Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

Diagnostic Testing for *Mycobacterium tuberculosis* complex

Updates from WSLH and the Field

Nate Simon
WI TB Laboratory Program Coordinator

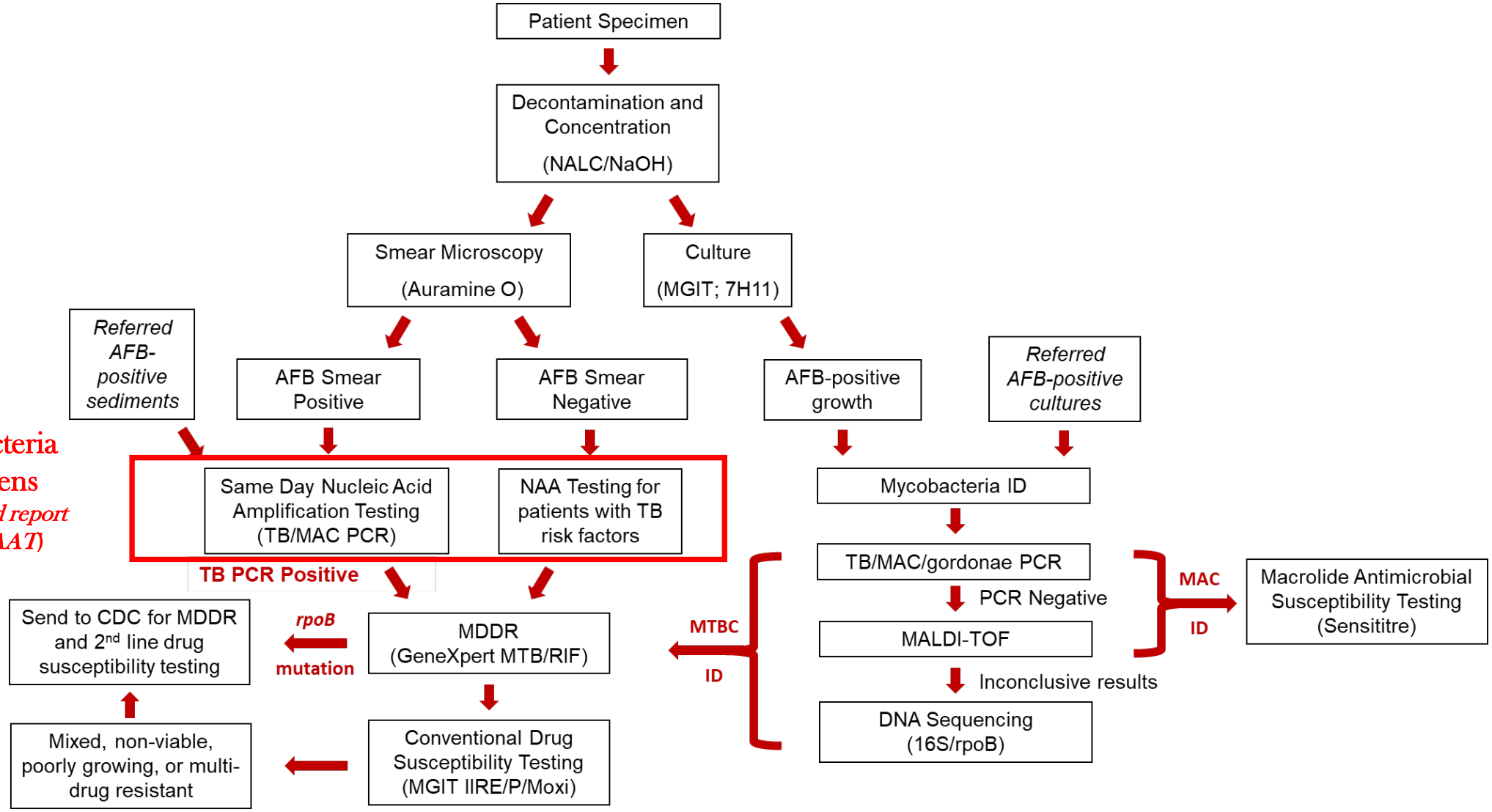
WI State Laboratory of Hygiene
04/07/2026

