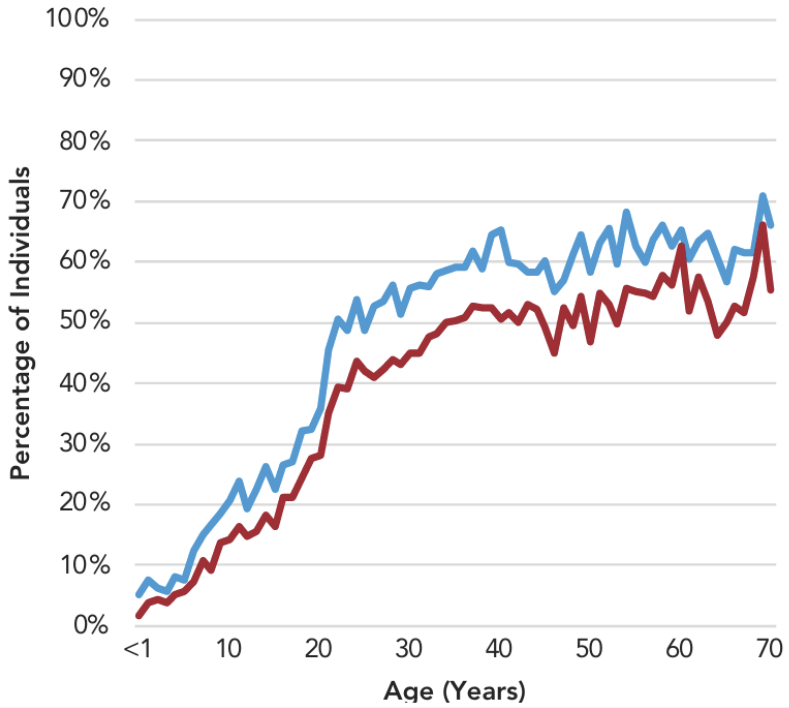
***M. avium* complex**	1,495	(80.1%)
M. chelonae/abscessus	225	(12.1%)
M. fortuitum	106	(5.6%)
M. kansasii	102	(5.5%)
M. simiae	53	(2.8%)
M. xenopi	33	(1.7%)

*KP Southern California, KP Southern Colorado, Group Health, Geisinger

Am J Respir Crit Care Med 2010 Prevots et al.

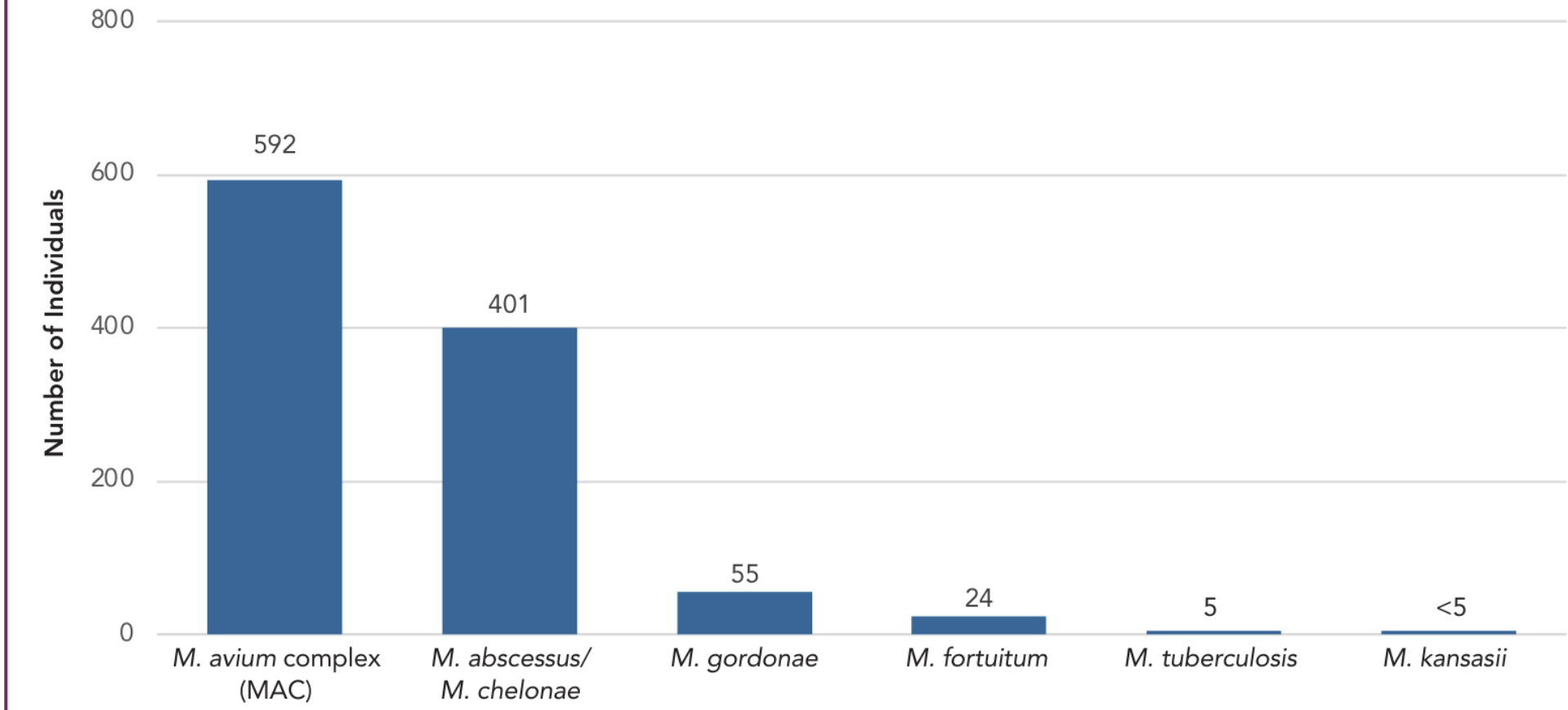
Sputum Produced and Mycobacterial Cultures by Age in Years, 2024





Of the 10,266 individuals who had a mycobacterial culture performed in 2024, 1,192 (11.6 percent) had a mycobacterial species isolated one or more times, a small increase from 10.1 percent in 2023. The prevalence of positive NTM cultures remained below that found in 2019 (13.8 percent) and earlier.

Mycobacterial Species Isolated, 2024



Data are not mutually exclusive. Some individuals had more than one species isolated in 2024.

Documenting Environmentally Acquired *Mycobacterium intracellulare* subsp. *chimaera* Pulmonary Disease Soon After Bronchiectasis Onset

Honda JR et al. Clin Infect Dis. 2026 Jan 10. Online ahead of print. PMID: 41518593

APHL Association of Public Health Laboratories

AUGUST 2025

Nontuberculous mycobacteria:
An Emerging Public Health Concern

Alana Sterkel, Wisconsin
Frances Jamieson, Ontario-Canada
Max Salfinger, Colorado
Sarah Totten, Colorado
Sphoorthy Rao, California
Theresa Savidge, Alaska
Tracy Stiles, APHL
Vincent Escuyer, New York
Reviewed by ID Committee

Table 2: Outbreaks of NTM in the US

Title	Facility	Organism (Cases)	Year(s)
Outbreak of nontuberculous mycobacteria joint prosthesis infections, Oregon, USA, 2010-2016[32]	Four hospitals	• *M. goodii* (2) • *M. fortuitum* (8)	• 2010 • 2013-2014
Clusters of nontuberculous mycobacteria linked to water sources at three Veterans Affairs medical centers[33]	Veterans Affairs medical centers	• *M. conceptionense* (41) • *M. porcinum* (4)	• 2012-2017 • 2018
Mycobacterium chimaera infections associated with heater-cooler unit during cardiopulmonary bypass surgery—Los Angeles County, 2012-2016[34]	Hospital	*M. chimaera* (20)	2013-2016
Use of statistical process control methods for early detection of healthcare facility-associated nontuberculous mycobacteria outbreaks: a single-center pilot study[35]	Tertiary care hospital	• *M. abscessus* (58) • *M. abscessus* (105) • *M. avium complex* (165)	• 2013-2014 • 2013-2016 • 2014-2016
Outbreak of tattoo-associated nontuberculous mycobacterial skin infections[25]	Tattoo studio	*M. fortuitum*, *M. abscessus* and *M. chelonae* (38)	2015
Mycobacterium avium pseudo-outbreak associated with an outpatient bronchoscopy clinic: lessons for reprocessing[36]	Outpatient clinic, tertiary care hospital	*M. avium* (N/A)	2015-2016
Invasive *Mycobacterium abscessus* outbreak at a pediatric dental clinic[37]	Pediatric dental clinic	*M. abscessus* subsp. *abscessus* (71)	2016
A bronchoscopy-associated pseudo-outbreak of *Mycobacterium mucogenicum* traced to use of contaminated ice used for bronchoalveolar lavage[38]	Tertiary care hospital	*M. mucogenicum* (15)	2017
Hospital water contamination associated with pseudo-outbreak of *Mycobacterium porcinum*—Wisconsin, 2016-2018[26]	Hospital	*M. porcinum* (16)	2017-2018
Mycobacterium porcinum skin and soft tissue infections after vaccination—Indiana, Kentucky, and Ohio, September 2018—February 2019[39]	Contractor providing vaccinations at workplaces	*M. porcinum* (101)	2018-2019
A bronchoscopy-associated pseudo-outbreak of *Mycobacterium chelonae* and *Mycobacterium mucogenicum* associated with contaminated ice machine water and ice[40]	Teaching community hospital	*M. chelonae* and *M. mucogenicum* (15)	2019
A cluster of three extrapulmonary *Mycobacterium abscessus* infections linked to well-maintained water-based heater-cooler devices[41]	Tertiary care hospital	*M. abscessus* (3)	2020
Nontuberculous mycobacteria infections after cosmetic surgery procedures in Florida—nine states, 2022-2023[42]	Plastic surgeon office	*M. abscessus* (15)	2020-2023
Mycobacterium abscessus outbreak related to contaminated water among ventilator-dependent residents of a pediatric facility[43]	Ventilator-dependent residents of a pediatric facility	*M. abscessus* (3)	2022

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- Health care provider suspects **TB disease**
  - TB-NAAT, AFB smear, culture
    - If TB-NAAT positive, screen for MDR-TB (molecular assay)
    - If TB-NAAT negative and AFB smear positive, perform NTM-NAAT

- Health care provider suspects **NTM disease**
  - NTM-NAAT, AFB smear, culture (including a selective NTM agar – inoculated before decontamination)
  - If NTM-NAAT negative, perform TB-NAAT

- Health care provider wishes to **rule out mycobacterial disease**
  - AFB smear, culture
    - If AFB smear is positive, perform TB-NAAT
    - If TB-NAAT is negative, perform NTM-NAAT

Fig. 3. Schematic of an ideal algorithm* for nontuberculous mycobacteria testing in clinical and public health laboratories (adapted from [43]).
*None of the current NTM-NAAT in use in the United States and in Canada are approved by a national regulatory body. The different laboratory developed tests have different performance characteristics. Therefore, it is of utmost importance that the laboratory be in contact with the ordering health care provider to discuss the use of NTM-NAAT. Ideally, the laboratory develops a local algorithm together with the health care providers at the institution based on the patient population seen.
TB, tuberculosis; AFB, acid-fast bacilli; NAAT, nucleic acid amplification test; NTM, nontuberculous mycobacteria; MDR, multidrug resistance

Brown-Elliott et al. Laboratory diagnosis of nontuberculous mycobacteria –an update –Part 3*: Molecular diagnostics, environmental testing, and notifiable conditions. Clin Micro Newsletter Vol. 52, No. 6 September 2025

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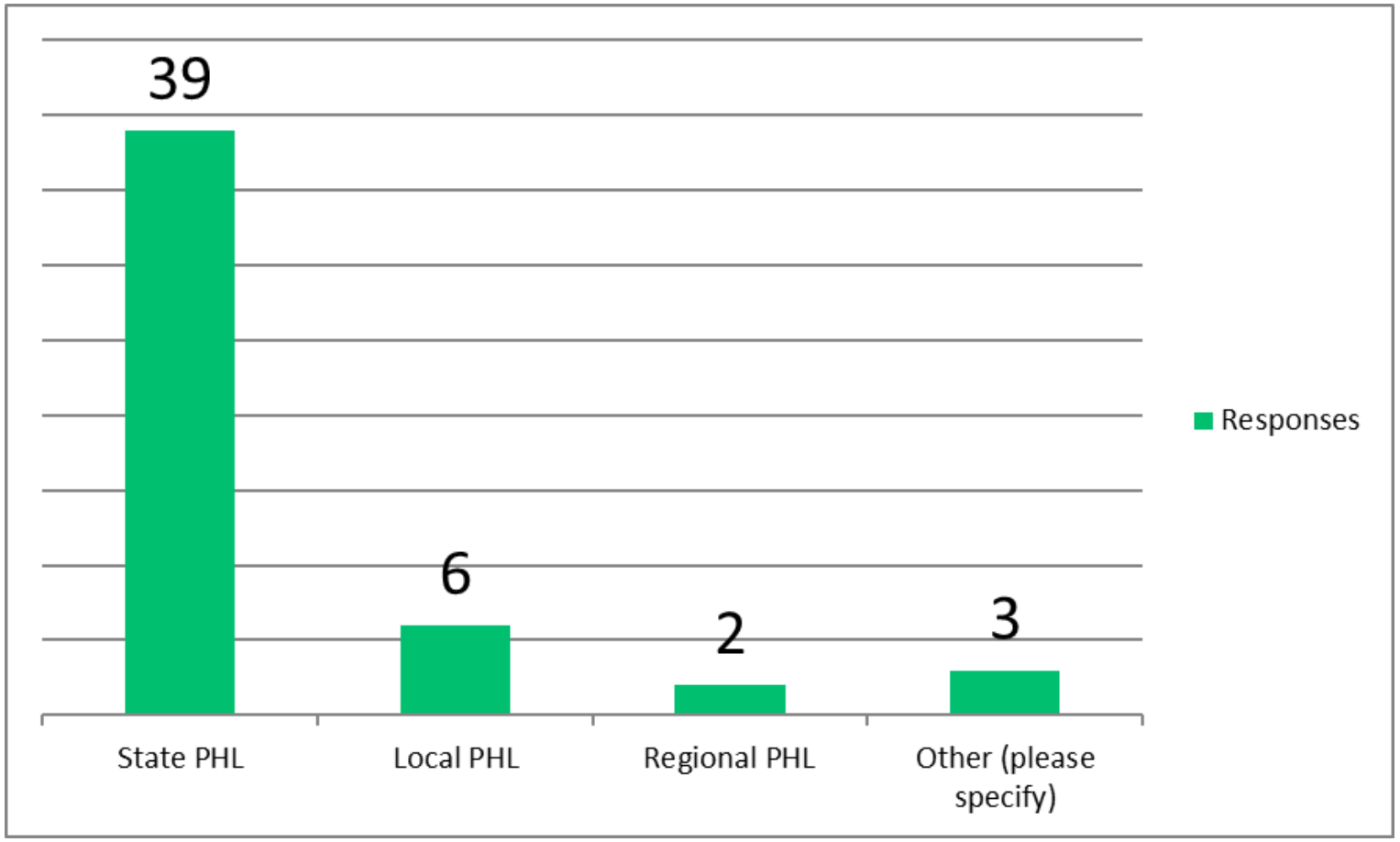
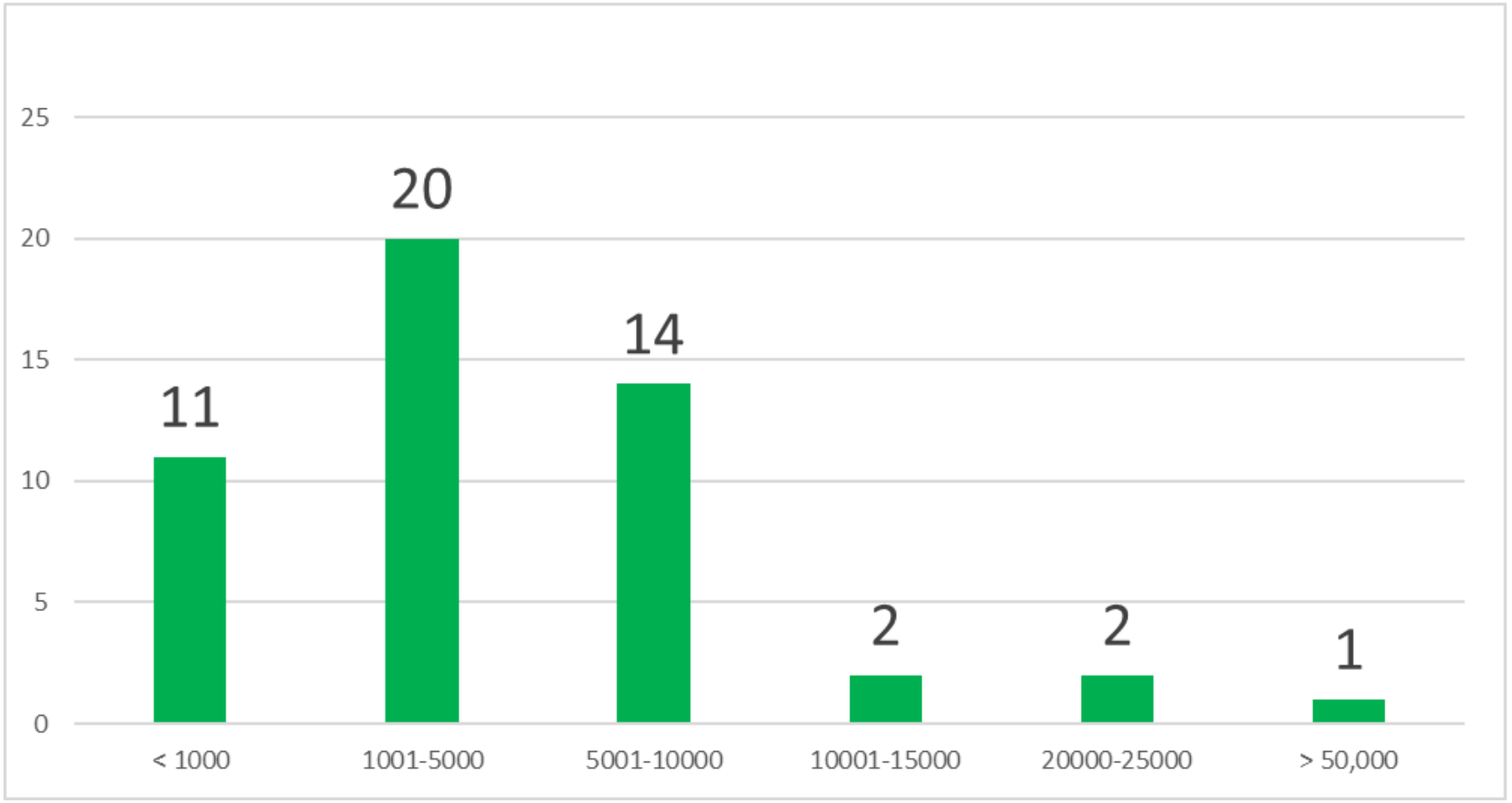
TB Conference Pre-Session Survey, N=50

