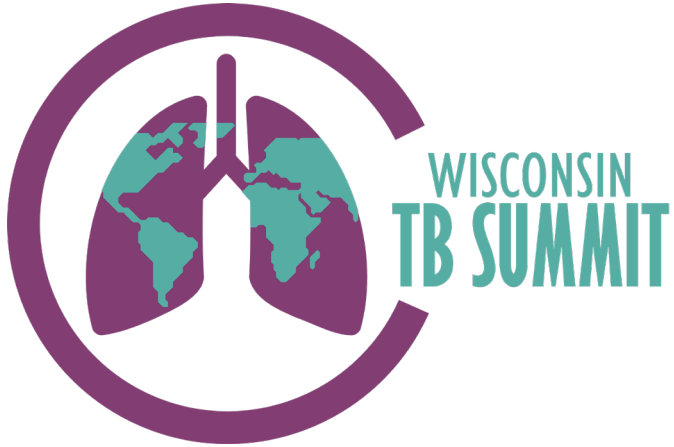


DIAGNOSTIC TESTING FOR MYCOBACTERIUM TUBERCULOSIS COMPLEX

UPDATES FROM WSLH AND THE FIELD

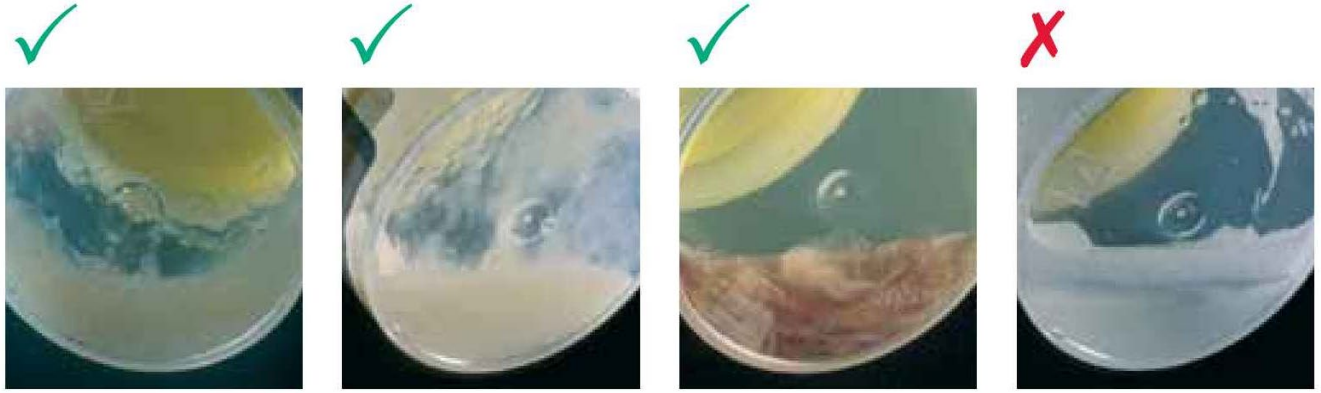
Nate Simon
WI TB Laboratory Program Coordinator

WI State Laboratory of Hygiene
04/01/2025

LEARNING OBJECTIVES

- Understand recommendations and rejection criteria for collecting, storing, and transporting sputum specimens for AFB smear and culture
- Understand turn-around times for various AFB testing and potential reasons for certain tests having delayed results
- WSLH testing updates

WSLH RECOMMENDATIONS FOR SPUTUM COLLECTION, STORAGE, AND TRANSPORT



LABORATORY DIAGNOSIS OF TUBERCULOSIS BY SPUTUM MICROSCOPY https://www.stoptb.org/sites/default/files/imported/document/TB_MICROSCOPY_HANDBOOK_FINAL.pdf