Learning objectives

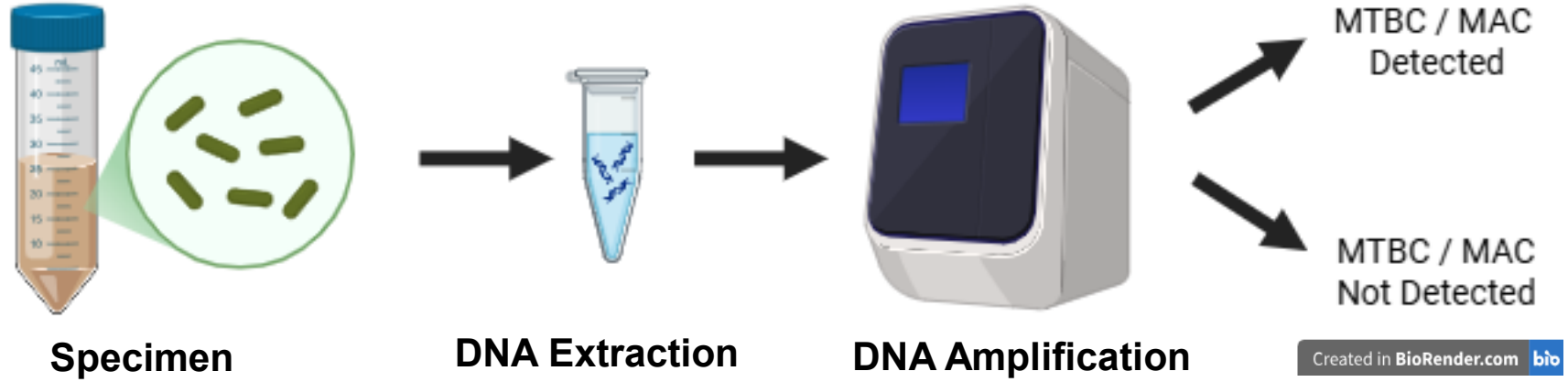
- Understand WSLH PCR algorithm and how to request TB PCR testing for patients suspected of having TB
- Understand GeneXpert MTB/RIF testing criteria and results interpretation
- WSLH testing updates

WSLH Mycobacteriology

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



Rapid ID of Mycobacteria from Primary Specimens: WSLH TB/MAC PCR



- Detect *M. tuberculosis* complex DNA and *M. avium* complex DNA directly from processed sediment (LDT)
- Sensitivity
  - >95% for AFB smear-positive, culture-confirmed TB patients
  - ~50% of AFB smear-negative, culture-confirmed TB patients
  - LOD: <1 MTBC bacillus/reaction (≈140 AFB/ml)

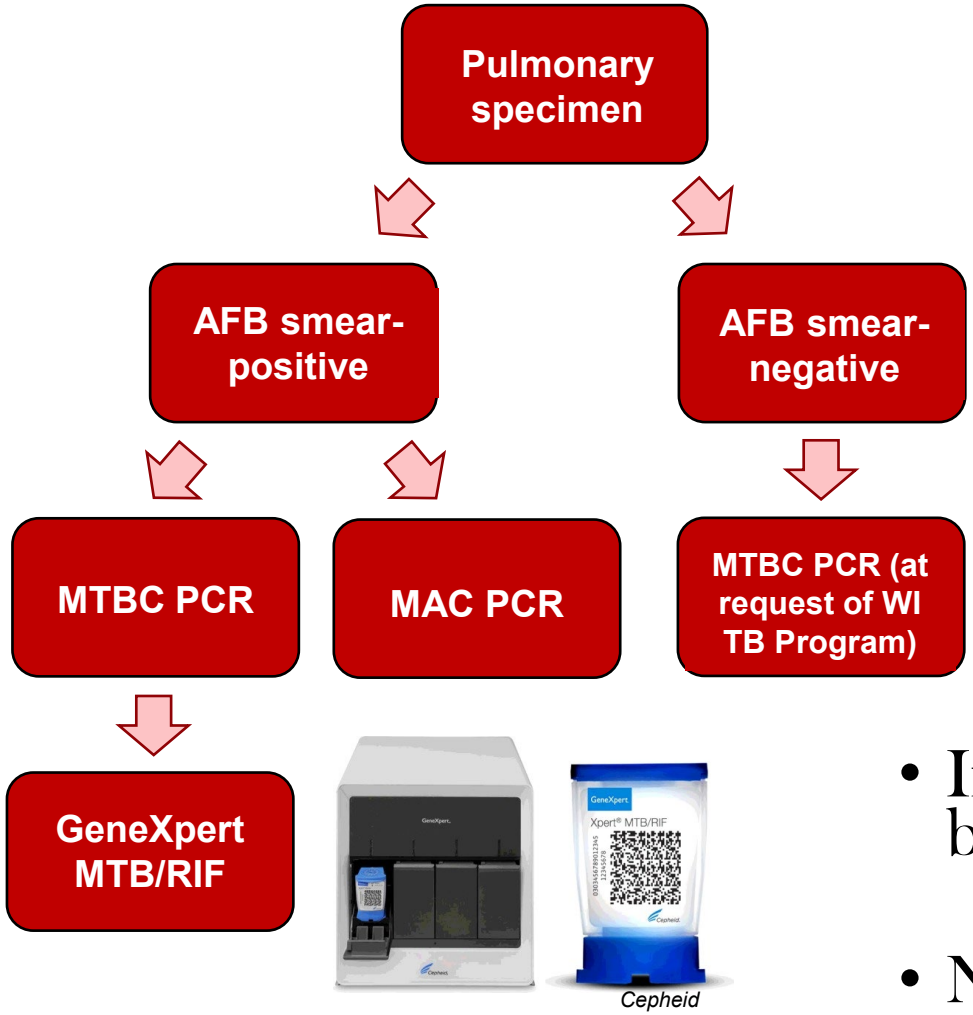
- CDC recommendation: test first sputum of all patients suspected to have TB for whom the test result would alter case management or TB control activities
  - Should not be routinely ordered when clinical suspicion of TB is low