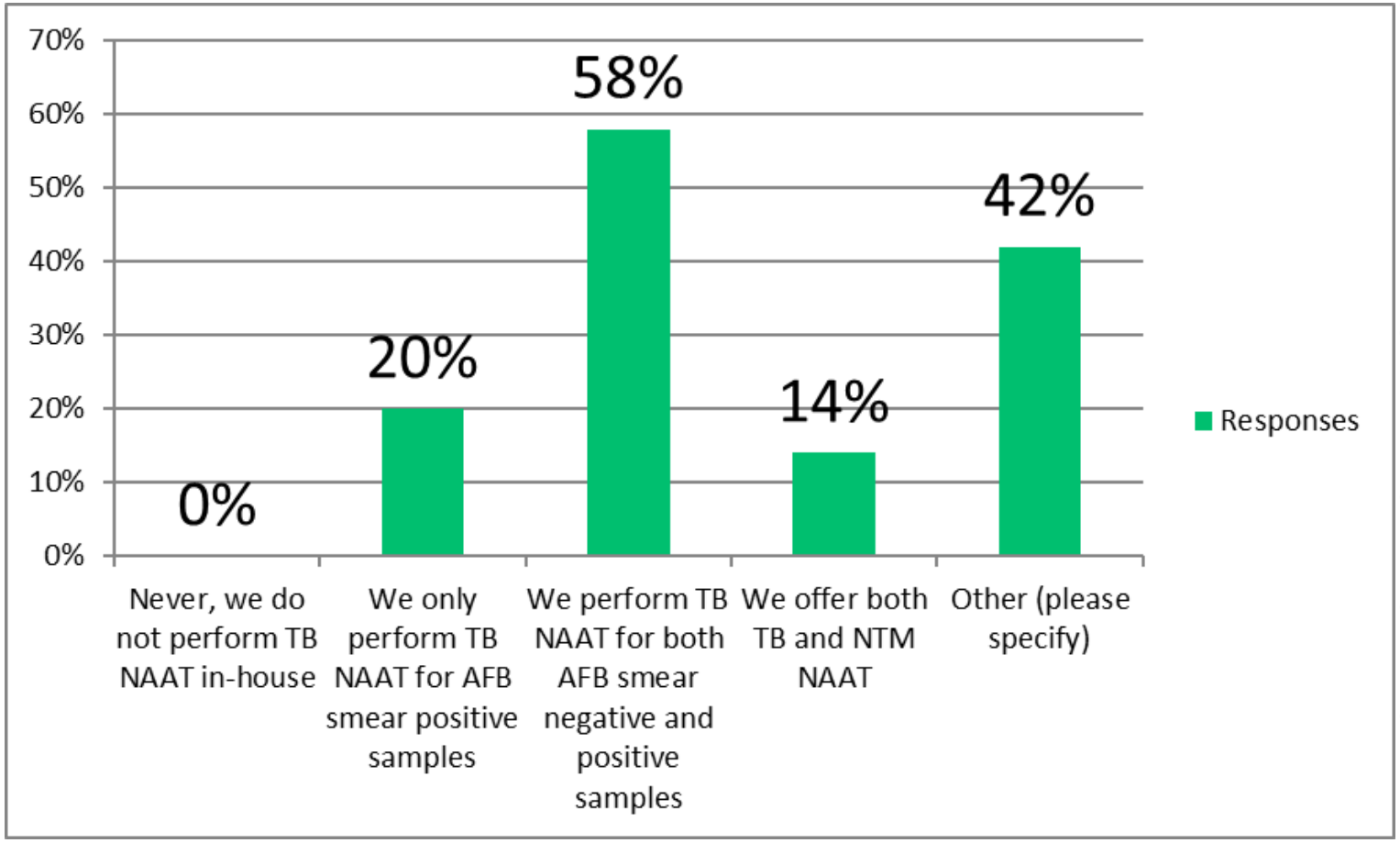


Thank you, **Sarah Buss,** for collecting and analyzing the responses.

Number of Specimens/Isolates Received in 2025, N=50

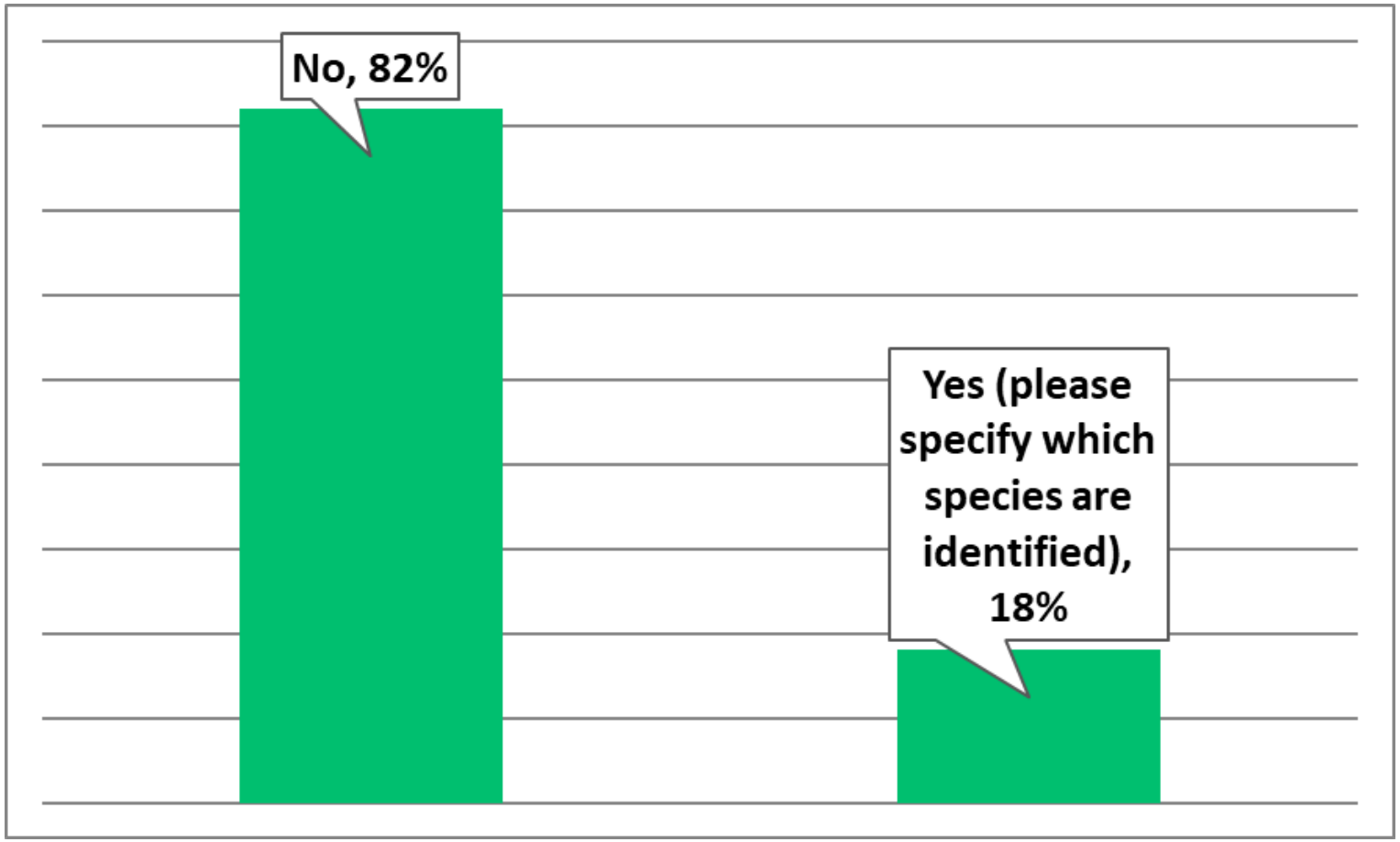


When does your laboratory perform TB NAAT? (Choose all that apply), N=50



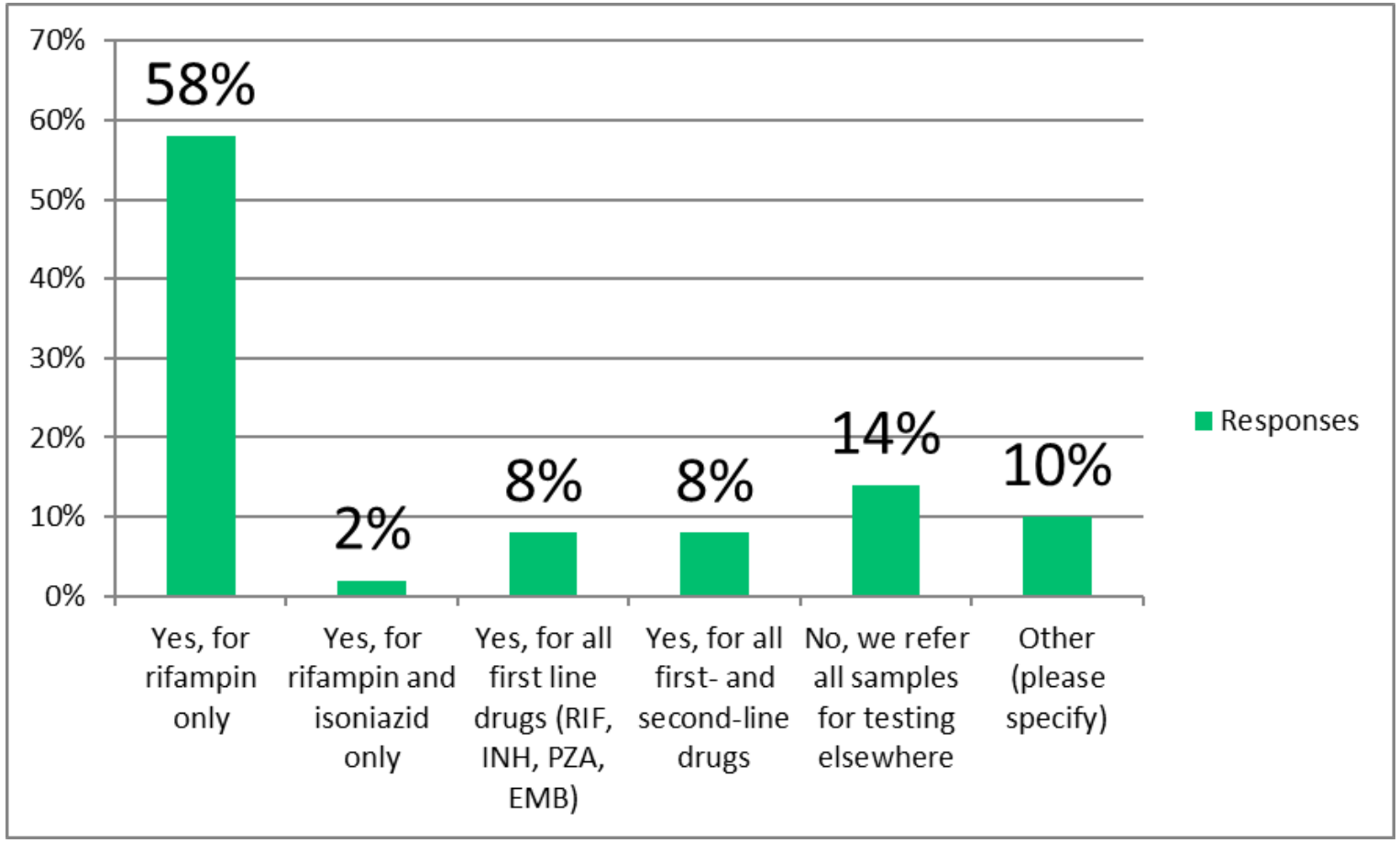
- NAAT on new smear positives and negatives by request.
- New smear positives and upon request
- TB NAAT is offered for diagnostic patients upon request before the smear is completed.
- We perform TB NAAT on the first specimen of each patient as long as they have not been on treatment.
- All smear-positive and smear-negative respiratory specimens by request only
- When the TB program requests NAATs on negative smears.
- NAAT on new patients identified by state TB Program.

Does your TB identification protocol differentiate species within the MTBC? N=50



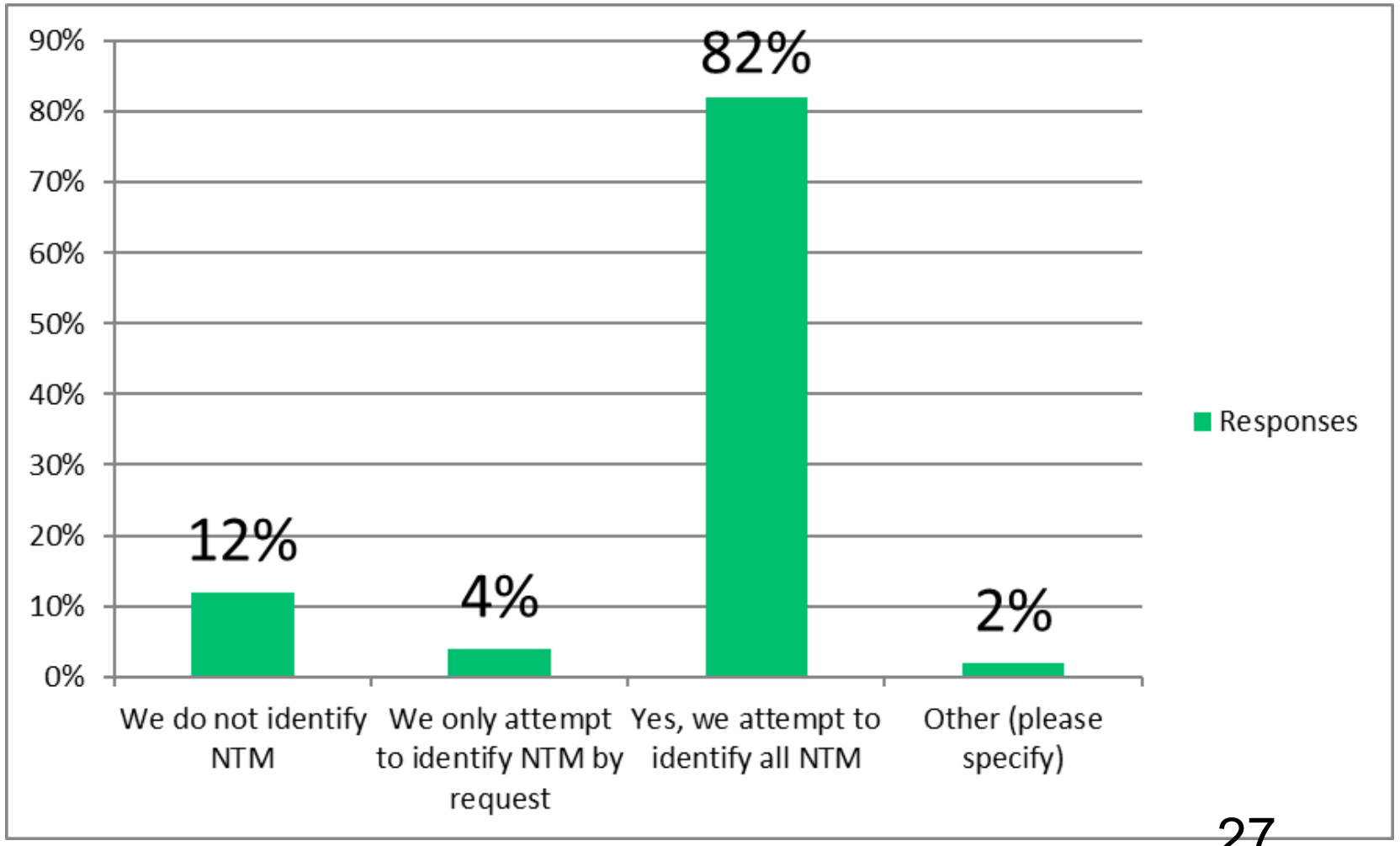
- All covered with PCR and WGS
- All species
- *M. tuberculosis, M. africanum, M. bovis, M. bovis* BCG, *M. canetti, M. caprae, M. microti*
- We refer all AFB positive samples for identification
- We have a separate molecular test for TB complex differentiation.
- We will ID *M. bovis* and *M. bovis* BCG

Do you perform in-house testing for molecular markers of drug resistance in M. tuberculosis? N=50

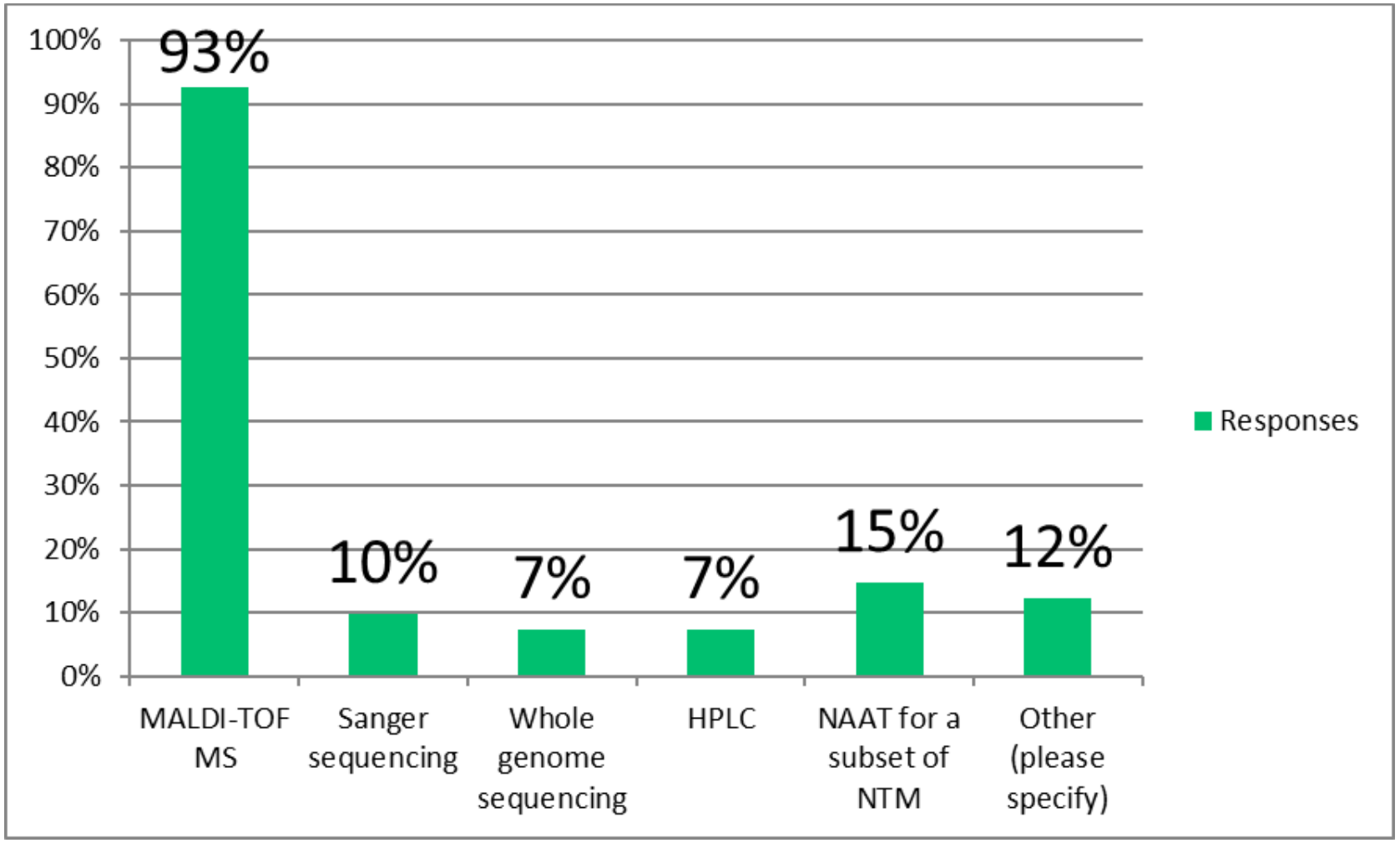


- Xpert on Clinicals for RIF only; all isolates getting a DST are sent for AFB Deeplex (or at least pncA previously)
- Validated WGS but not yet gone-live, aiming for go-live June 2026
- Yes, for Rifampin and Ethambutol
- We test for RIF, INH, EMB
- Send to CDC when requested