Sputum Quality and Rejection Criteria

- High-quality specimens are thick/mucopurulent
- Poor quality specimens are thin and watery
- **Saliva and nasal secretions are unacceptable and will be rejected**
- 3-5 ml in volume ideal
  - **<1 ml sputum will be rejected**
- **Specimens >7 days from collection will be rejected**

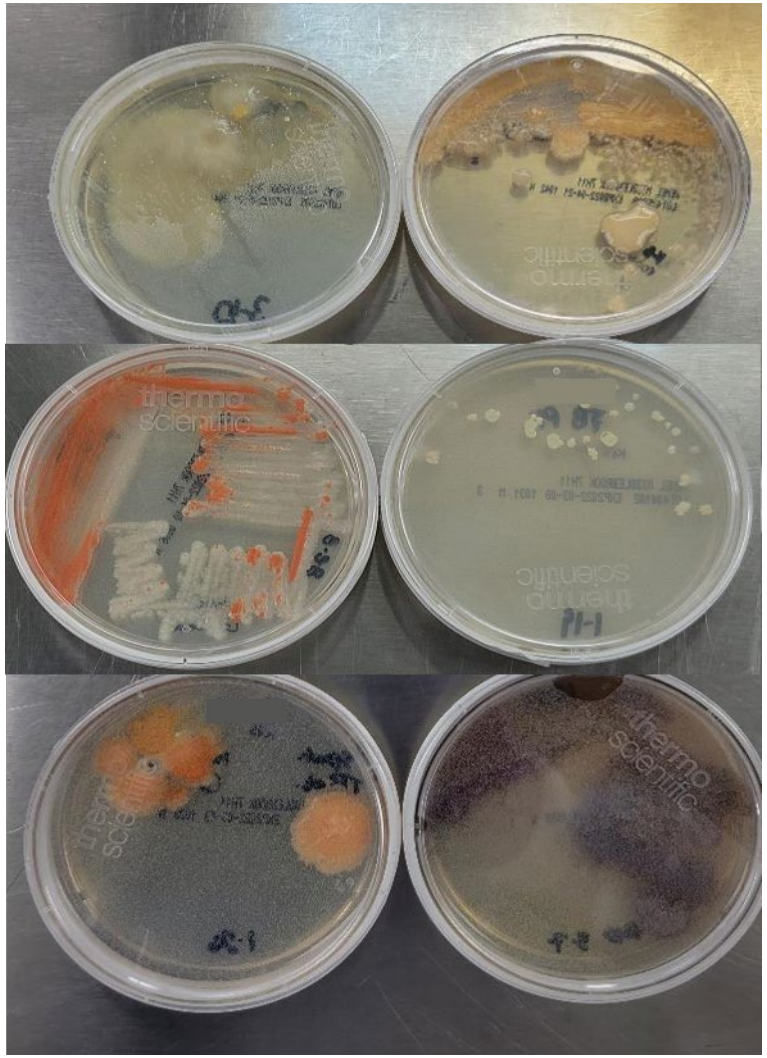
WHAT ARE THE RECOMMENDED STORAGE AND SHIPPING CONDITIONS FOR SPUTUM COLLECTED FOR AFB SMEAR/CULTURE TESTING?

A	Freeze specimens after collection; deliver specimens as they are collected
B	Refrigerate specimens after collection; collect full set of specimens before delivering
C	Refrigerate specimens; deliver specimens as they are collected
D	No specific storage conditions needed; collect full set of specimens before delivering