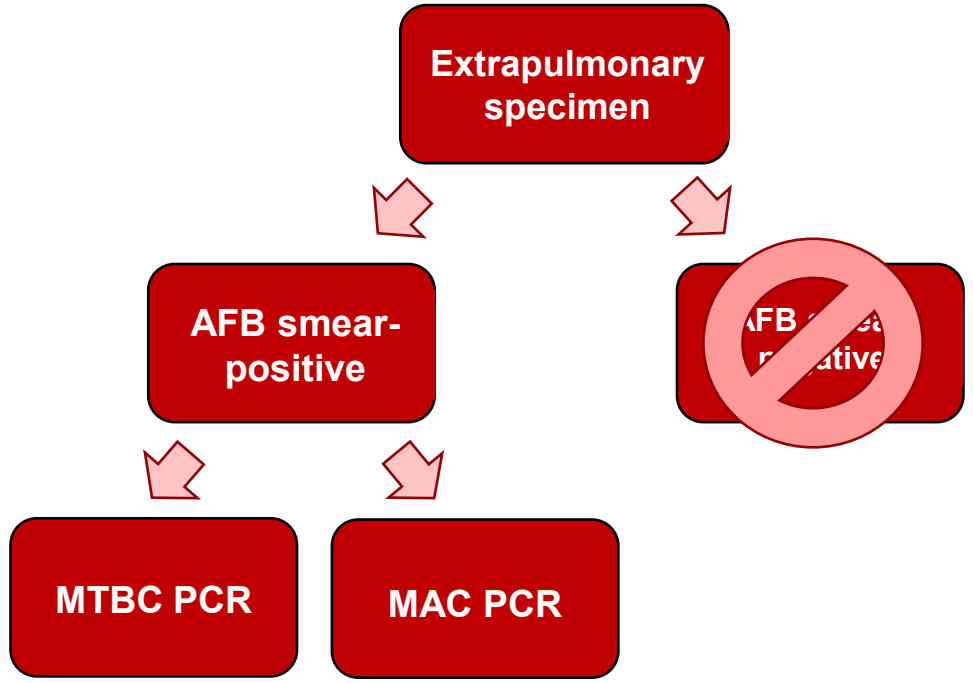
WSLH TB PCR: Fee-exempt testing criteria

- Patient must have signs and symptoms of pulmonary TB
- Patient must be reported to the local or state public health department as a suspect TB case as required by Wisconsin Statute Chapter 252.05 and Wisconsin Administrative Code Chapter HFS 145.04 (3)(a)
- Patient must be in respiratory isolation (for pulmonary disease)

- Patient must not have been diagnosed with TB or a non-tuberculous mycobacterial infection within the last 12 months
- Patient must have received ≤7 days of anti-mycobacterial therapy or no such treatment within the last 12 months