Do you identify NTM? N=50

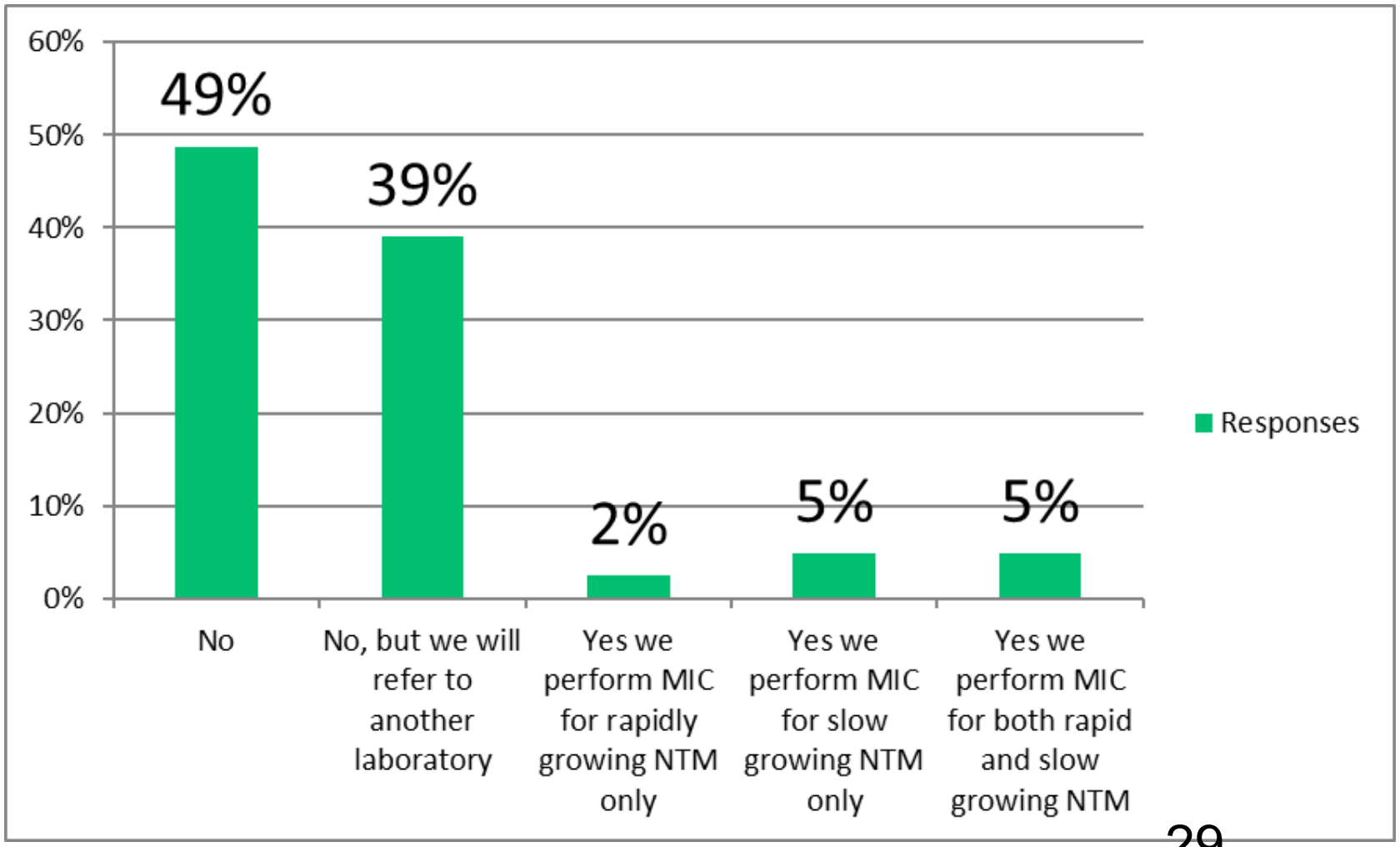


What method(s) do you use for NTM identification? (select all that apply), N=41



- Fujirebio INNO-LiPA
- ABI 7500 FAST
- LDT tNGS
- Line Probe

Do you perform phenotypic AST for NTM, N=41



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Change…

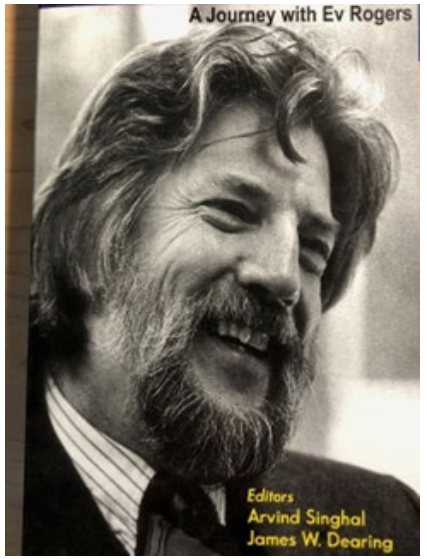
Said is not heard

Heard is not understood

Understood is not implemented

Implemented is not retained

Everett Rogers

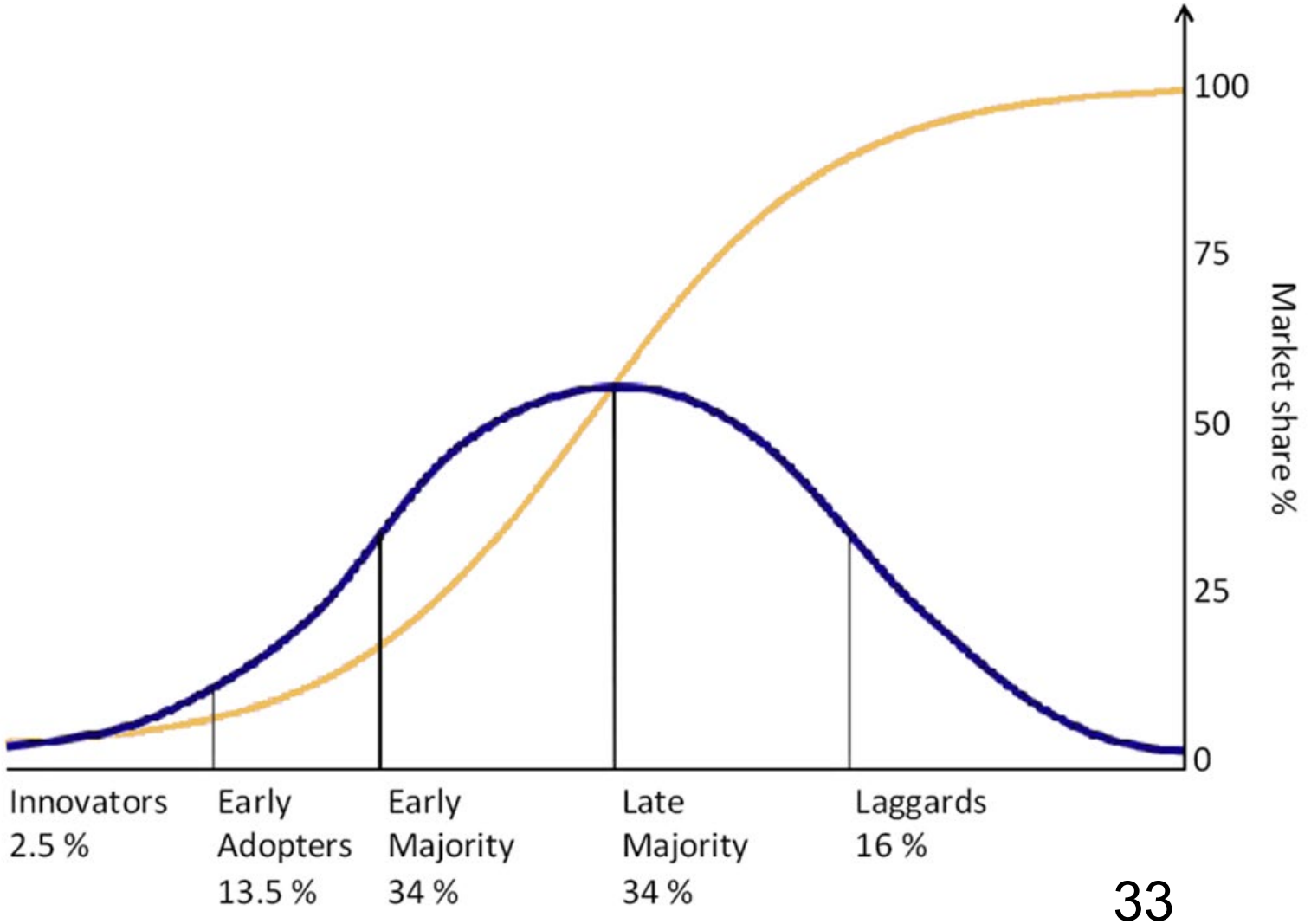


March 6, 1931 – October 21, 2004

An eminent American communication theorist and sociologist, who originated the diffusion of innovations theory and introduced the term ***early adopter***.

1962-The first edition of the Diffusion of Innovations – at the age of 31 years

Diffusion of innovations



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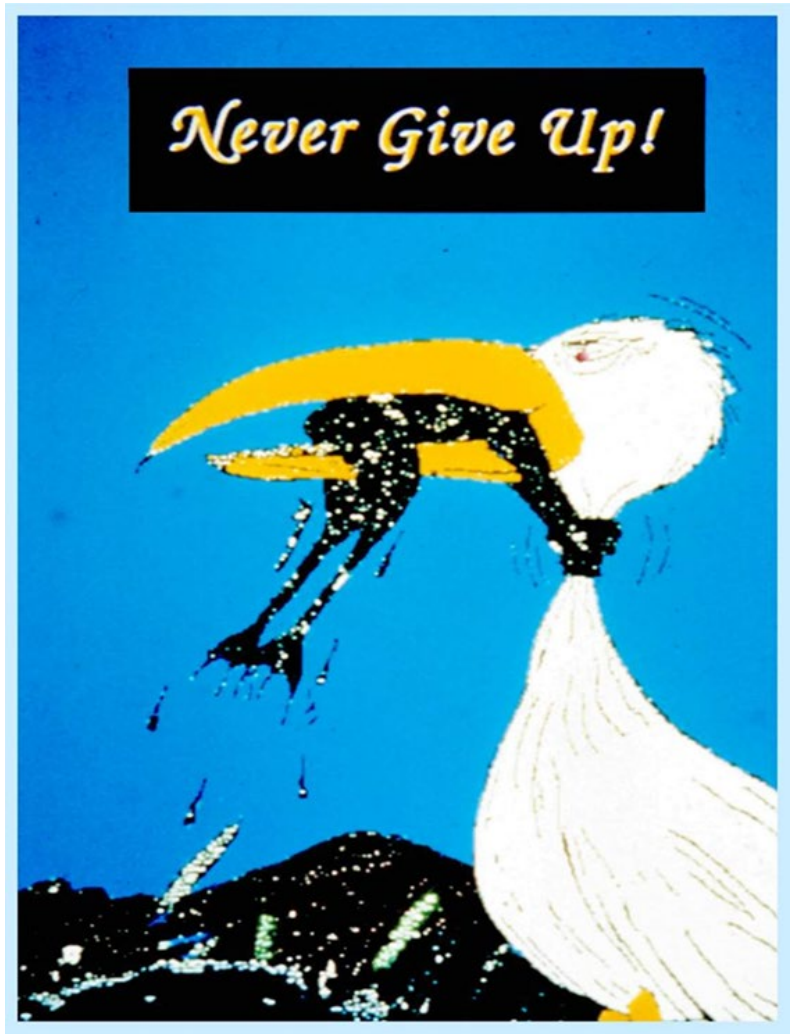
Every laboratory is different…

- Percent TB positive, i.e. population served
- Charging for services or not
- Public health law, rules and regulations
- Specimens load
- Equipment/ laboratory facility (i.e., BSL-3)
- Staffing
- Expertise/experience
- Work hours/days



Fighting TB
and being a change agent!

Thank you!

max@usf.edu or
904-476-6783 (text)

Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

Not the Usual Suspects

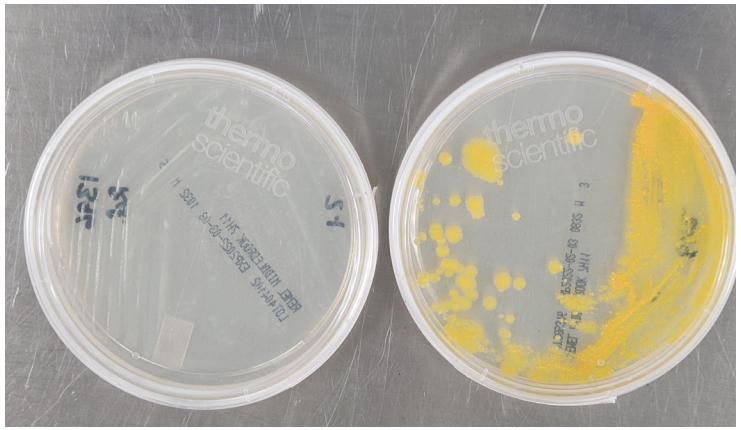
Public Health Laboratory Testing for Non-Tuberculous Mycobacteria

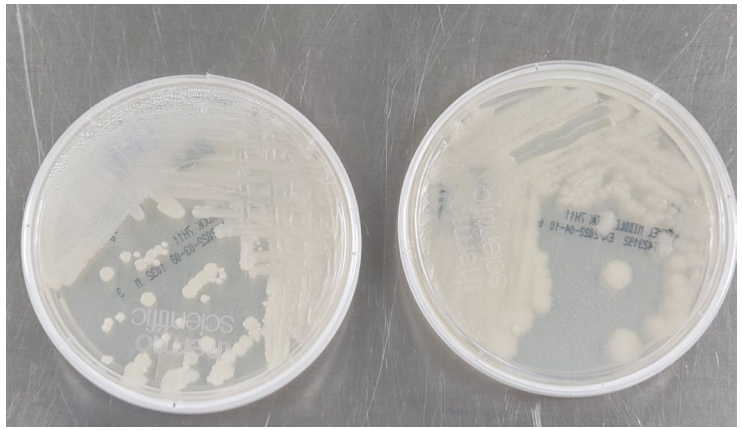
Nate Simon
WI TB Laboratory Program Coordinator
WI State Lab of Hygiene – Communicable Disease Division
03/11/2026

Public Health Mycobacteriology Testing

- Primary roles:
  - Rapid ID of MTBC
  - MTBC susceptibility testing
  - Outbreak investigation
- US rate 3.0/100,000 population (CDC, 2024)
- MTBC disease standardized mortality ratio ~3

- Expanded role for non-tuberculous mycobacteria (NTM) testing?

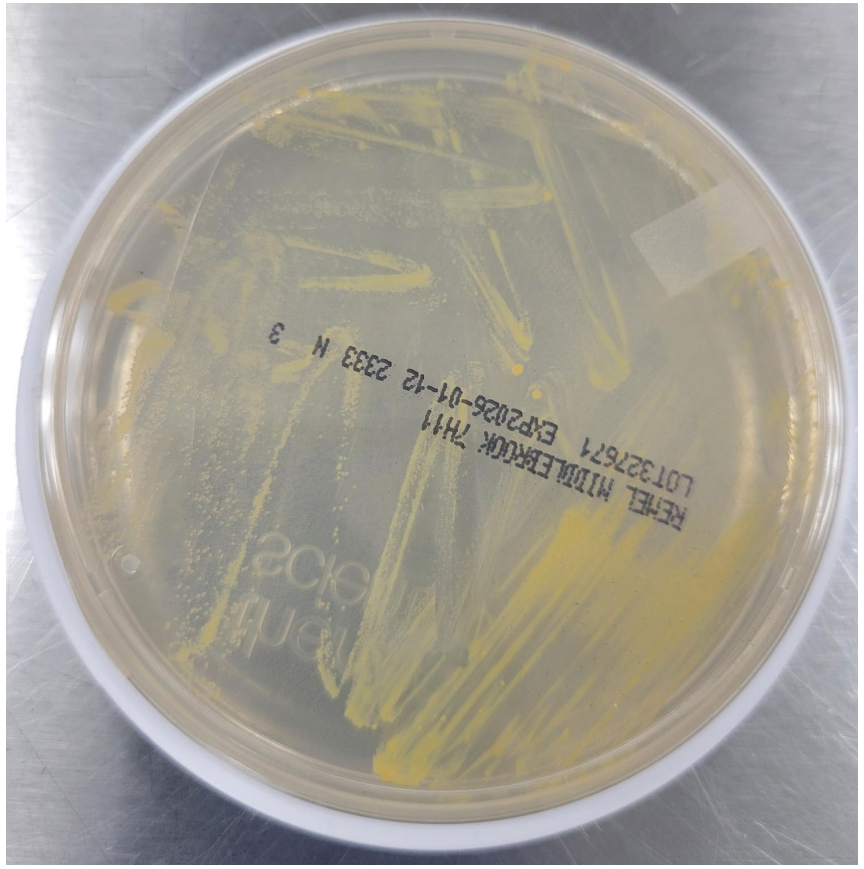




Non-tuberculous mycobacteria

- Environmental organisms, common in water and soil
- Nosocomial transmission due to biofilm capabilities
  - Water systems
  - Medical equipment
- Positive cultures can be true disease, contamination, colonization

- Range of pathogenicity
  - *M. gordonae* → *M. abscessus, M. kansasii*
- Often highly antibiotic resistant
  - Poor correlation between *in vitro* activity and clinical outcome
- NTM disease SMR range 2-4 (species dependent)



APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES

AUGUST 2025

Nontuberculous mycobacteria:
An Emerging Public Health Concern

Nontuberculous mycobacteria (NTM) infections are an increasing public health concern. This document establishes the need to improve the public health response with a proposed addition of NTM to the nationally notifiable conditions list. It also describes the current role of public health laboratories and how their role could be enhanced through better early identification of outbreaks and improved surveillance and disease estimates if submission of isolates to public health was mandated.

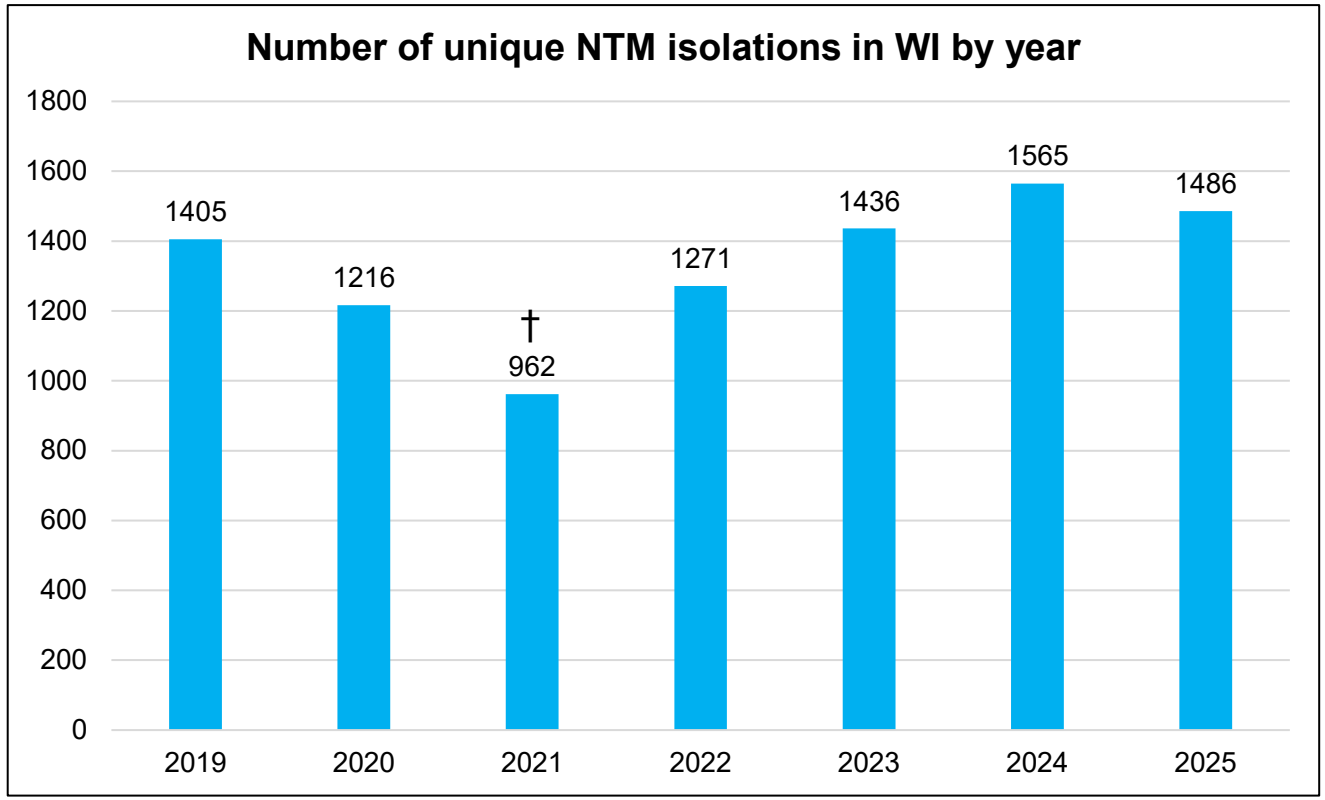
- Add NTM to national and state disease reporting
- Coordinate with clinical laboratories to ensure all NTM cultures are characterized
- Improve testing of municipal water systems
- Develop sequencing techniques to allow linking environmental isolates to patient isolates

Wisconsin Mycobacteriology Laboratory Network (WMLN)

- Offshoot of WCLN, started in 1998
  - Mission: *Ensure state-wide access to rapid and reliable TB testing*
- Coordinated by WSLH, collaboration between public health and clinical labs, local and state public health, and other healthcare professionals
  - Infrastructure for communication
  - Education and outreach activities
  - Assessment of state lab capacity
  - Annual conference
  - **Laboratory-based surveillance**

WMLN: Non-Tuberculous Mycobacteria Epidemiology

Wisconsin Mycobacteriology Laboratory Network Data Report | **2025**



NTM isolations are self-reported to the Wisconsin State Laboratory of Hygiene by mycobacteriology labs in the state of Wisconsin. These data represent one unique isolation per organism per patient.