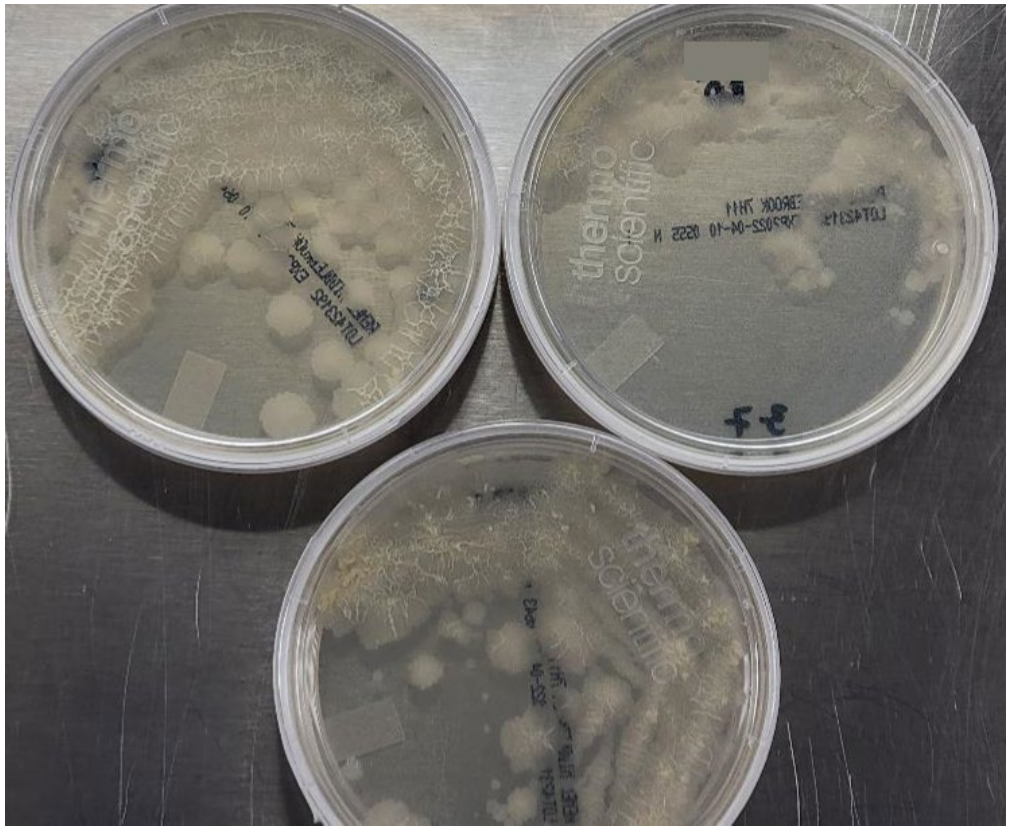
WSLH RECOMMENDATIONS FOR SPUTUM COLLECTION, STORAGE, AND TRANSPORT

- **Sputum samples should be refrigerated**
  - Minimize overgrowth of normal flora
  - Maximize viability of AFB
- **Deliver specimens to the laboratory as soon as possible** – try not to batch 3x sputa!
  - CDC goal: delivery within 24 hours of collection
  - Rapid turn-around times
    - <24 hour for AFB smear, <48hr for TB PCR
    - 6-8 weeks for AFB culture negative result