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





- If patient is TB suspect, smear-negative PCR can be run with WI TB Program approval
  - Contact WI TBP → Will send approval to WSLH
- No PCR run on smear-negative extrapulmonary specimens

Rapid ID of Mycobacteria from Primary Specimens: GeneXpert MTB/RIF

- Amplifies DNA from decontaminated sputum sediment
  - Target: *rpoB* gene (RIF-resistance)
- LOD: ≈130 AFB/ml
- FDA-approved for sputum only
  - All other specimen types require lab-specific validation

Cepheid

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

Interpreting GeneXpert MTB/RIF results

- 3 main results based on the absence/presence of *rpoB* mutations
  - MTB Not Detected → No MTB, or level of MTB is below limit of detection (ie: AFB smear-negative specimens)
  - MTB Detected, No RIF-resistance Detected
    - No mutations detected, patient MTB likely susceptible to RIF
    - Some RIF-resistance mutations outside target area, so still need phenotypic susceptibilities!
  - MTB Detected, RIF-resistance Detected
    - *rpoB* mutation detected, isolate potentially RIF-resistant
      - Could be any mutation, not specifically one conferring RIF-resistance
    - Needs to be confirmed by either DNA sequencing or phenotypic testing (or both)!

Use of NAAT results in WI

NAAT testing is diagnostic only! Not to be used in place of WI TB Program guidelines for removing patients from isolation!

TB PCR

- Positive TB PCR results are confirmatory for tuberculosis
- Two negative TB PCRs from two smear-positive specimens likely rule out TB
- **Negative TB PCR results do not rule out TB in smear-negative specimens**

GeneXpert

- If no *rpoB* mutations are detected by GeneXpert, patient likely treatable with RIF
- **GeneXpert detection of RIF-resistance should be treated as true resistance until sequencing or phenotypic testing results available**

Release from isolation

- At this time, WI is not using GeneXpert or other NAAT results for release from isolation
- A negative NAAT should not be used to make a "patient does not have infectious TB" decision

Advantages of NAAT

- More rapid diagnosis in AFB smear-positive patients; diagnosis in smear-negative patients
  - Initiation of earlier treatment
    - TB vs MAC
  - Cost savings for patient isolation
    - Release MAC+ from isolation
  - Faster reporting to TB programs
    - Contact tracing
    - LTHD

Goal: Fewer transmissions

Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

WSLH Mycobacteriology Testing Updates

WSLH Testing Updates: MTBC First-line Drug Susceptibility Testing

Historically, WSLH has performed phenotypic testing for INH, RIF, EMB, PZA by MGIT method

- FDA-approved test, so stringent guidelines for testing must be followed
- Requires actively growing pure MTBC culture
  - Mixed or contaminated cultures
  - Poorly growing isolates
- Only one manufacturer, so reagent issues can severely impact testing

Modified from https://www.bd.com/en-menat/products-and-solutions/products/product-families/bd-bactec-mgit-susceptibility-testing-reagents

WSLH Testing Updates: Pyrazinamide DST

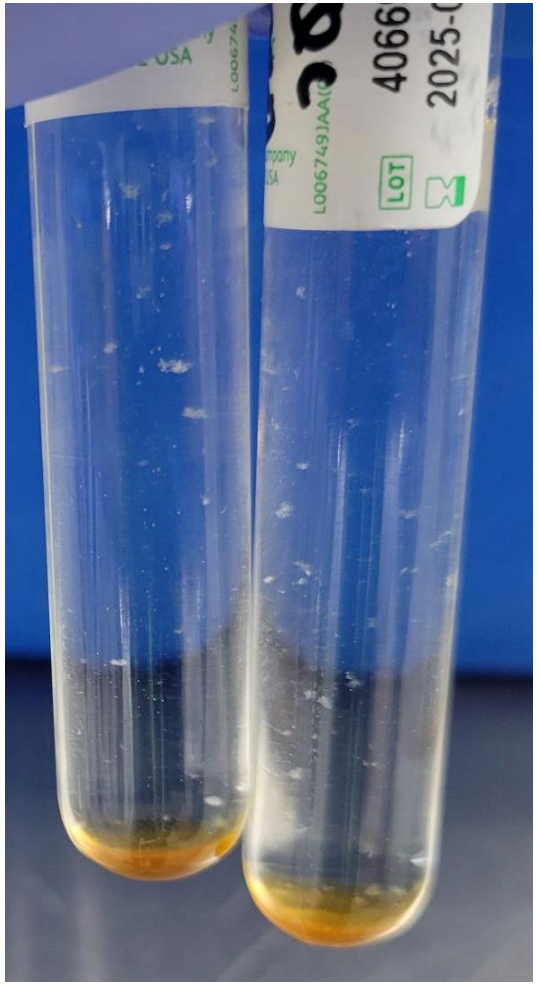
Mid-2023: WSLH seeing repeated PZA QC failures (false-resistance)