†Please note: Isolate data for 2021 is incomplete, as one WMLN lab was unable to submit NTM isolation data that year.

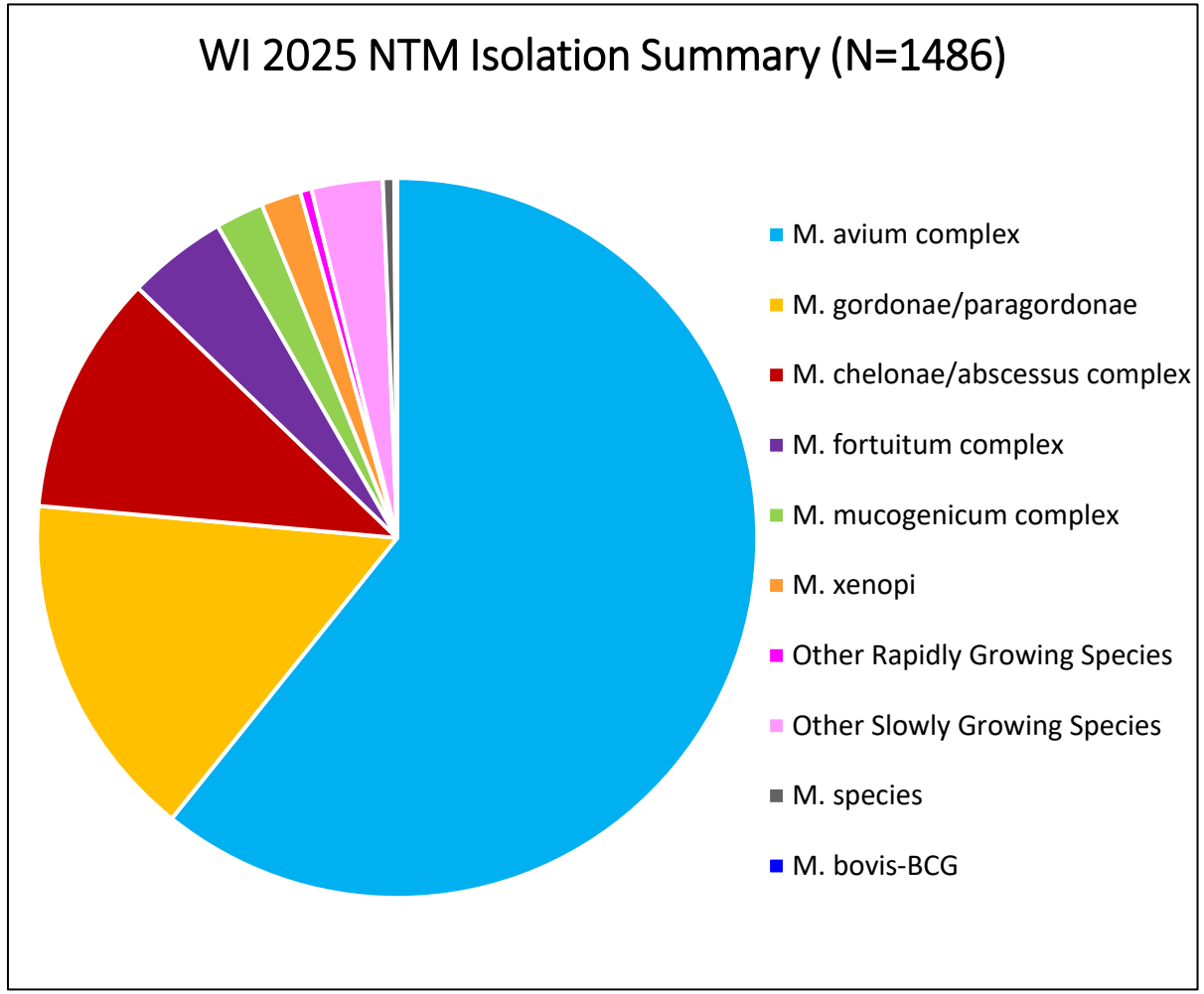
NTM Infection rate

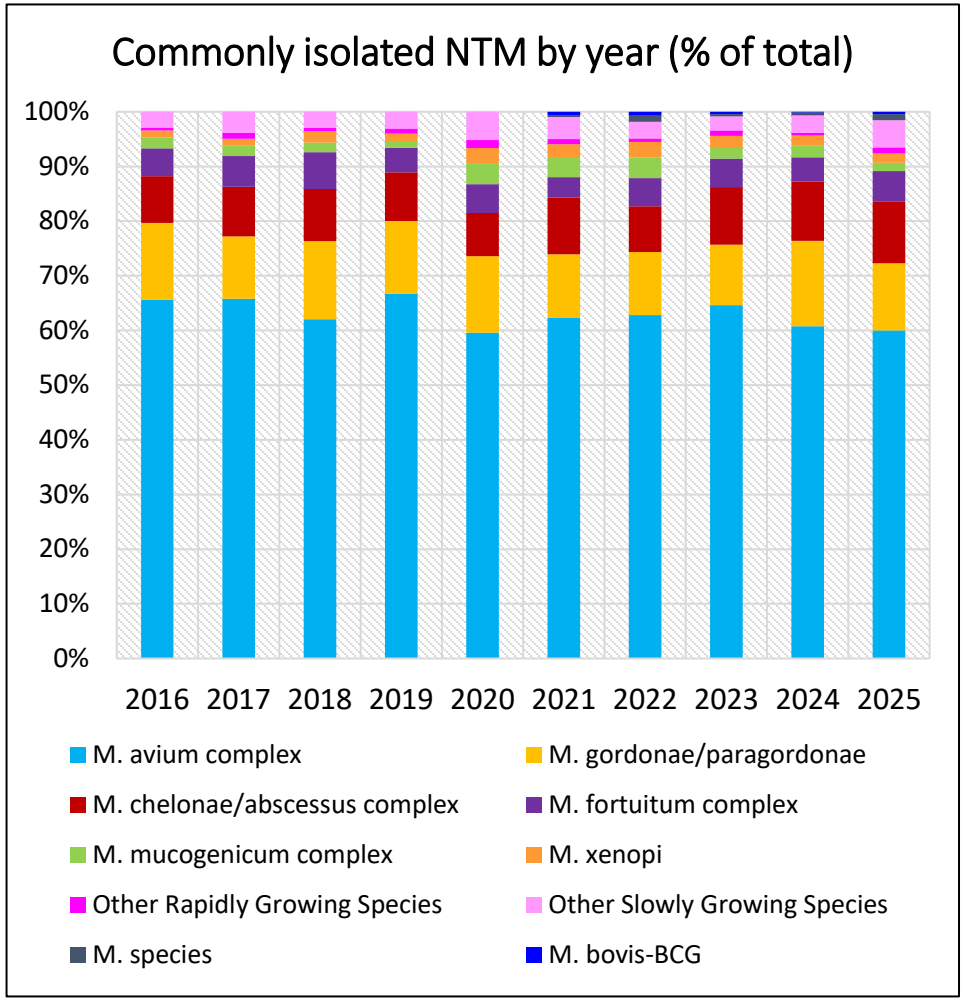
APHL/CDC four state pilot study (2020): 3.8 per 100,000 population

WI (2025): 25 per 100,000

WMLN: Non-Tuberculous Mycobacteria Epidemiology

Wisconsin Mycobacteriology Laboratory Network Data Report | **2025**





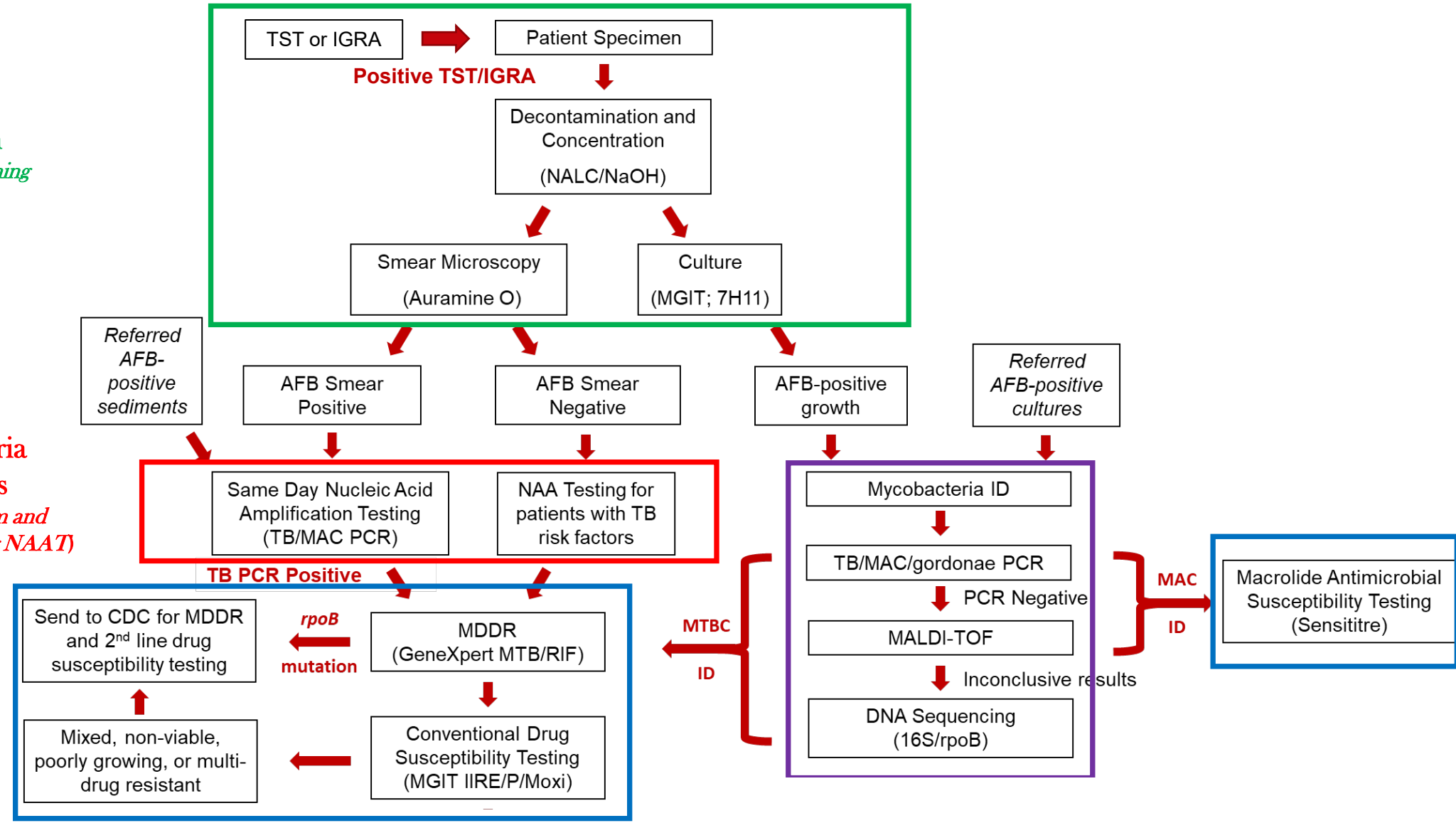
Modern Mycobacteriology

Rapid ID of potential mycobacteria infection
(*Use fluorescent acid-fast staining and promptly transmit results*)

Rapid ID of mycobacteria from primary specimens
(*Reduce average time to confirm and report mycobacteria cases using NAAT*)

ID of mycobacteria from positive culture
(*Use rapid methods to identify and report isolates*)

Access to antimicrobial susceptibility testing
(*Determine susceptibilities of initial isolates to first-line drugs*)



Mycobacteriology at WI State Laboratory of Hygiene

- Primary diagnostic facility receiving clinical specimens from:
  - 2 local hospitals
  - LTHD (state-wide)
- Reference laboratory
  - Mycobacteria ID and NAAT testing for clinical labs in WI
- Public health laboratory
  - TB DST, WGS, genotyping

Almost any source is acceptable for AFB smear and culture

- **Sputum**
- Bronchial washing
- BAL
- Gastric aspirate

>95% of NTM+ cultures (2023-2025)

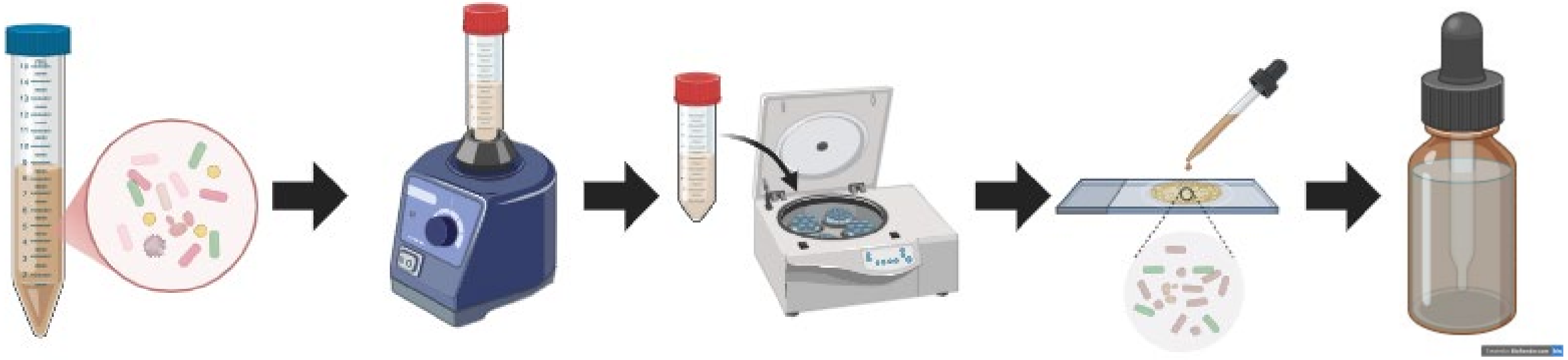
- Fresh tissue
- Bone
- Blood
- Bone Marrow
- CSF
- Body fluids
- Abscess
- Stool
- Urine
- Skin

<5% of NTM+ cultures (2023-2025)

Rapid ID of potential Mycobacteria infection

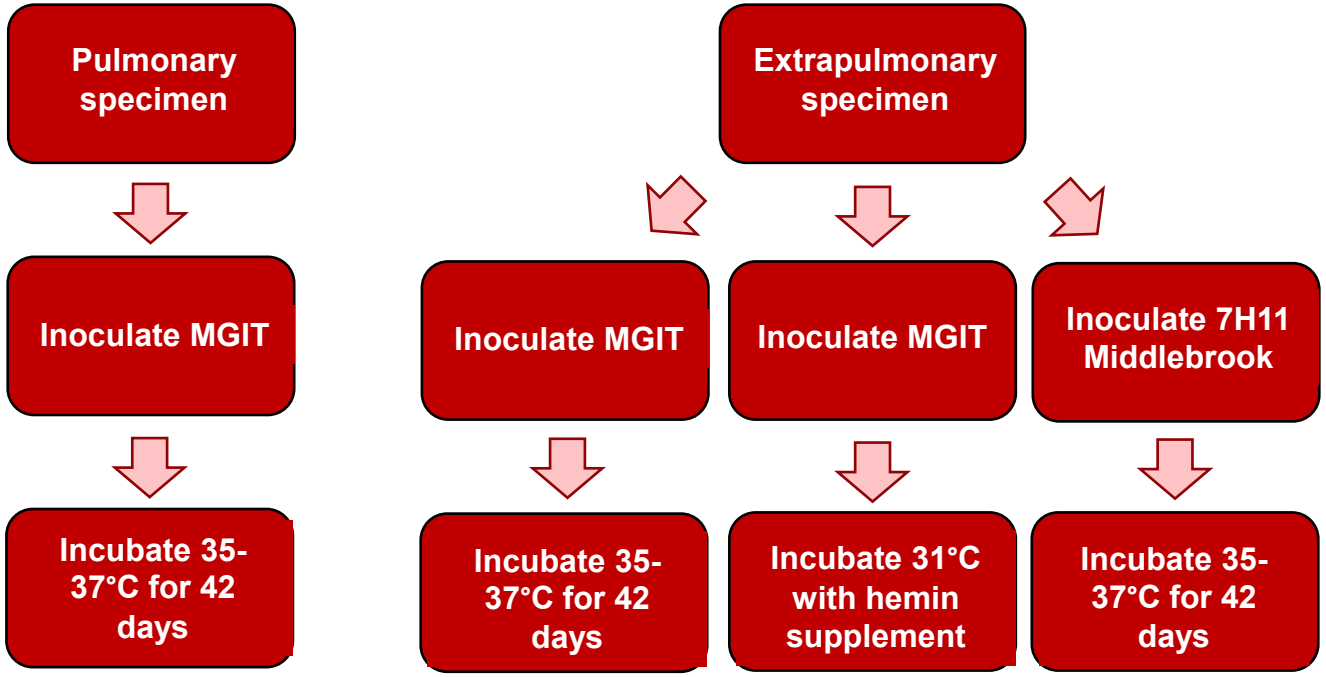
AFB smear testing

Respiratory specimen → Decontaminate: NALC/NaOH → Centrifugation concentration → Prepare AFB smear from 1-2 drops concentrated sediment → Stain slides