MYCOBACTERIAL CULTURE: RE-DECONTAMINATION



Re-decontamination

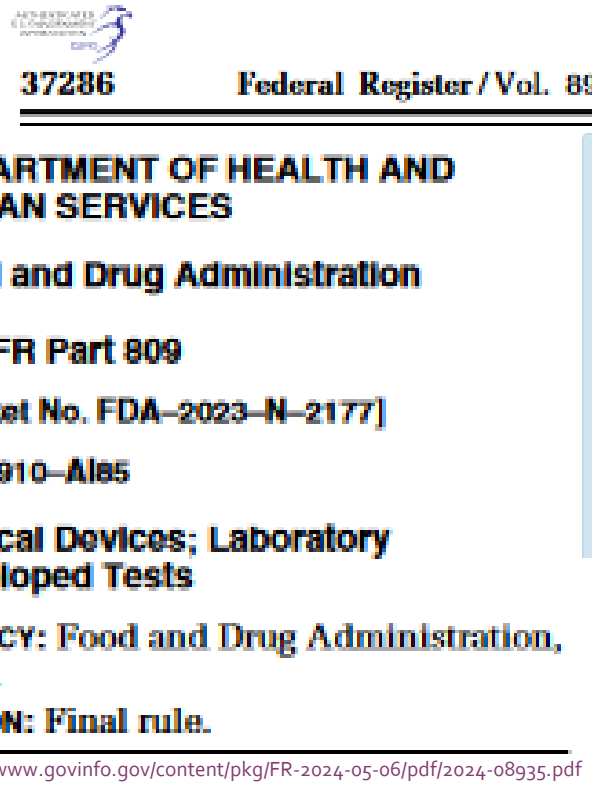


**Restarts the 6 week culture incubation

WISCONSIN TB SUMMIT

WSLH MYCOBACTERIOLOGY TESTING UPDATES

TB LABORATORY DIAGNOSTICS AND LABORATORY DEVELOPED TESTING (LDT)



37286 Federal Register / Vol. 89, No. 88 / Monday, May 6, 2024 / Rules and Regulations

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 809

[Docket No. FDA–2023–N–2177]

RIN 0910–AI85

Medical Devices; Laboratory Developed Tests

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

https://www.govinfo.gov/content/pkg/FR-2024-05-06/pdf/2024-08935.pdf

April 29, 2024: The FDA announced a final rule aimed at helping to ensure the safety and effectiveness of laboratory developed tests (LDTs). The rule amends the FDA's regulations to make explicit that in vitro diagnostic products (IVDs) are devices under the Federal Food, Drug, and Cosmetic Act (FD&C Act) including when the manufacturer of the IVD is a laboratory. Along with this amendment, the FDA is finalizing a policy under which the FDA will provide greater oversight of IVDs offered as LDTs through a phaseout of its general enforcement discretion approach for LDTs over the course of four years, as well as targeted enforcement discretion policies for certain categories of IVDs manufactured by laboratories. https://www.fda.gov/medical-devices/in-vitro-diagnostics/laboratory-developed-tests

LDT: clinical tests "designed, manufactured, and used within a single clinical laboratory certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA)"

FDA RULE TO REGULATE LDT – PUBLISHED 05/06/2024

- Basic summary: All laboratory-performed clinical tests that have not yet received FDA approval for use in human diagnostic testing are now subject to FDA regulatory oversight
  - New "device" requirements include: adverse event reporting, LDT registration/listing, labeling requirements (use, limitations, performance),quality system requirements, **premarket review**

- **FDA-approved TB diagnostic tests**:
  - Cepheid GeneXpert MTB/RIF (sputum only)
  - BD BACTEC MGIT SIRE/PZA kit and VersaTREK Myco Susceptibility Kit
  - IGRA
- **TB LDT**:
  - AFB smear/culture
  - TB/MAC PCR
  - MALDI-TOF identification
  - DNA sequencing
  - GeneXpert MTB/RIF on any material other than sputum
  - TB whole-genome sequencing

SO WHAT DOES THIS MEAN FOR TB TESTING AT WSLH?

"If an IVD was offered as an LDT prior to 5/6/2024 and is not modified in such a way that changes the indications for use, alters the operating principle, includes significantly different technology, or adversely changes the performance or safety specifications of the IVD, FDA intends to exercise enforcement discretion and generally not enforce premarket review and QS requirements"