2024

- July: Phenotypic PZA testing discontinued nationwide due to manufacturer recall
  - PZA-resistance testing moved to *pncA* sequencing
- September: manufacturer began issuing new lots of PZA drug and supplement

2025

- February: WSLH began performing phenotypic PZA DST again
- May: WSLH once again discontinues phenotypic PZA testing due to second manufacturer recall



WSLH Testing Updates: Pyrazinamide DST

- November 2025: BD announces that new PZA kits are available
  - Modification of protocol and alteration of lot outdates from 18 to 13 months

None of the protocol modifications would change previous WSLH protocol

- WSLH will not be evaluating new PZA kits at this time

For the foreseeable future, specimens will be sent to CDC for *pncA* sequencing

- Estimated 90-97% of PZA-resistant isolates have mutation in *pncA* gene or promoter
- Negative predictive value of *pncA* sequencing for non-MDR TB >99%

Centers for Disease Control and Prevention

National Tuberculosis Reference Laboratory

Pyrazinamide (PZA)	Result	Interpretation
PZA interpretation		Cannot rule out PZA resistance.
pncA	No mutation	

Pyrazinamide (PZA)	Result	Interpretation
PZA interpretation		PZA resistant. The His57Asp mutation is common to M. bovis/BCG.
pncA	His57Asp	

Report Comments and Disclaimers

Results from molecular drug resistance testing determined by targeted next generation sequencing assay.

Results for molecular drug resistance assays were developed, and the performance characteristics determined by the DTBE Reference Laboratory. They have not been cleared or approved by the Food and Drug Administration.

A negative result (e.g., no mutations) does not rule out contributory mutations present elsewhere in the genome.

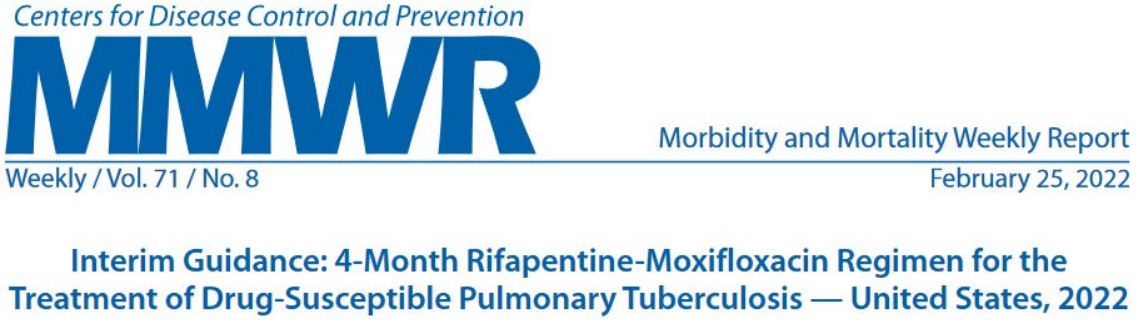
Notable CDC TB Testing Updates:

- Phenotypic PZA susceptibility testing permanently discontinued
- MTBC speciation testing is still unavailable
- CDC Infectious Diseases Pathology Branch is performing ***Evaluation of Fixed Tissues for Possible Infectious Etiologies*** and is now again reflexing TB-positive specimens to MDDR testing
    - MTBC PCR from fixed specimens (FFPE blocks)
    - Useful in cases where patients are:
        - culture-negative
        - have tissue blocks available from biopsy
        - MTBC confirmation is important for patient care
- Finishing evaluation of broth microdilution for susceptibilities to MOX, LEV, LZD, CLF, BDQ, PRT

WSLH Testing Updates: Moxifloxacin DST

Phenotypic moxifloxacin susceptibility testing for MTBC

- WSLH has completed validation of MGIT-based phenotypic moxifloxacin susceptibility testing
  - Testing at critical concentration of 0.25µg/ml