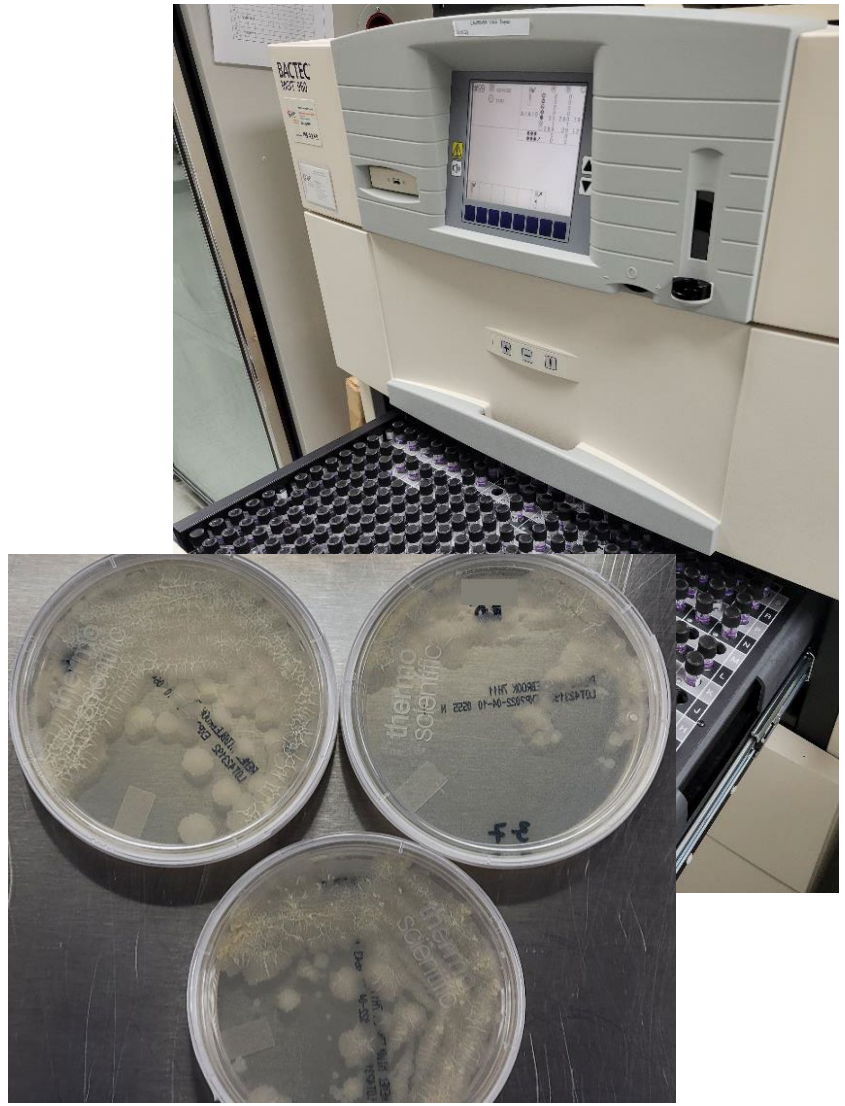


Rapid ID of potential Mycobacteria infection

AFB culture



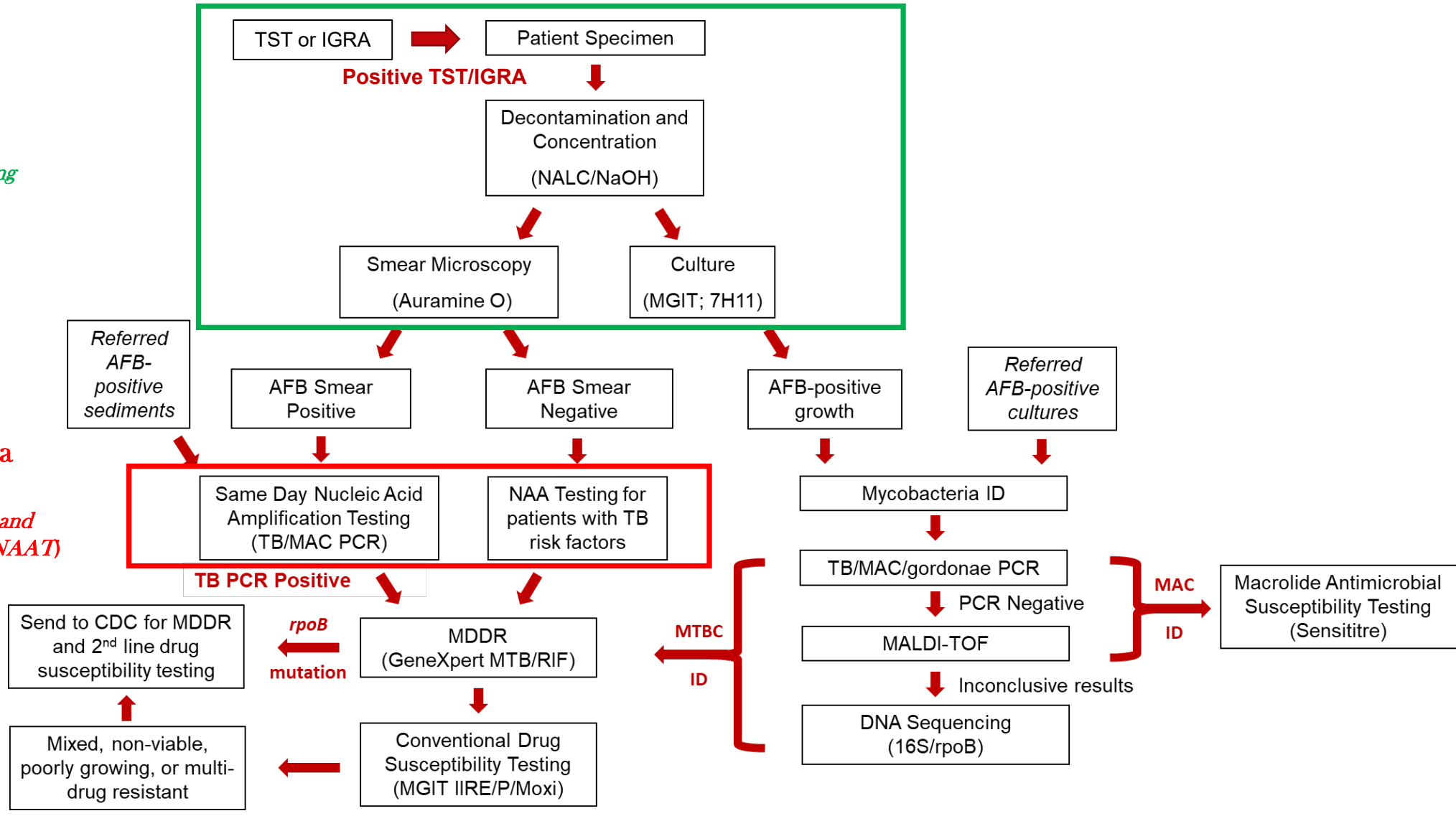
Upon submitter request or with suspicion of fastidious NTM:
Mycobactin J supplementation, 42°C incubation



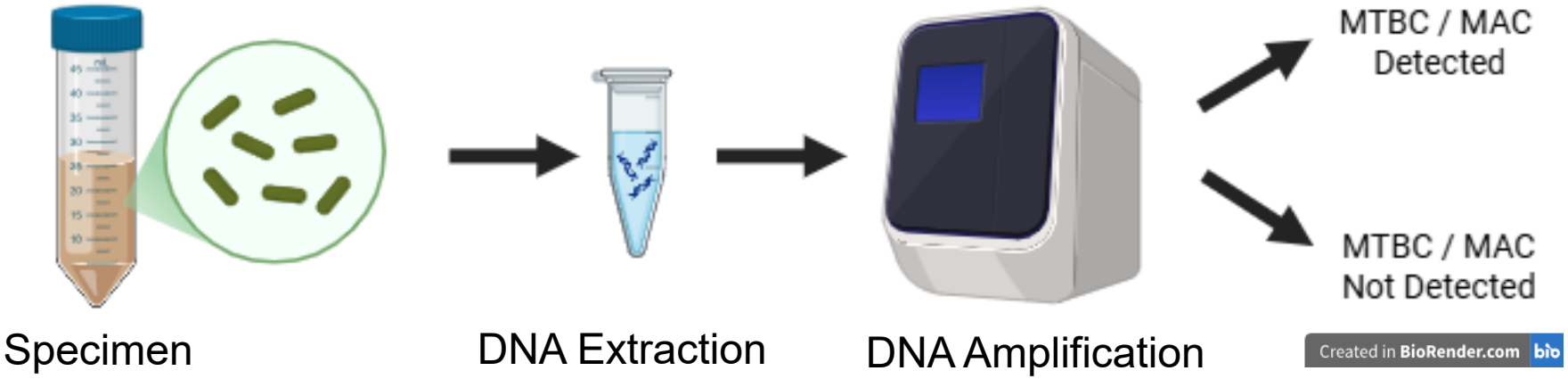
Modern Mycobacteriology

Rapid ID of potential mycobacteria infection
(*Use fluorescent acid-fast staining and promptly transmit results*)

Rapid ID of mycobacteria from primary specimens
(*Reduce average time to confirm and report mycobacteria cases using NAAT*)

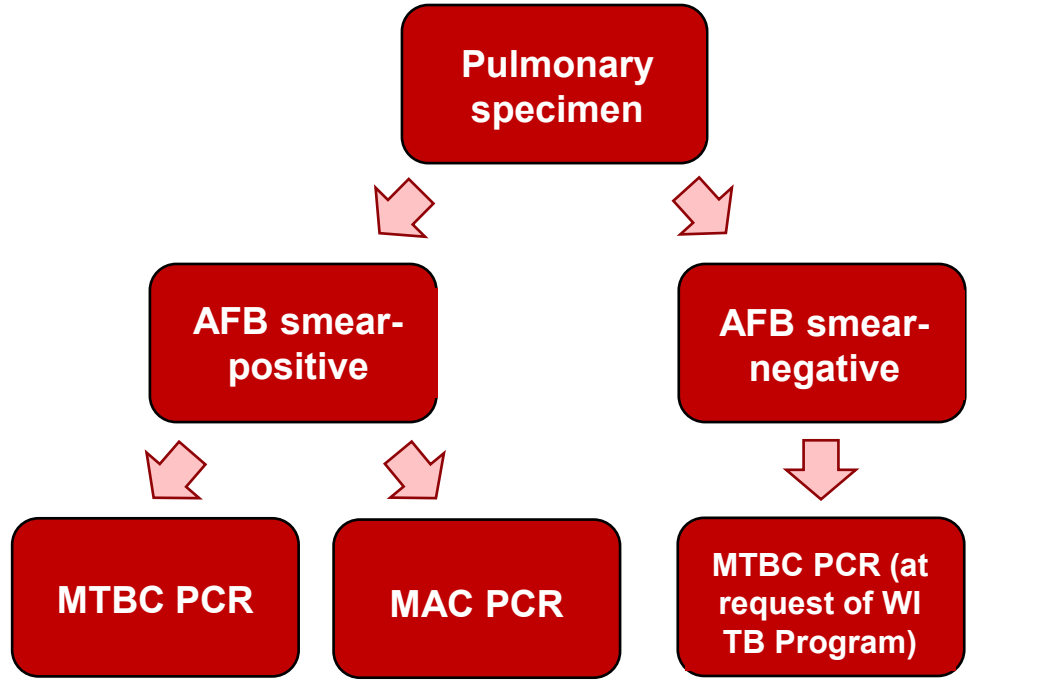


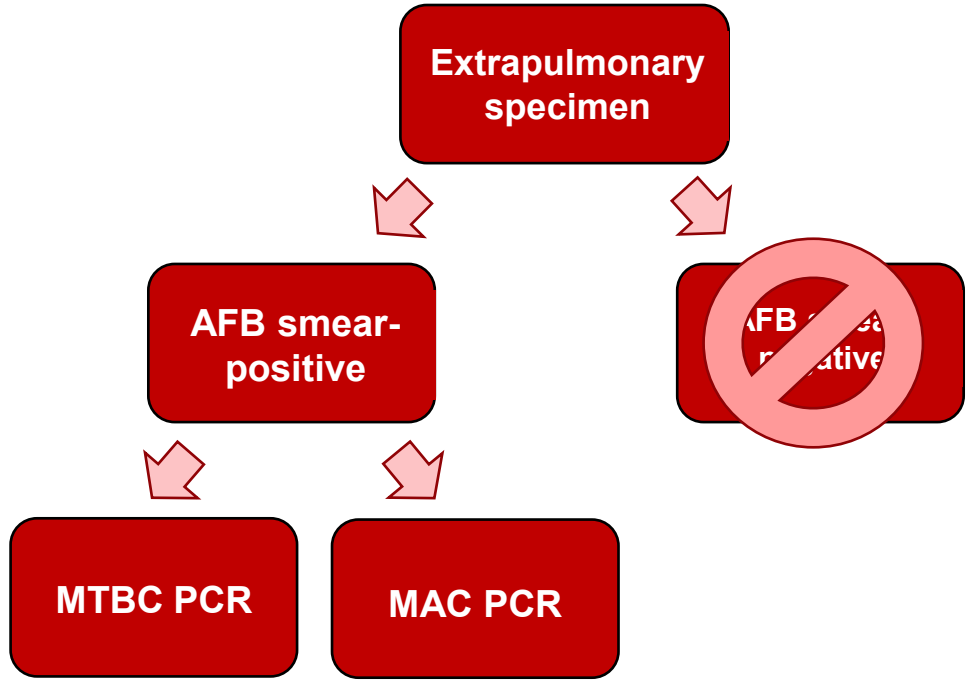
Rapid ID of Mycobacteria from Primary Specimens: WSLH LDT PCR and GeneXpert MTB/RIF



- MTBC real-time PCR: IS6110 target
- *M. avium* complex real-time PCR: 16S-23S internal transcribed spacer
  - 5 reverse primers, 2 probes
  - LOD: roughly 10^4 CFU/ml
- DNA extraction via spin-column removes PCR inhibitors and concentrates specimen

Rapid ID of Mycobacteria from Primary Specimens: WSLH LDT PCR and GeneXpert MTB/RIF





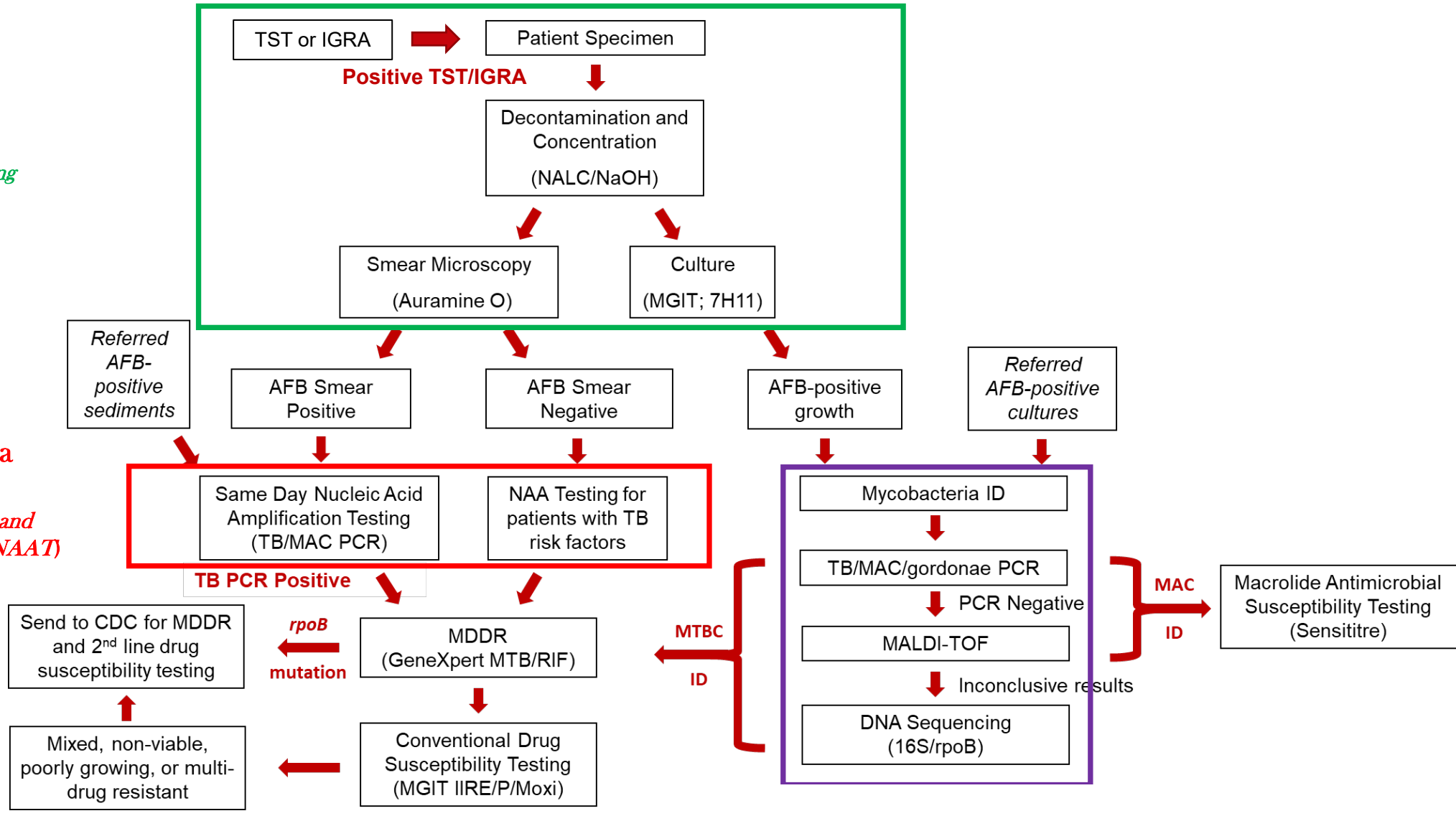
- GeneXpert performed only on MTBC-positive pulmonary specimens
  - *rpoB* mutation result
  - MTBC confirmation/second target in the event LDT PCR is inconclusive

Modern Mycobacteriology

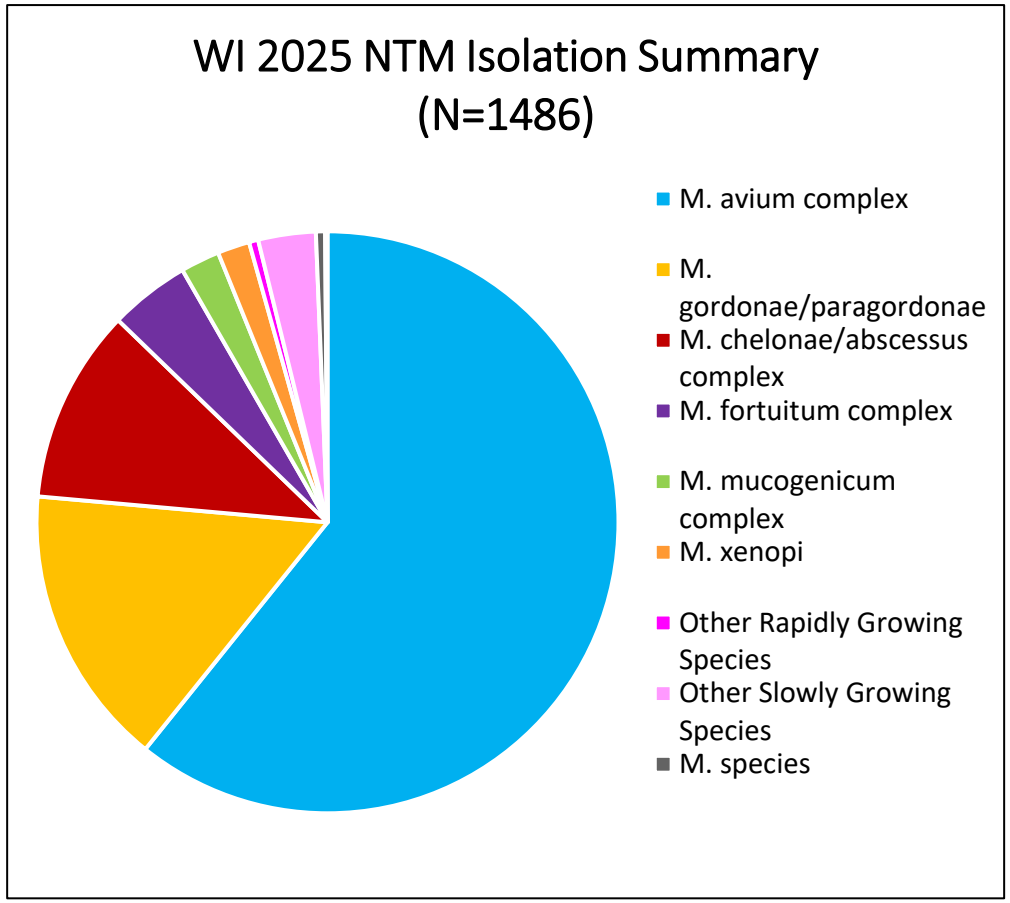
Rapid ID of potential mycobacteria infection
(*Use fluorescent acid-fast staining and promptly transmit results*)