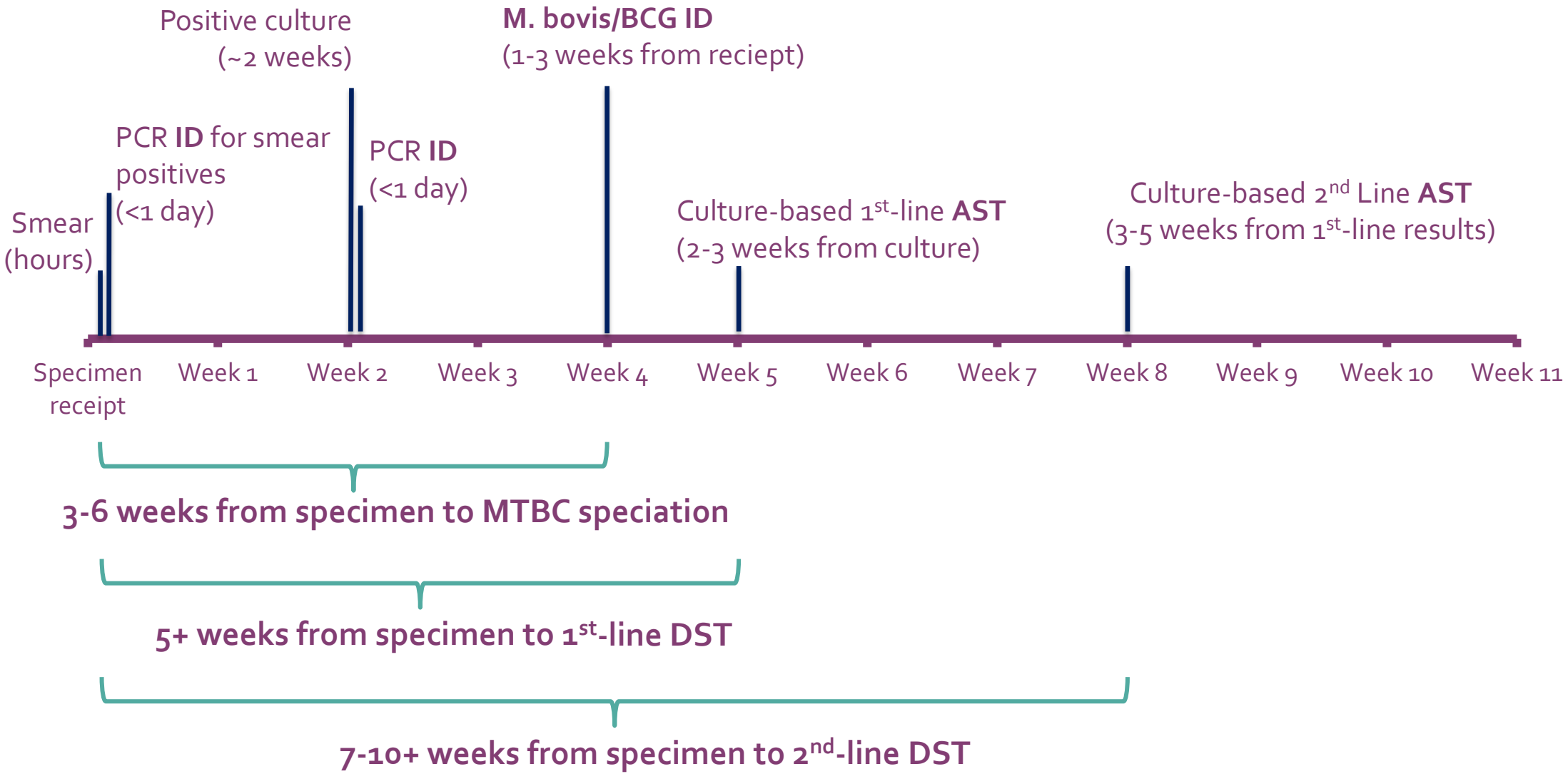
**Generally interpreting the new rule to say that any test validated/in-use prior to 5/6/2024 will fall under this exemption and will continue to receive enforcement discretion

**However, any new tests or any changes to currently performed tests will require review by WSLH Clinical Director to determine if the change may require application for FDA 510K approval.

WHAT IS YOUR COMFORT LEVEL WITH INTERPRETING AND UNDERSTANDING RESULTS FROM MTBC DIAGNOSTIC TESTING?