MOX susceptibilities by MGIT is now part of standard 1st-line DST at WSLH

- Do not need to order separately, will automatically be performed alongside INH, RIF, EMB susceptibilities

Susceptibility

	Mycobacterium tuberculosis complex [C1]
	MYCOBACTERIAL SUSCEPTIBILTY
Isoniazid (0.2 mcg/mL)	Susceptible
Isoniazid (1.0 mcg/mL)	Susceptible
Rifampin (1 mcg/mL)	Susceptible
Ethambutol (5 mcg/mL)	Susceptible
Moxifloxacin (0.25 mcg/mL)	Susceptible
Pyrazinamide (100 mcg/mL)	Not performed

WSLH Testing Updates: TB Whole Genome Sequencing

- WSLH has sequenced close to 400 unique MTBC isolates over the past 6 years
- Validation focused on MTBC species ID, 1st-line drug susceptibility prediction (INH, RIF, EMB, PZA)
  - ID accuracy: 100%
  - DST accuracy: 100% (RIF), 88.2% (INH), 100% (EMB), 84.2% (PZA)
- WSLH Sequencing has switched extraction and sequencing instruments to improve speed and cost
  - Re-validating test using the MiSeq i100 and Omni Bead Ruptor
- WSLH Bioinformatics has built a new analysis pipeline and is ready to move into production

WSLH Testing Updates: TB Whole Genome Sequencing

M. tuberculosis complex WGS (Final result)

ID:	25MM000113	Type/Src:	Sputum
		Result	**Units**
MTBC Identification		Mycobacterium tuberculosis complex	
Isoniazid DST Prediction		No high-confidence mutations associated with resistance to INH have been detected.	
Rifampin DST Prediction		No high-confidence mutations associated with resistance to RIF have been detected.	
Ethambutol DST Prediction		No high-confidence mutations associated with resistance to EMB have been detected.	
Pyrazinamide DST Prediction		No high-confidence mutations associated with resistance to PZA have been detected.	

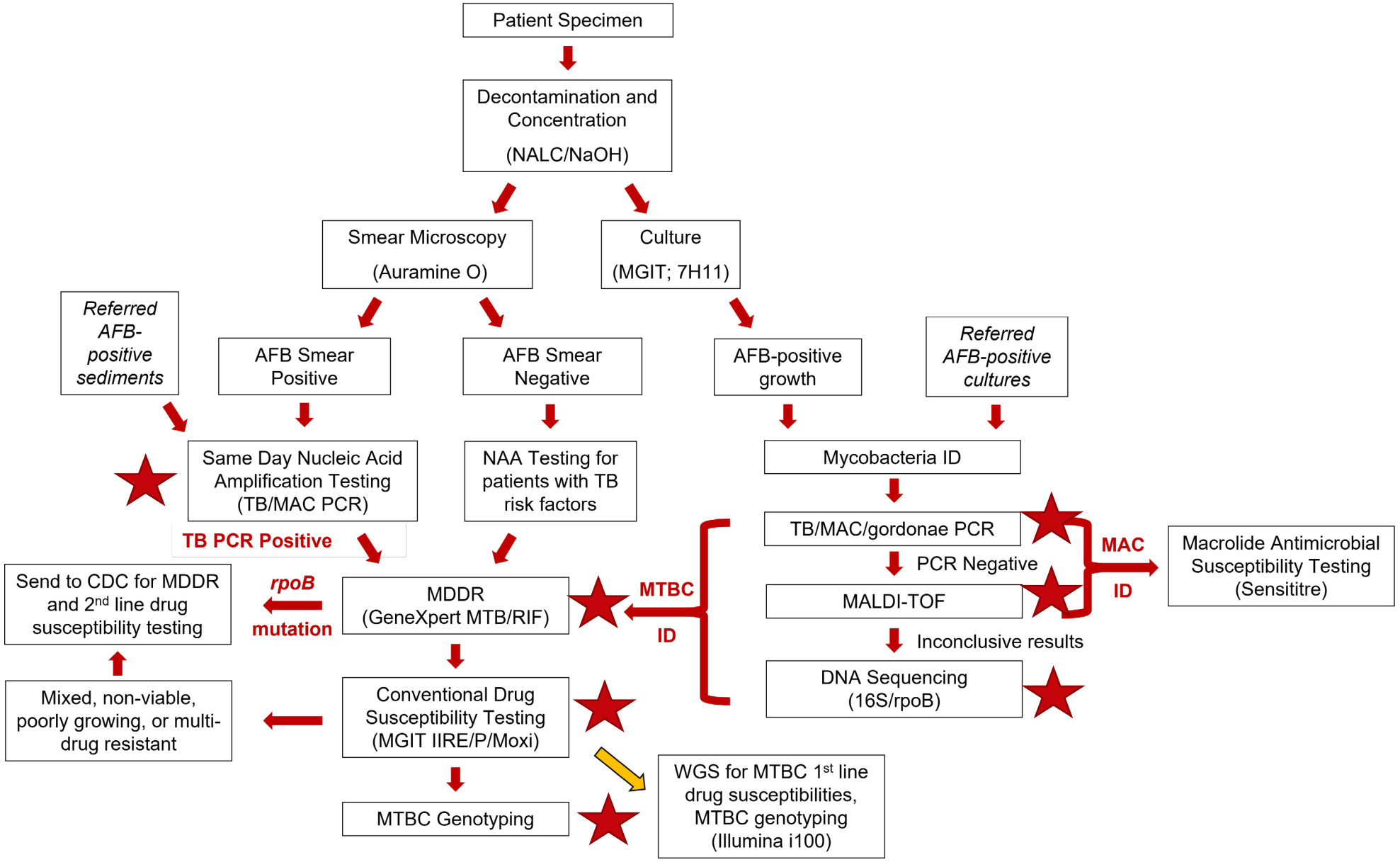
Comments:
This next-generation sequencing (NGS)-based test is designed for the identification and prediction of drug susceptibility in Mycobacterium tuberculosis complex (MTBC) isolates. This test uses the APHL Datapult TB_V5 pipeline for analysis of sequence data.