Rapid ID of mycobacteria from primary specimens
(*Reduce average time to confirm and report mycobacteria cases using NAAT*)

ID of mycobacteria from positive culture
(*Use rapid methods to identify and report isolates*)



ID of Mycobacteria from Positive Culture: LDT real-time PCR



All cultures not obviously contaminated:

- MTBC real-time PCR (27% of positive cultures)
- MAC PCR (45%)

If both TB/MAC negative:

- *M. gordonae* real-time PCR (8%)
  - Unique 16S rRNA sequence
  - Cannot differentiate between *M. gordonae/M. paragordonae*

- DNA extraction via simple heat-lysis
- Not multiplexed, but all 3 reactions use same cycling conditions

ID of Mycobacteria from Positive Culture: MALDI-TOF

Bruker

M. timonense

M. scrofulaceum/parascrofulaceum

M. gordonae

M. avium

M. chimaera-intracellulare

M. mageritense

M. paragordonae

M. smegmatis

M. neoaurum/bacteremicum

M. llatzerense

M. iranicum

M. abscessus

M. franklinii

M. wolinskyi

M. neoaurum

M. marinum

M. paraffinicum

M. chelonae

M. arupense

M. tuberculosis complex

M. xenopi

M. malmoense

M. immunogenum

M. goodii

M. haemophilum

M. kansasii

M. septicum

M. porcinum

M. szulgai

M. kumamotonense

M. kubicae

M. peregrinum

M. fortuitum

M. saskatchewanense

M. simiae

M. shimoidei

M. nebraskense

M. triplex

M. heraklionense

M. holsiaticum

M. lentiflavum

M. mucogenicum/phocaicum

M. celatum

ID of Mycobacteria from Positive Culture: MALDI-TOF

Bruker

- Bruker Mycobacteria extraction, Bruker Mycobacteria Library v7.0
  - Validated both solid media (7H11, 7H10, LJ) and broth media (7H9)
    - Limited success with primary MGIT cultures
  - >1.0 McFarland initial suspension
  - For 7H9, perform wash step after initial pelleting
  - Subculture age matters: RGM 5-7 days, SGM 7-14

- Any NTM unable to ID by MALDI → Sanger sequencing (16S rRNA, *rpoB*)

Modern Mycobacteriology

Rapid ID of potential mycobacteria infection
(*Use fluorescent acid-fast staining and promptly transmit results*)

Rapid ID of mycobacteria from primary specimens
(*Reduce average time to confirm and report mycobacteria cases using NAAT*)

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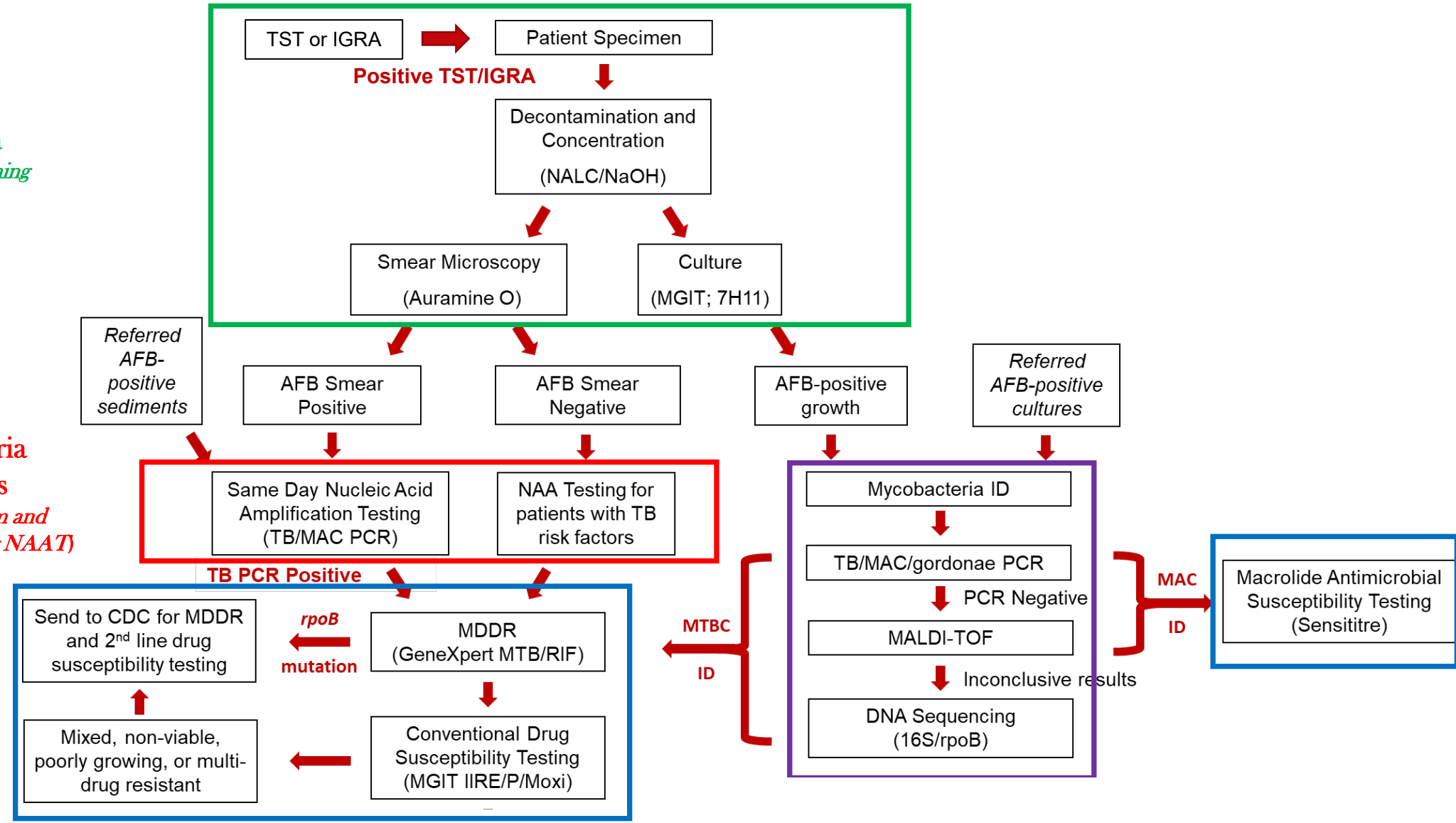


NTM Antimicrobial Susceptibility Testing

CLSI recommended method: Broth microdilution

- AST recommended for clinically significant isolates
- CLSI guidelines for only MAC, *M. kansasii, M. marinum*, RGM
  - Limited correlation of *in vitro* results with clinical response
- WSLH: Sensititre CLR susceptibilities on MAC by request
  - Repeated after 6 months if patients still positive
- All other organisms, drugs, molecular testing requests sent to a national reference lab

NTM Outbreak Investigations

A bronchoscopy-associated pseudo-outbreak of *Mycobacterium chelonae* and *Mycobacterium mucogenicum* associated with contaminated ice machine water and ice

Drew W. Engers MD[1], Rajeev Swarup MD[2,6], Cheryl Morrin CIC[3], Mica Blauw MPH[3,5], Miles Selfridge CHFM[4], Pierre Gonyon CHSP[4], Janet E. Stout PhD[7,8] and Anurag N. Malani MD[1,3]

https://www.cambridge.org/core/services/aop-cambridge-core/content/view/722973E9A74F8CB6D01083EE26732427/S0899823X23001010a.pdf

Mycobacterium abscessus Outbreak Related to Contaminated Water Among Ventilator-Dependent Residents of a Pediatric Facility — Pennsylvania, 2022

Jenna N. Sinkevitch, MSPH[1,2]; Julie Paoline, MA[1]; Aaron Smee, MPH[1]; Sophie Jones, PhD[3,4]; Kevin Spicer, MD, PhD[3]; Paige Gable, MPH[3]; Hollis Houston[3]; Valerie Stevens[3]; Cara Bicking Kinsey, PhD[1]

https://www.cdc.gov/mmwr/volumes/72/wr/pdfs/mm7242a5-H.pdf

Mycobacterium chimaera Contamination of Heater-Cooler Devices Used in Cardiac Surgery — United States

Kiran M. Perkins, MD[1]; Adrian Lawsin, MS[1]; Nabeeh A. Hasan, PhD[2]; Michael Strong, PhD[2]; Alison L. Halpin, PhD[1]; Rachael R. Rodger, MPH[2]; Heather Moulton-Meissner, PhD[1]; Matthew B. Crist, MD[1]; Suzanne Schwartz, MD[3]; Julia Marders, MS[3]; Charles L. Daley, MD[2]; Max Salfinger, MD[2]; Joseph F. Perz, DrPH[1]

https://www.cdc.gov/mmwr/volumes/65/wr/pdfs/mm6540a6.pdf

Mycobacterium abscessus Cluster in Cardiac Surgery Patients Potentially Attributable to a Commercial Water Purification System

Michael Klompas, MD, MPH; Chidiebere Akusobi, MD, PhD; Jon Boyer, ScD, CIH; Ann Woolley, MD; Ian D. Wolf; Robert Tucker, MPH, CIC; Chanu Rhee, MD, MPH; Karen Fiumara, PharmD; Madelyn Pearson, DNP; Charles A. Morris, MD, MPH; Eric Rubin, MD, PhD; and Meghan A. Baker, MD, ScD

https://www.acpjournals.org/doi/epdf/10.7326/M22-3306

Mycobacterium chelonae outbreak investigation at a quaternary pediatric hospital following the opening of a LEED-certified critical care tower: where does water sustainability intersect with infection control?

Andrea L. Ankrum MS, MS, MT(ASCP), CIC, FAPIC[1], Silvia M. Caceres MSc[2,3], Michael Torsell BA[1], Elaine Epperson PhD[4], Vinicius Calado Nogueira de Moura MSc[4], Jennifer J. Gilick BS[4], Michael Strong PhD[4], Qingyun Liu PhD[3], Matthew J. Strand PhD[5], Rachel N. Wilsey BS[6], Jennifer R. Honda PhD[6], Jane E. Gross MD, PhD[2,3] and Felicia A. Scaggs-Huang MD, MSc[1,7,8]

https://www.cambridge.org/core/services/aop-cambridge-core/content/view/03832C73EDE4FD3B587D3050193682CC/S0899823X25103449a.pdf

Outbreak of Tattoo-associated Nontuberculous Mycobacterial Skin Infections

Isabel Griffin,[1] Ann Schmitz,[2,3] Christine Oliver,[4] Scott Pritchard,[2] Guoyan Zhang,[1] Edhelene Rico,[1] Emily Davenport,[5] Anthoni Llau,[1] Emily Moore,[1] Danielle Fernandez,[1] Alvaro Mejia-Echeverry,[1] Juan Suarez,[1] Pedro Noya-Chaveco,[1] Samir Elmir,[4] Reynald Jean,[1] James B. Pettengill,[6] Katherine A. Hollinger,[6] Kyson Chou,[6] Donna Williams-Hill,[6] Sherif Zaki,[7] Atis Muehlenbachs,[7] M. Kelly Keating,[7] Julu Bhatnagar,[7] Marie-Claire Rowlinson,[8] Calin Chiribau,[8] and Lillian Rivera[1]

https://watermark02.silverchair.com/ciy979.pdf

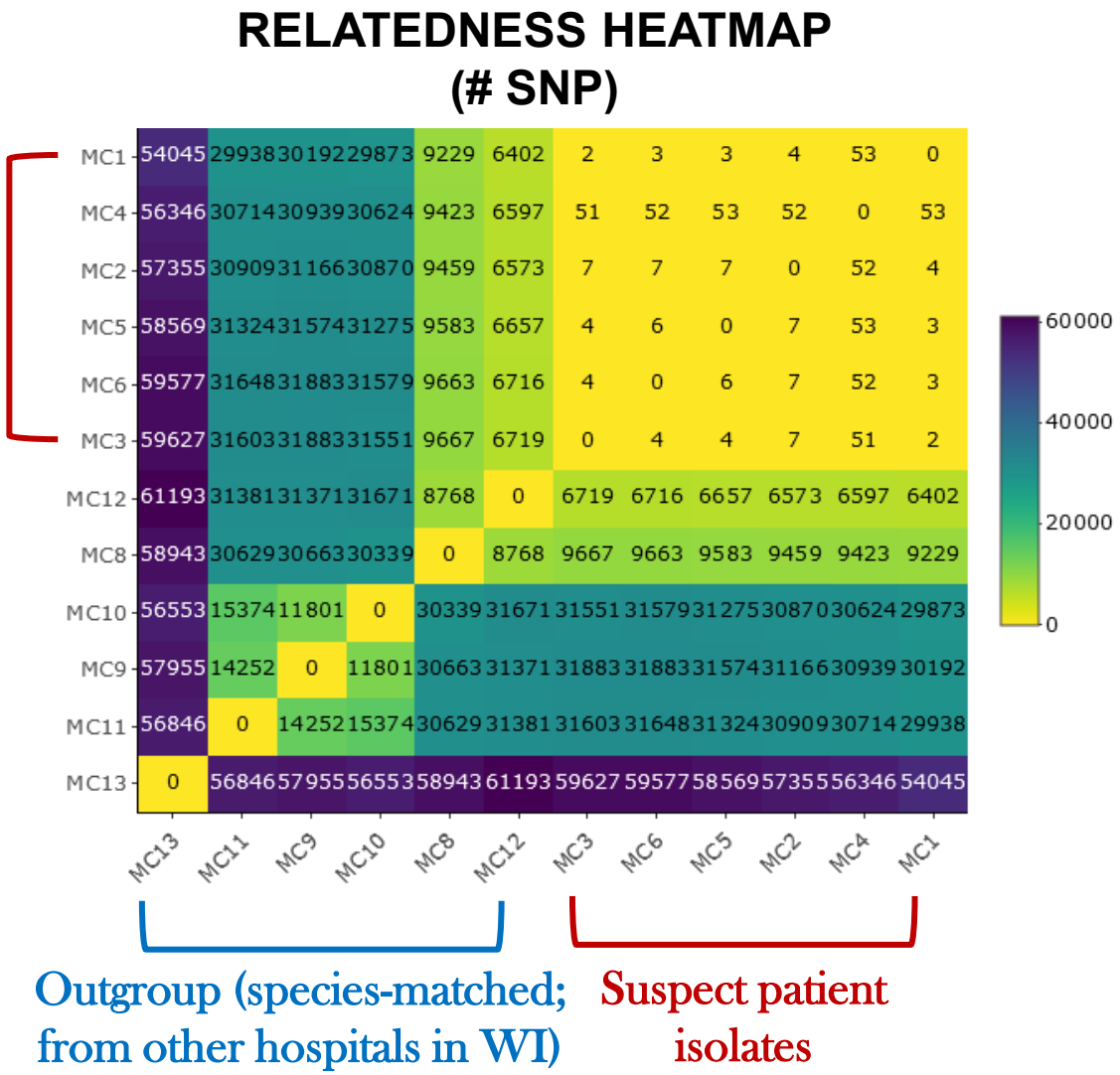
Hospital Water Contamination Associated with a Pseudo-Outbreak of *Mycobacterium porcinum* — Wisconsin, 2016–2018

Heather Kloth[1]; Lina I. Elbadawi, MD[1,2]; Allen Bateman, PhD[3]; Laura Louison, MLS[3]; Nijika Shrivastwa, PhD[1]

https://www.cdc.gov/mmwr/volumes/68/wr/pdfs/mm6849a4-H.pdf

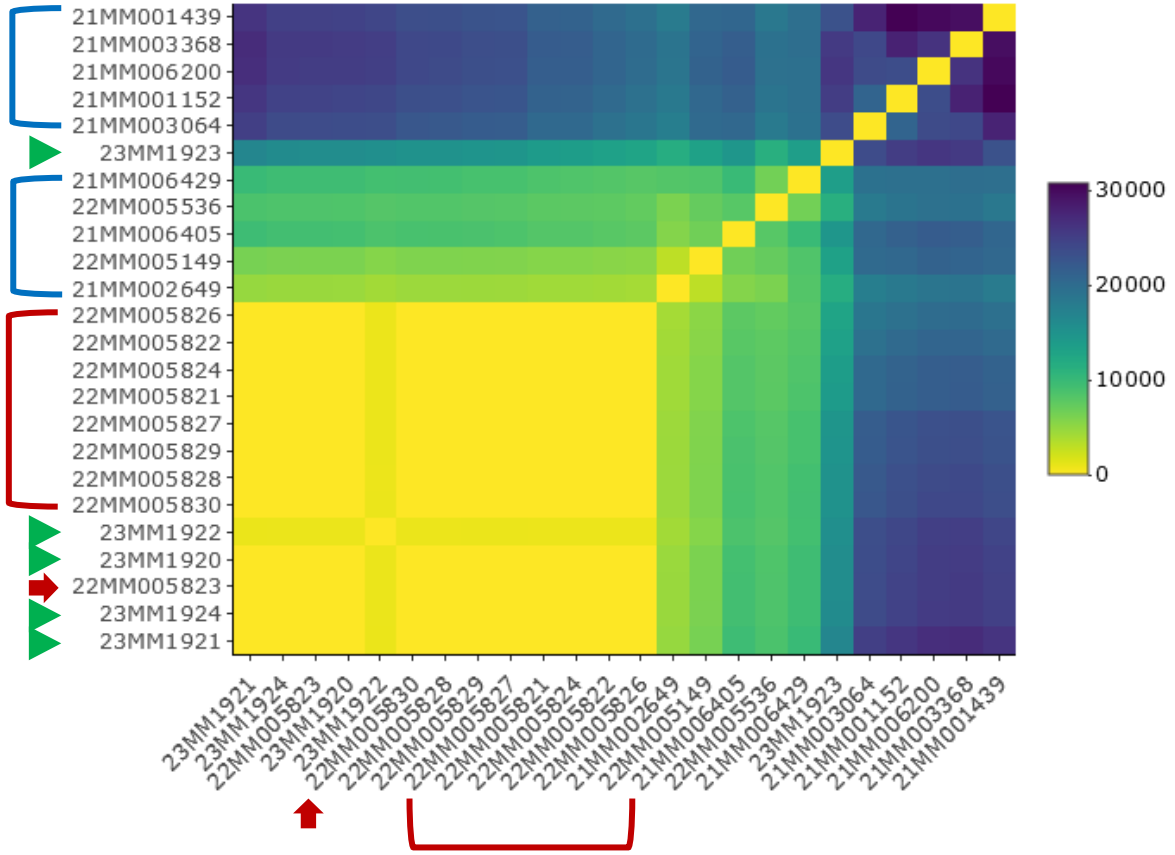
Non-tuberculous Mycobacteria: Outbreak Investigation A

- Clinical laboratory noticed abnormal number of patient cultures positive for *M. chelonae*
  - Could WSLH perform relatedness analyses?
- DNA extracted from NTM isolates using TB WGS extraction
  - Sequenced on Illumina NextSeq
  - WGS data processed with tbQC and Dryad genome analysis pipelines