A	Novice: I'm new to TB. I can read the results, but don't really know what they mean
B	Intermediate: I understand the majority of the results, but I require assistance interpreting certain tests (ie: molecular testing, susceptibility testing, molecular susceptibility signatures)
C	Proficient: Test results are clear to me; I understand how to interpret molecular signatures and DST results
D	Expert: I can run the tests myself

TESTING FOR TB DRUG RESISTANCE IS SLOOOOW



MTBC PHENOTYPIC DRUG RESISTANCE TESTING

- Principle: Incubate a standardized concentration of MTBC isolate with a "critical concentration" of drug and observe for growth or inhibition of growth

- 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)
  - 3 failures over 5 weeks, eventually 15 over 26 weeks
- Months of troubleshooting: either the supplied drug concentration is incorrect, or the drug is less active than it should be
- January 2024: extended backorders of product
- Company said product was passing all internal QC… until July 18, 2024

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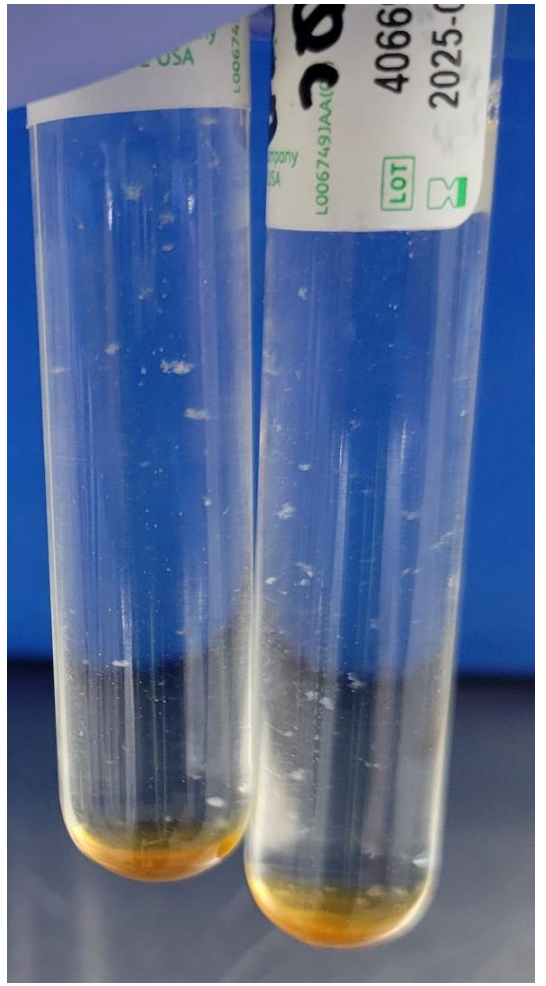
URGENT: Medical Device Correction - UPDATE
Type of Action: Product Removal – discarded by customer
BD BACTEC™ MGIT™ 960 PZA Kit

- July 2024: Phenotypic PZA testing discontinued nationwide
  - PZA-resistance testing moved to *pncA* sequencing

WSLH TESTING UPDATES: PYRAZINAMIDE DST

- September 2024: BD began issuing new lots of PZA drug and supplement
- November 2024: CDC announces permanently discontinuing all phenotypic PZA testing
- January 2025: WSLH has completed evaluation of new kits
  - QC passed for 100% of sets
    - Other labs around the country are still seeing intermittent false resistance
  - *pncA* sequencing turn-around time averaging 6-8 weeks



WSLH TESTING UPDATES: PYRAZINAMIDE DST

- February 2025: WSLH began performing phenotypic PZA DST again
- PZA-sensitive isolates will be reported as "Susceptible"
  - No issues with false-susceptibility have been noted
- **Patient isolates scored as "Resistant" to PZA by MGIT method will now be reported as PZA "Indeterminate"**. These isolates will be sent to CDC for *pncA* sequencing to confirm the presence of resistance mutations.

"Due to intermittent quality issues with phenotypic PZA testing reagents, MTBC isolates displaying indeterminate PZA resistance will be sent to the Centers for Disease Control and Prevention (CDC) for pncA sequencing to determine if mutations known to confer PZA resistance are present.