The sequence-based approach can only predict drug susceptibility by detecting well-characterized mutations. It may not identify novel mutations or other genetic factors that could contribute to drug resistance outside of these known regions.

This test was developed and its performance characteristics determined by the Wisconsin State Laboratory of Hygiene, a Clinical Laboratory Improvement Amendments (CLIA) certified, high complexity clinical laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration.

Phenotypic susceptibility testing remains the gold standard for detecting antimicrobial resistance. Phenotypic susceptibility results to follow.

Mycobacteriology Testing at WSLH



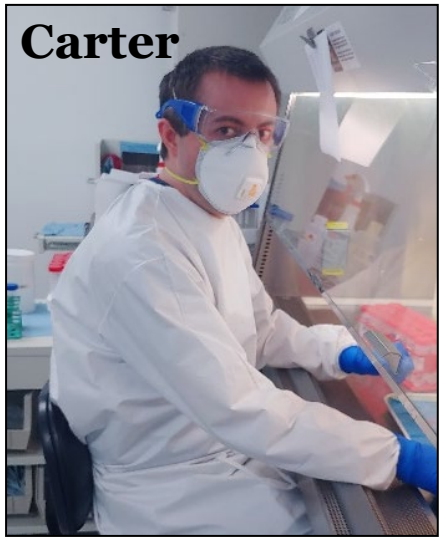
WSLH Testing Updates: Other New Instrument Validations

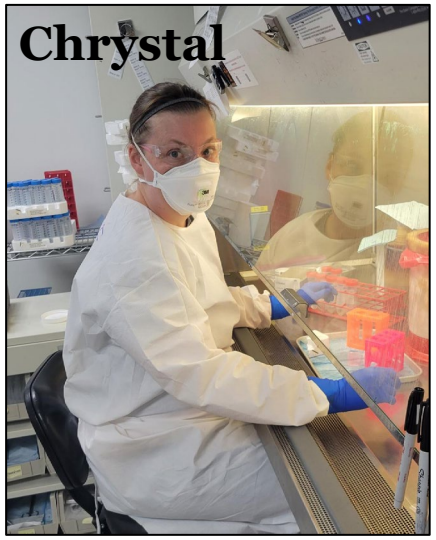
- Mycobacteria Identification
  - Validated 39 different MTBC/NTM species; broth, slant, and plate solid media on new Bruker MALDI-TOF machine
  - Validated Eppendorf Mastercycler for 16S rRNA and *rpoB* Sanger DNA sequencing for identification of NTM and other bacterial species
- Mycobacteria Culture and TB DST
  - Validated new MGIT320 machine to increase lab capacity
- TB/MAC PCR
  - In progress: Validation of Quantstudio 5 instrument for PCR from primary specimen and positive cultures
  - In progress: Evaluating Promega Maxwell for DNA extraction from primary specimen
- GeneXpert MTB/RIF
  - In Progress: Validation of GeneXpert from MTBC-positive tissues and body fluids