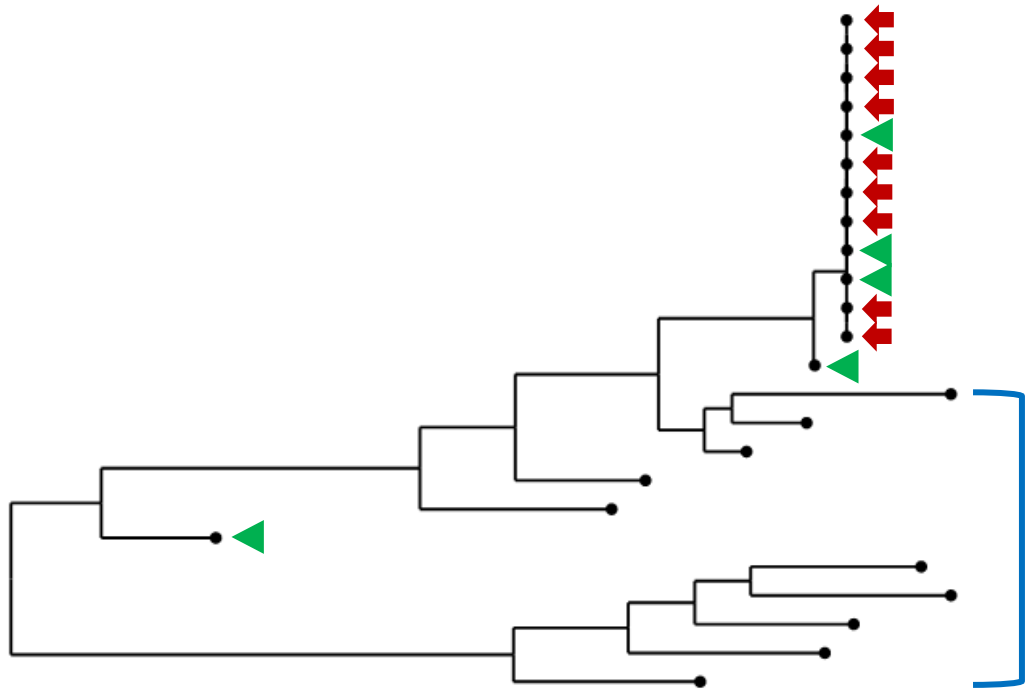


Non-tuberculous Mycobacteria: Outbreak Investigation B

RELATEDNESS HEATMAP (# SNP)



PHYLOGENETIC TREE



➡ Suspect patient isolates

▶ Environmental isolates (surgical equipment/suite)

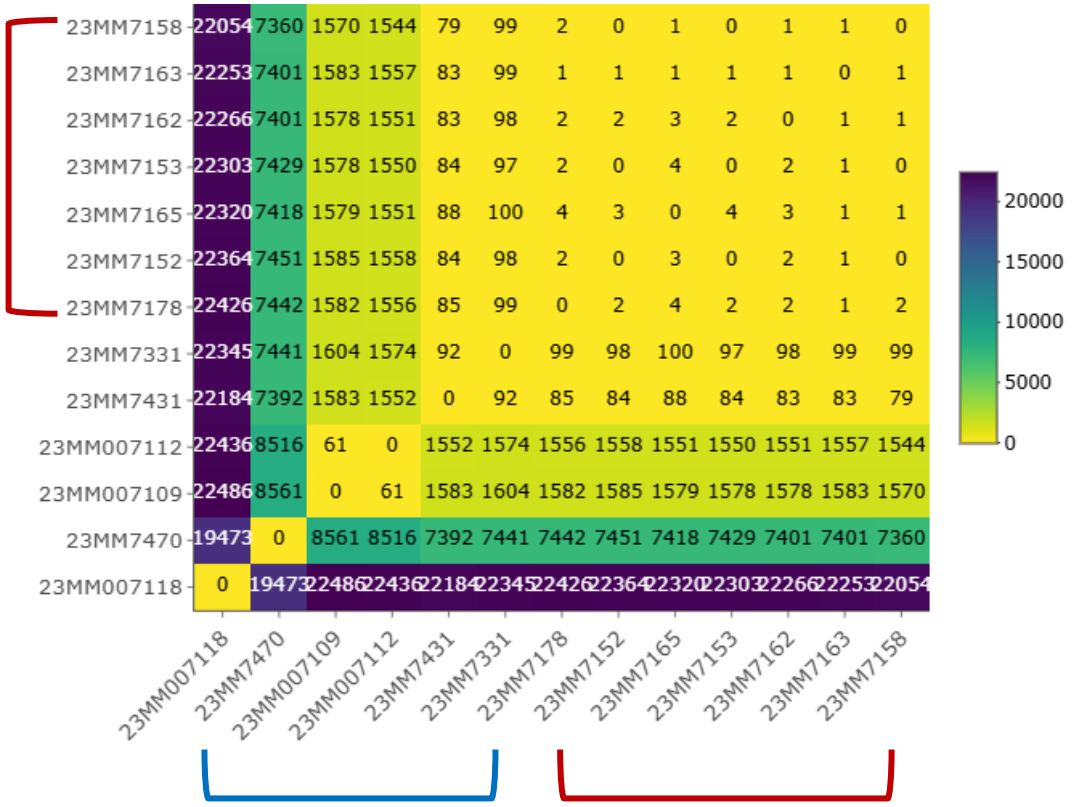
Outgroup (species-matched; from other hospitals in WI)

Use of WGS to identify NTM cross-contamination

- Clinician questioned accuracy of a couple of MAC-positive cultures
  - Thigh fluid from 10yo
  - Brain abscess fluid
- Specimens processed on same day
  - Upon review, 7/16 specimens on that smear/culture batch positive for MAC
- Pulled all potential contaminant isolates for DNA extraction, NextSeq WGS
  - MAC+ processed specimens, other MAC+ subcultured on same day as suspect false-positives
  - Outgroup-referred MAC+ cultures from WI clinical labs

Specimen ID	Source	WGS species
23MM007151	sputum	Mycobacterium intracellulare
23MM007152	**BAL**	Mycobacterium avium
23MM007153	**tissue**	Mycobacterium avium
23MM007155	BAL	negative
23MM007156	pleural fluid	negative
23MM007157	sputum	negative
23MM007158	**Sputum**	Mycobacterium avium
23MM007162	**thigh fluid**	Mycobacterium avium
23MM007163	**abscess**	Mycobacterium avium
23MM007164	wound	negative
23MM007165	**BAL**	Mycobacterium avium
23MM007166	BAL	negative
23MM007167	neck fluid	negative
23MM007168	BAL	negative
23MM007169	tissue	negative
23MM007170	fluid	negative

Use of WGS to identify NTM cross-contamination



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

Suspect patient isolates

Specimen ID	Source	WGS species	#SNP (23MM007152)
23MM007152	BAL	Mycobacterium avium	-
23MM007153	tissue	Mycobacterium avium	0
23MM007158	Sputum	Mycobacterium avium	0
23MM007162	thigh fluid	Mycobacterium avium	0
23MM007163	abscess	Mycobacterium avium	0
23MM007165	BAL	Mycobacterium avium	2
23MM007431	Referred subculture	Mycobacterium avium	82
23MM007331	Primary subculture	Mycobacterium avium	98
23MM007470	Referred subculture	Mycobacterium avium	7362
23MM007109	Primary subculture	Mycobacterium avium	1585
23MM007112	Primary subculture	Mycobacterium avium	1558
23MM007118	Primary subculture	Mycobacterium avium	22364

Role of Public Health in NTM ID and Surveillance

- NTM incidence probably higher than appreciated
- While likely not classically “transmissible”, morbidity and mortality can approach that of MTBC infection; outbreaks do occur
- Public Health laboratories are uniquely set up to handle these specimens
  - Existing workflows rapidly ID TB; if not TB, then likely NTM
  - Existing relationships with clinical partners to obtain specimens
  - Increasing NGS capacities at many public health labs allow for enhanced ID and surveillance capabilities
    - Still need well developed bioinformatics platforms
    - Potential uses for molecular AST?
- Build partnerships with clinical labs to enhance testing and surveillance!

Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

ACKNOWLEDGEMENTS

WI DHS TB Program

WI Mycobacteriology Laboratory Network

WSLH Communicable Disease Division

- *WSLH Mycobacteriology Laboratory*
- *WSLH Sequencing Group*
- *WSLH Bioinformatics Group*

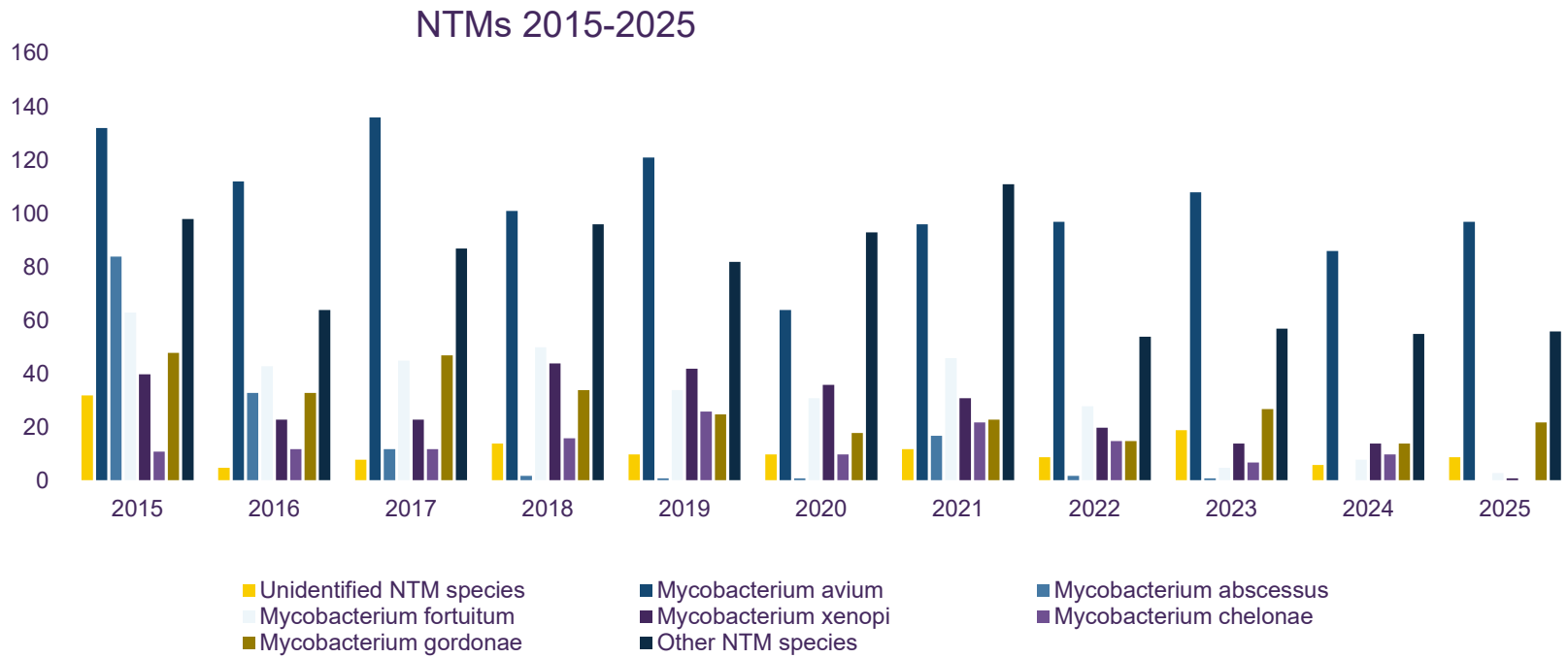
NEW YORK STATE

Department of Health
Wadsworth Center

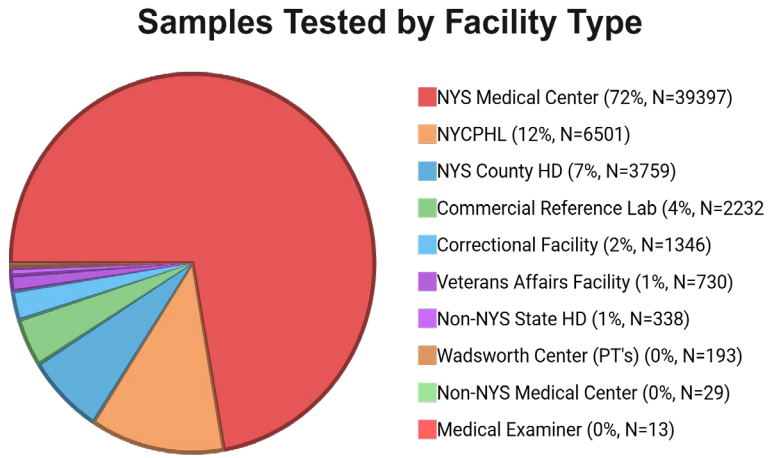
Nontuberculous Mycobacteria Testing at Wadsworth Center

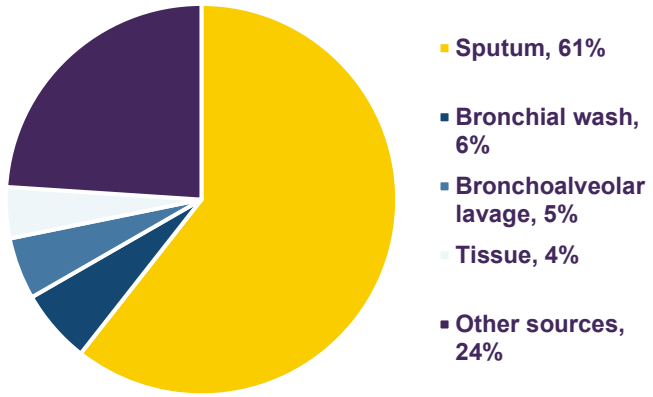
Vincent Escuyer, Pharm.D., Ph.D.
Director, Mycobacteriology Laboratory

Some numbers…



NTM= Nontuberculous Mycobacteria





NTM are not reportable in New York State

NEW YORK STATE | Department of Health
Wadsworth Center

NTM identification

AccuProbes	No longer available
HPLC, PRA	No longer recommended
MALDI-TOF MS*	**FDA cleared**
Sanger sequencing**	Laboratory developed test
Whole genome sequencing	Laboratory developed test
MicroSeq500 16S rDNA kit	Not FDA cleared
GenoType NTM-DR	Not FDA cleared
GenoType Mycobacterium CM/AM	Not FDA cleared
INNO-LiPA Mycobacteria	Not FDA cleared
Deeplex-MycTB (tNGS)***	Not FDA cleared

https://clsi.org/shop/standards/m24/

*MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

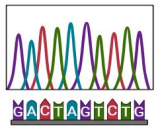
***16S* rRNA, ITS, *hsp65*, *rpoB* genes

*** tNGS: Targeted Next generation sequencing

Eras of Molecular NTM Testing at Wadsworth Center

2005 — 2010 — 2015 — 2020 — 2025

Broad Range PCR w/Sanger Sequencing & Sequence Analysis: *rpoB, hsp65,* 16S rRNA

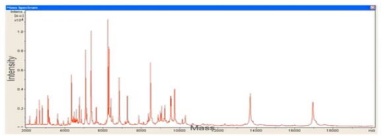


AccuProbe (Hologic):
MAC, *M. gordonae*

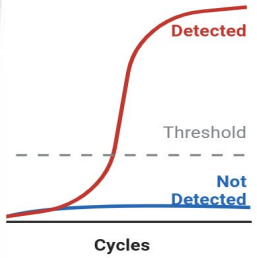
M. abscessus real-time PCR

MAC real-time PCR

MALDI-TOF MS (Bruker)

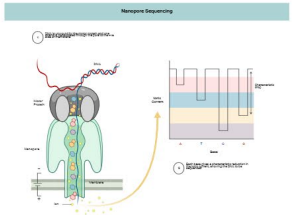


Real time PCR:
M. fortuitum
M. kansasii
M. xenopi
M. chelonae



16S Minion tNGS

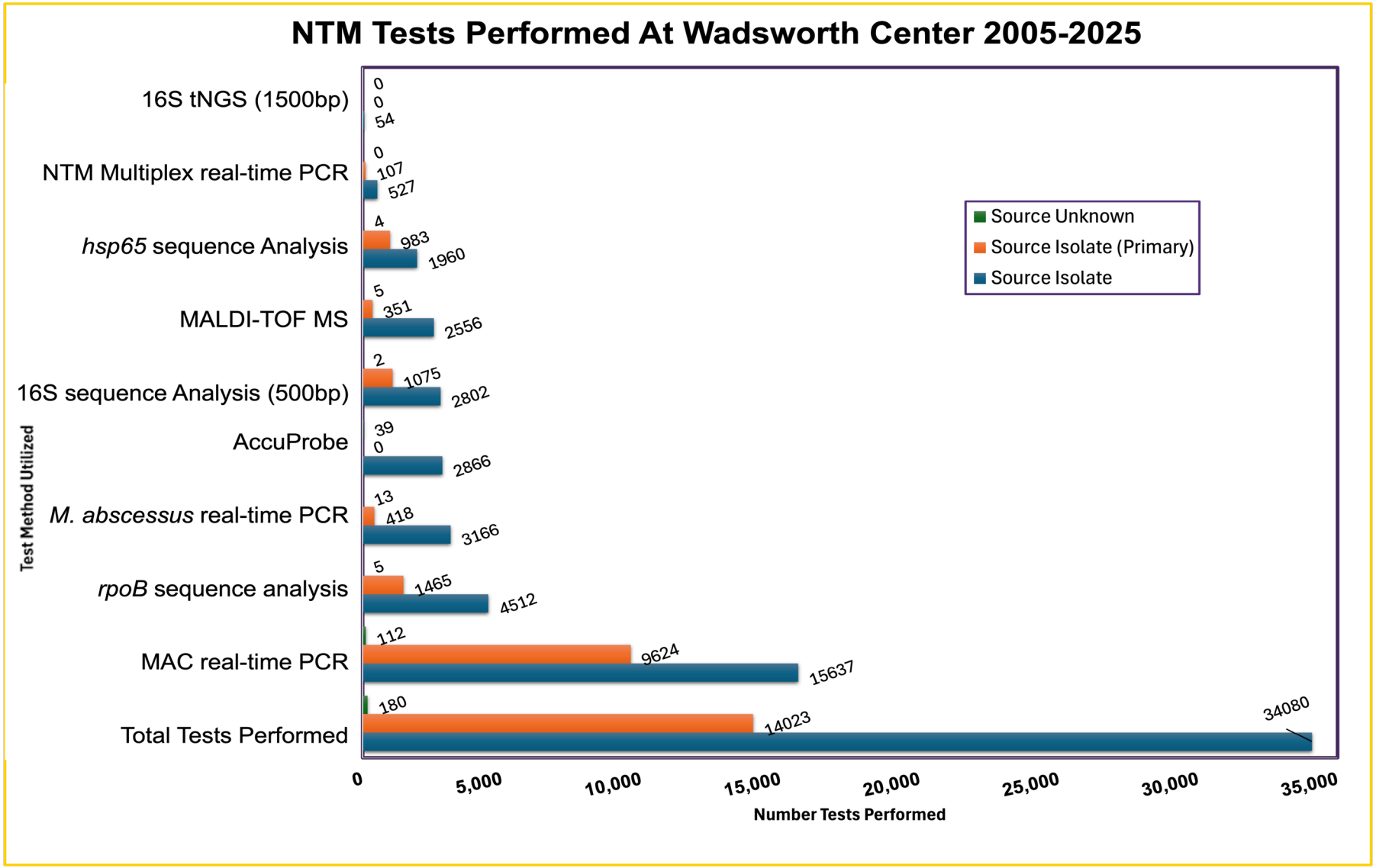
The Next Era: tNGS + in-house curated pipeline *rpoB, atpD* (ATP synthase β subunit), EF-Tu (elongation factor thermo unstable), *hsp65, 16S* rRNA, *23S* rRNA