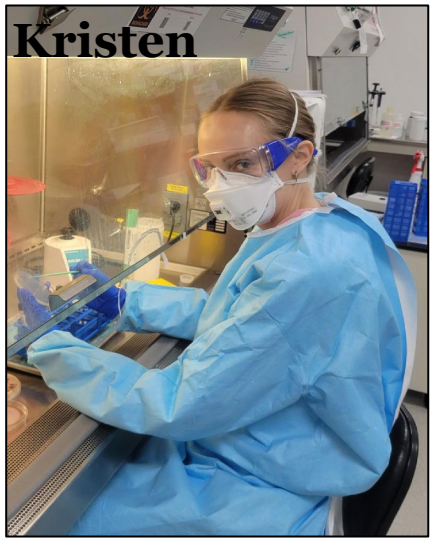
WSLH Testing Updates

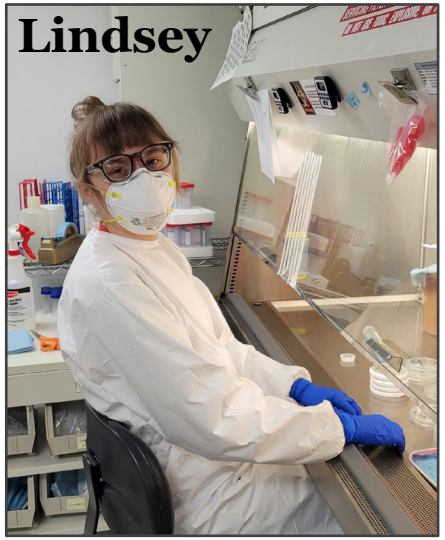
- Phenotypic PZA testing at WSLH has been discontinued indefinitely
  - Isolates will be sent to CDC for *pncA* sequencing until WSLH TB WGS is live
- Phenotypic moxifloxacin susceptibility testing validation is complete
  - Test is now part of TB 1st-line DST, does not need to be ordered separately
- MTBC whole-genome sequencing for MTBC speciation and 1st-line susceptibility prediction validation is now (finally) completed
  - New extraction and sequencing platform validation is complete
  - WSLH Bioinformatics new analysis pipeline is complete
  - Training staff and wrapping up final workflow and clerical details before going live!
- Multiple validations in progress to improve testing speed and reliability, add additional specimen type to current tests

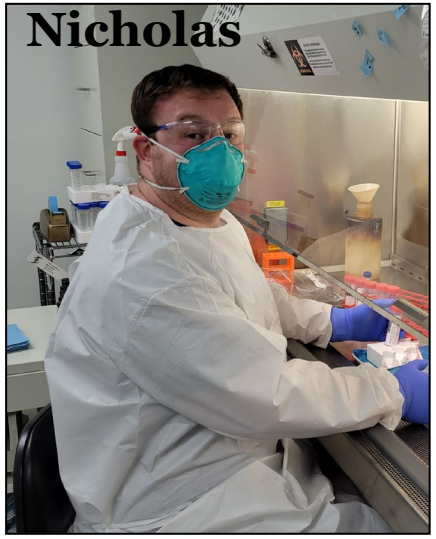
WSLH TB Laboratory Team











Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

WISCONSIN DEPARTMENT of HEALTH SERVICES

WSLH Communicable Disease Division

Allen Bateman
Alana Sterkel
Laura Louison
Michael Mamerow

WSLH Mycobacteriology Laboratory

Carter Seehafer
Chrystal Egstad
Kristen Wendtland
Lindsey Moderski
Nicholas Mack

WSLH Sequencing

Alicia Mooney
Ava Gehrke-Laundrie
Claire LaFleur

WSLH Bioinformatics

Kelsey Florek
Abigail Shockey
Madeline Topf (APHL)

WI DHS TB Program

Claire Leback
Andrea Liptack
Mary Rashka

WI Mycobacteriology Laboratory Network

WI Clinical Laboratory Network

Centers for Disease Control and Prevention

Department of TB Elimination
DTBE TB Laboratory

Association of Public Health Laboratories