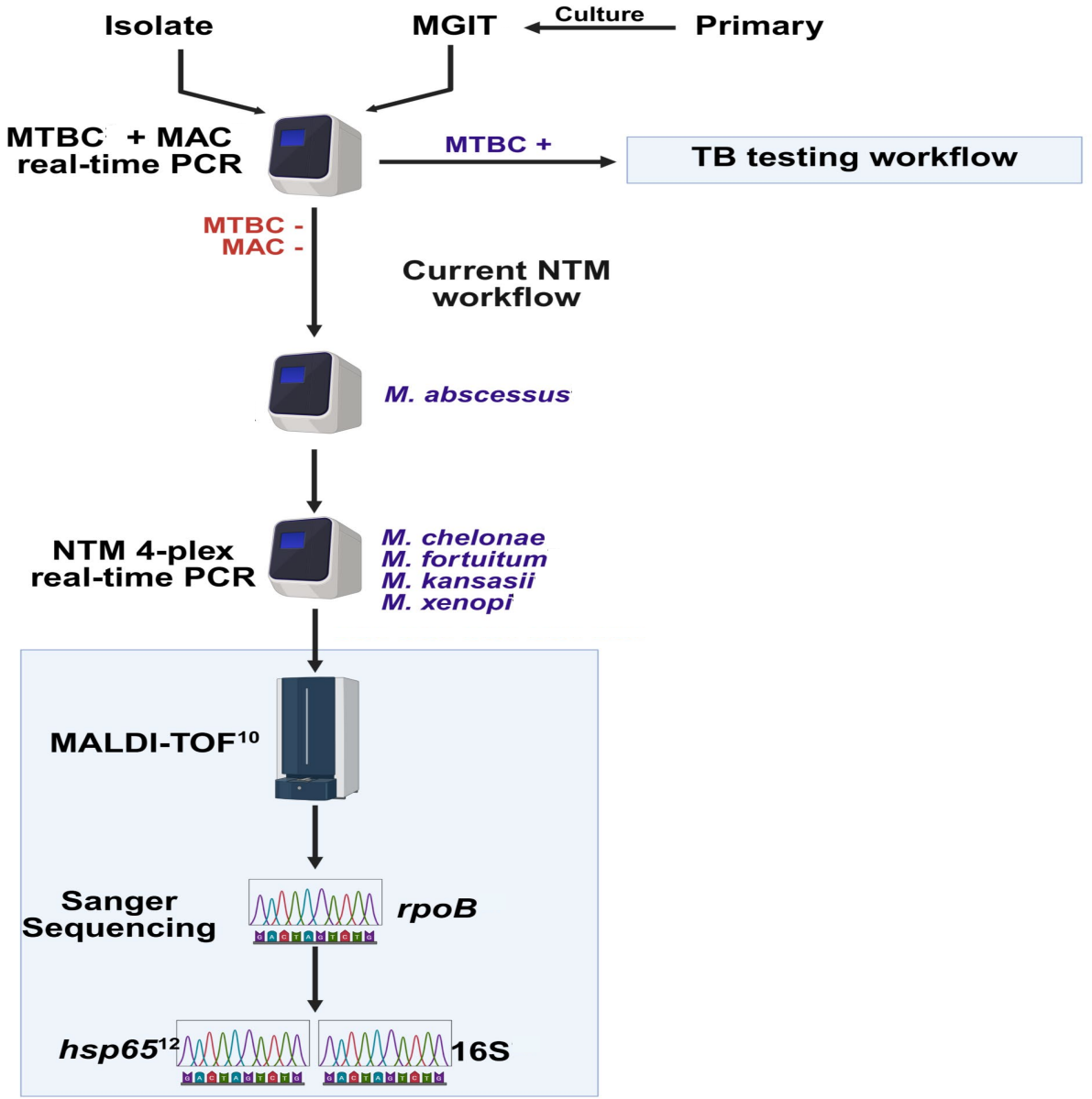


tNGS performed on Oxford Nanopore Technologies (ONT) Minion device

MAC: Mycobacterium avium complex
PCR: Polymerase Chain Reaction

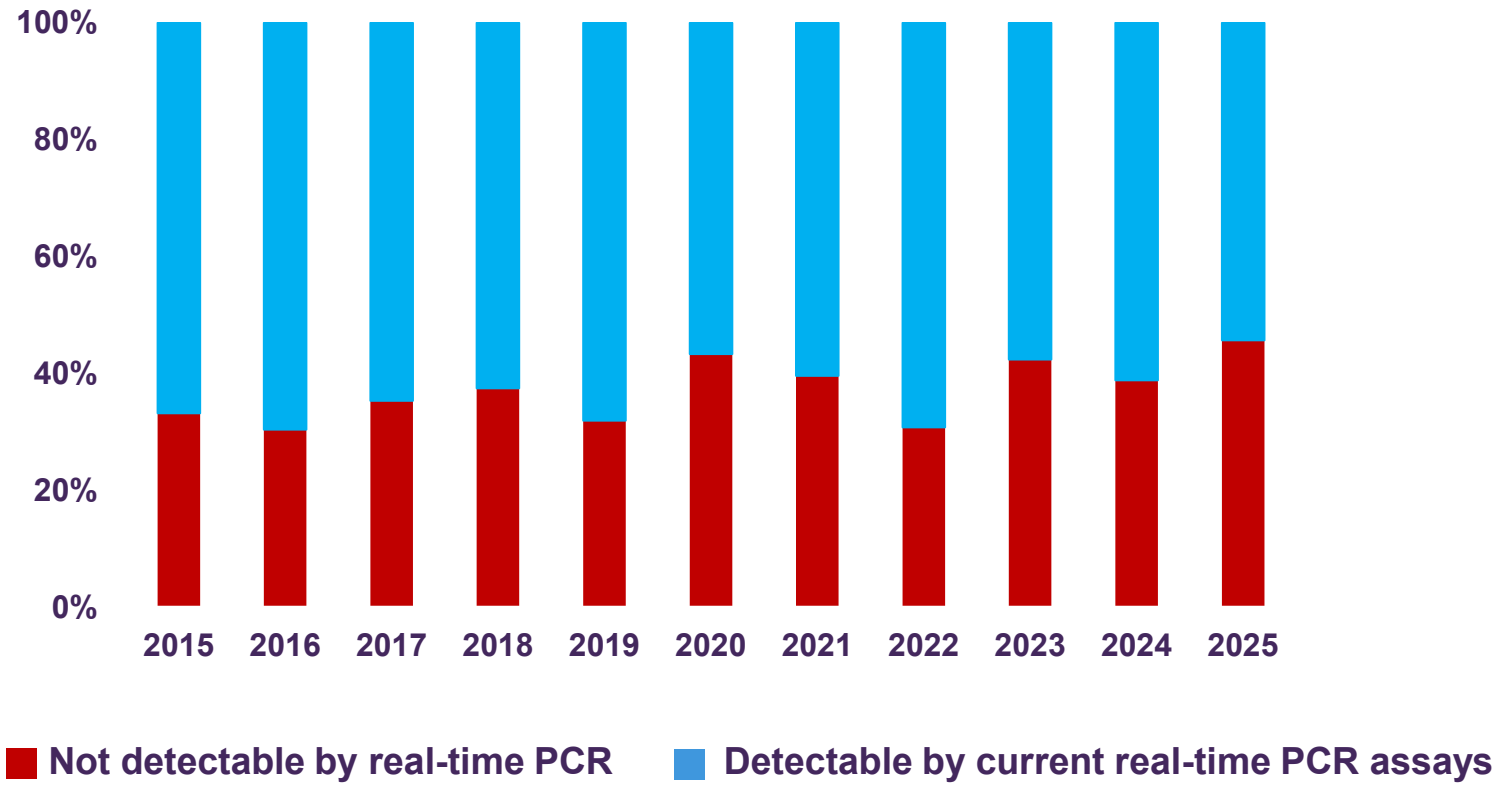
Biorender.com


NTM Tests Performed At Wadsworth Center 2005-2025
16S tNGS (1500bp)
NTM Multiplex real-time PCR
hsp65 sequence Analysis
MALDI-TOF MS
16S sequence Analysis (500bp)
AccuProbe
M. abscessus real-time PCR
rpoB sequence analysis
MAC real-time PCR
Total Tests Performed
Test Method Utilized
0
0
54
0
107
527
4
983
1960
5
351
2556
2
1075
2802
39
0
2866
13
418
3166
5
1465
4512
112
9624
15637
180
14023
34080
Source Unknown
Source Isolate (Primary)
Source Isolate
0
5,000
10,000
15,000
20,000
25,000
30,000
35,000
Number Tests Performed



Biorender.com

NTM grouped by real-time PCR detection status



Limitations of MALDI-TOF MS

Bruker image

- Mycobacterial cell wall that requires special extraction methods
- Low sensitivity; Needs enough bio-masse; Problem with slow growers
- Limitations of databases. Need to be validated as they are Research Use Only (RUO)
- Cannot discriminate between very closely related species or subspecies: *M. intracellulare/chimerae*; *M. abscessus complex*, …

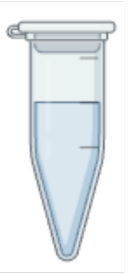
Succes rate in our laboratory ~70%: 30% need to reflex to Sanger sequencing

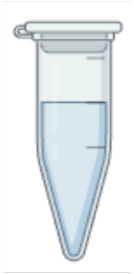
NTM identification: The next generation

PCR Amplification

Pool A

16S
efTu
atpD
hsp65





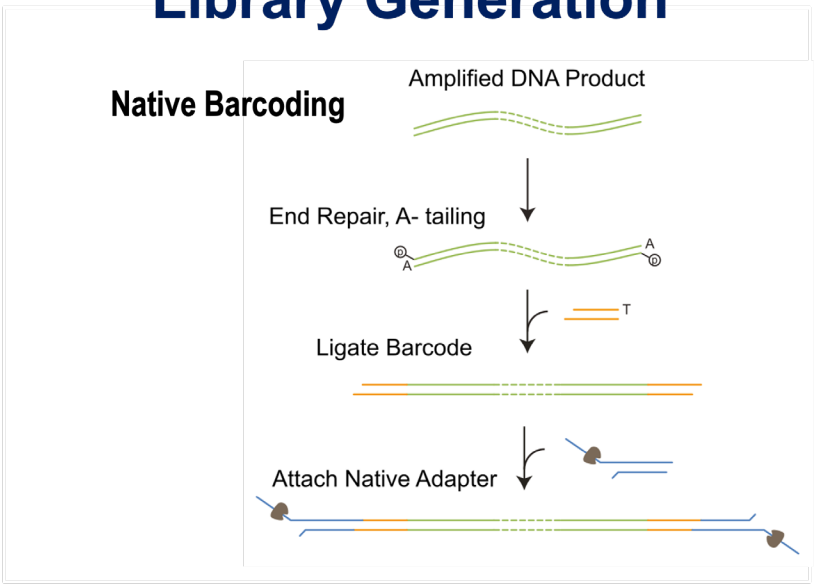
Pool B

23S
rpoB

~ 800 – 1,200 bp

~ 2,300 – 2,900 bp

Library Generation



Nanopore Sequencing

Library build time: ~ 6-8 hours (1.5 days)

Sequencing time: ~1-2 hours

Throughput: up to 16 samples per run

Wadsworth cost - reagents and flow cells: ~$36 per sample

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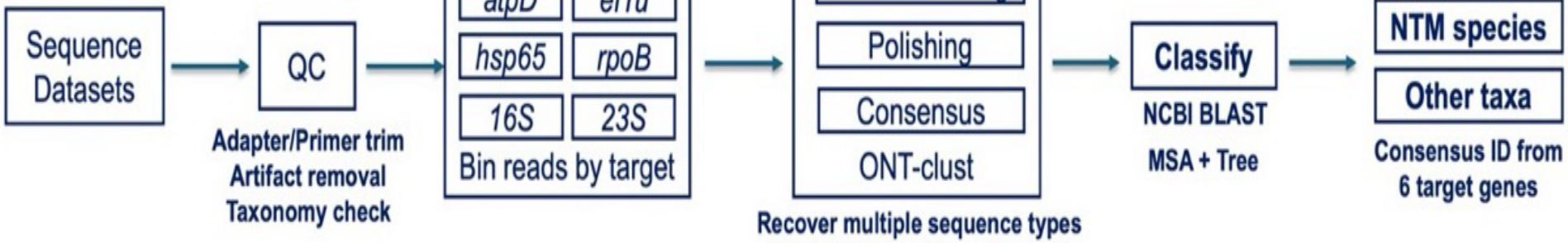


Abbreviations
NTM: Nontuberculous Mycobacteria
MGIT: Mycobacteria Growth Indicator Tube
ONT: Oxford Nanopore Technologies
tNGS: Targeted next-generation sequencing
MSA: Multiple sequence alignment

Images from https://www.biorender.com

Come see our poster!

Poster 9

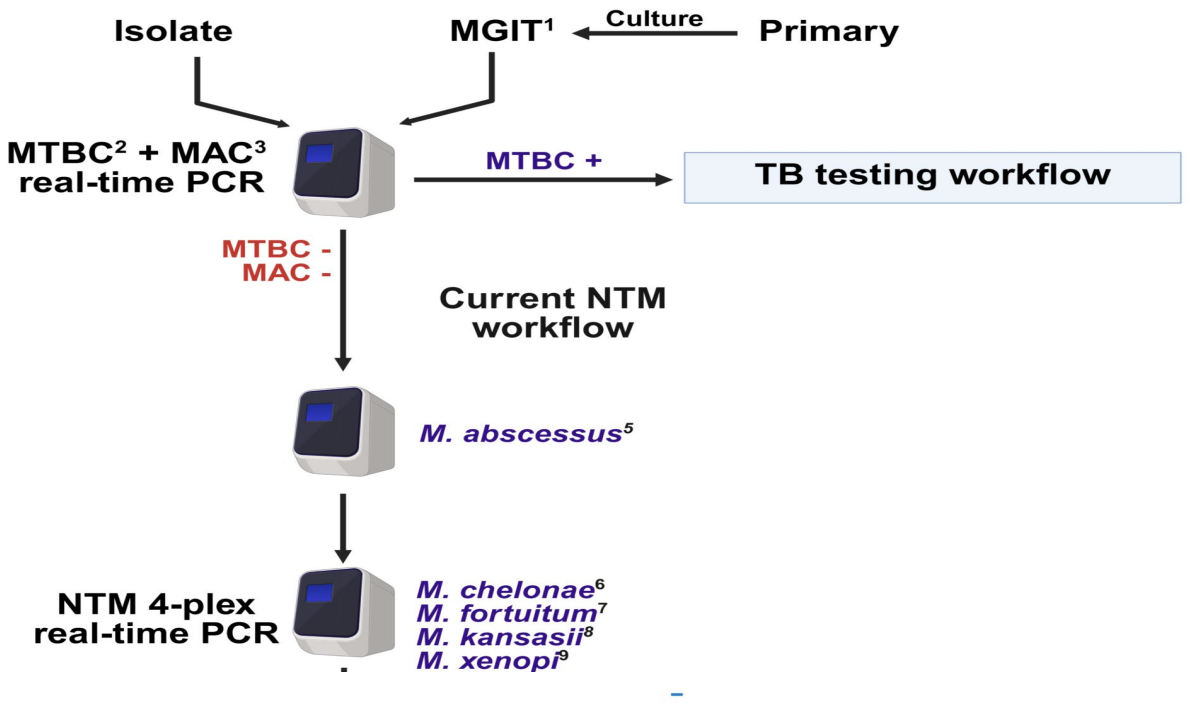
Development and Validation of a Targeted Next Generation Sequencing Assay for Identification of Nontuberculous Mycobacteria

Carol D Smith, Krithivasan Sankaranarayanan, Joseph Shea, Michelle Dickinson, Donna Kohlerschmidt, Vincent Escuyer, Kimberlee Musser, New York State Department of Health, Wadsworth Center

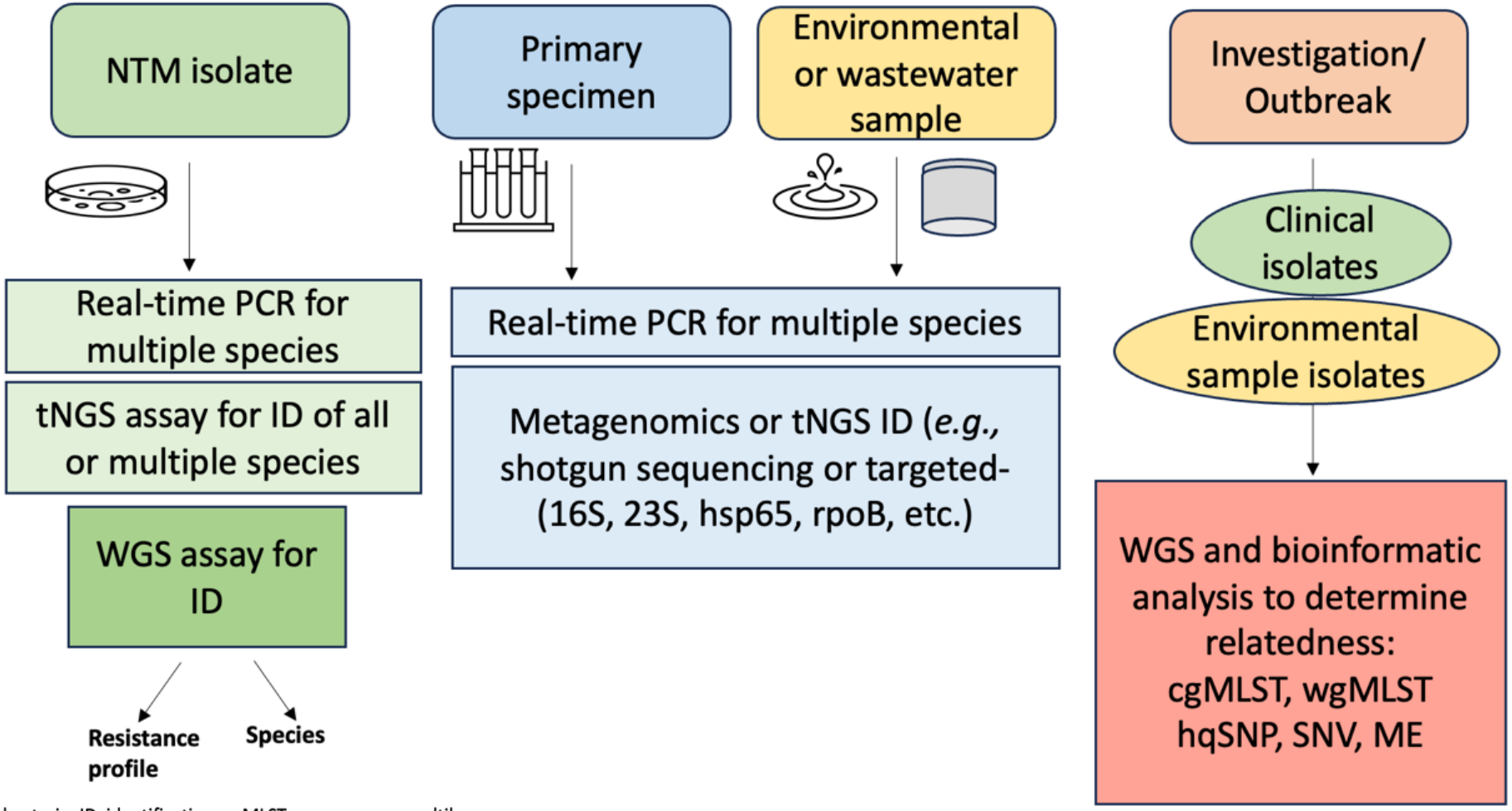
23 | 14th National Conference on Laboratory Aspects of Tuberculosis | #APHLTB

Presenter: Carol Smith, carol.smith@health.ny.gov

Soon in a theater near you…



Comprehensive NTM testing in the Public Health Laboratory



NTM, nontuberculous mycobacteria; ID, identification; cgMLST, core genome multilocus sequence typing; wgMLST, whole genome multilocus sequence typing; hqSNP, high quality single nucleotide polymorphism; SNV, single nucleotide variant; ME, mutation event

Investigation of a *Mycobacterium abscessus* outbreak in a hospital setting

9 patients isolates
18 environmental isolates

5 clusters: **4 involving both patient and environmental isolates**
1 involving exclusively environmental isolates

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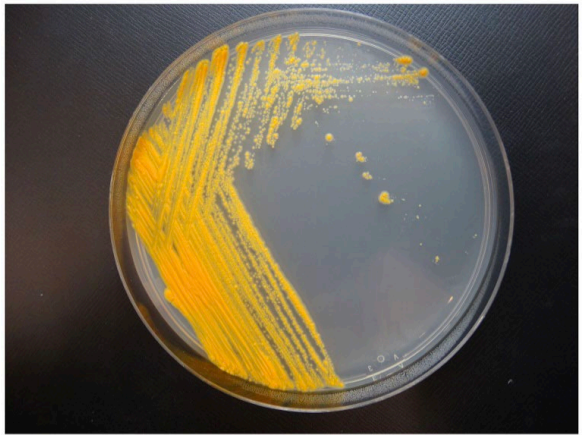

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