The MGIT test used here has been approved by the U.S. Food and Drug Administration and is a World Health Organization recommended method. However, MGIT and other growth-based susceptibility tests also have known issues with false resistance and reproducibility of pyrazinamide. PZA mono-resistance is rare outside of M. bovis or M. bovis-BCG. Consider patient history while interpreting resistant results."

Centers for Disease Control and Prevention

National Tuberculosis Reference Laboratory

Pyrazinamide (PZA) – Sanger	**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 Sanger sequencing assay.

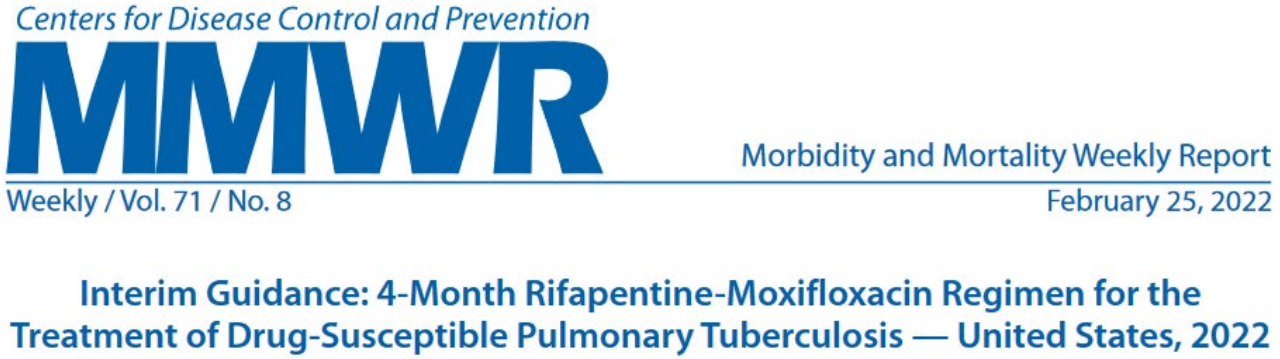
Results for molecular drug resistance assays were developed, and the performance characteristics determined by the DTBE Reference Laboratory. They have not been cleared or approved by the Food and Drug Administration.

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

OTHER NOTABLE CDC TESTING UPDATES

- Phenotypic PZA susceptibility testing permanently discontinued
- MTBC speciation testing is still unavailable
- MTBC genotyping service (TB-GIMS) is back online as of 2/28/25 – major software update
- CDC Infectious Diseases Pathology Branch still performing **Evaluation of Fixed Tissues for Possible Infectious Etiologies**
    - MTBC PCR from fixed specimens (FFPE blocks)
    - Useful in cases where patients are culture-negative, but may have tissue blocks available from biopsy and MTBC confirmation is important to care
    - **Recommend working with state or local Public Health Lab to submit these specimens, as the requirements and paperwork are very specific**

WSLH TESTING UPDATES: MOXIFLOXACIN DST



Phenotypic moxifloxacin susceptibility testing for MTBC

- Currently send all phenotypic MXF susceptibility requests to California Microbial Diseases Laboratory
- WSLH is finalizing validation of MGIT-based phenotypic moxifloxacin susceptibility testing
  - If possible, will become part of standard 1st-line DST at WSLH (LDT)

BPAL/BPAL(M) TESTING OPTIONS

- **Molecular Detection of Drug Resistance**
  - Targeted next-generation sequencing (tNGS)
  - 15 gene targets covering 11+ drugs
    - RIF, INH, EMB, PZA, FQ, AMK, CAP, KAN, **BDQ, CLF, LZD**
  - Molecular data confirmed with phenotypic testing for majority of drugs

Pre-approval required for all testing

Centers for Disease Control and Prevention
National Tuberculosis Reference Laboratory

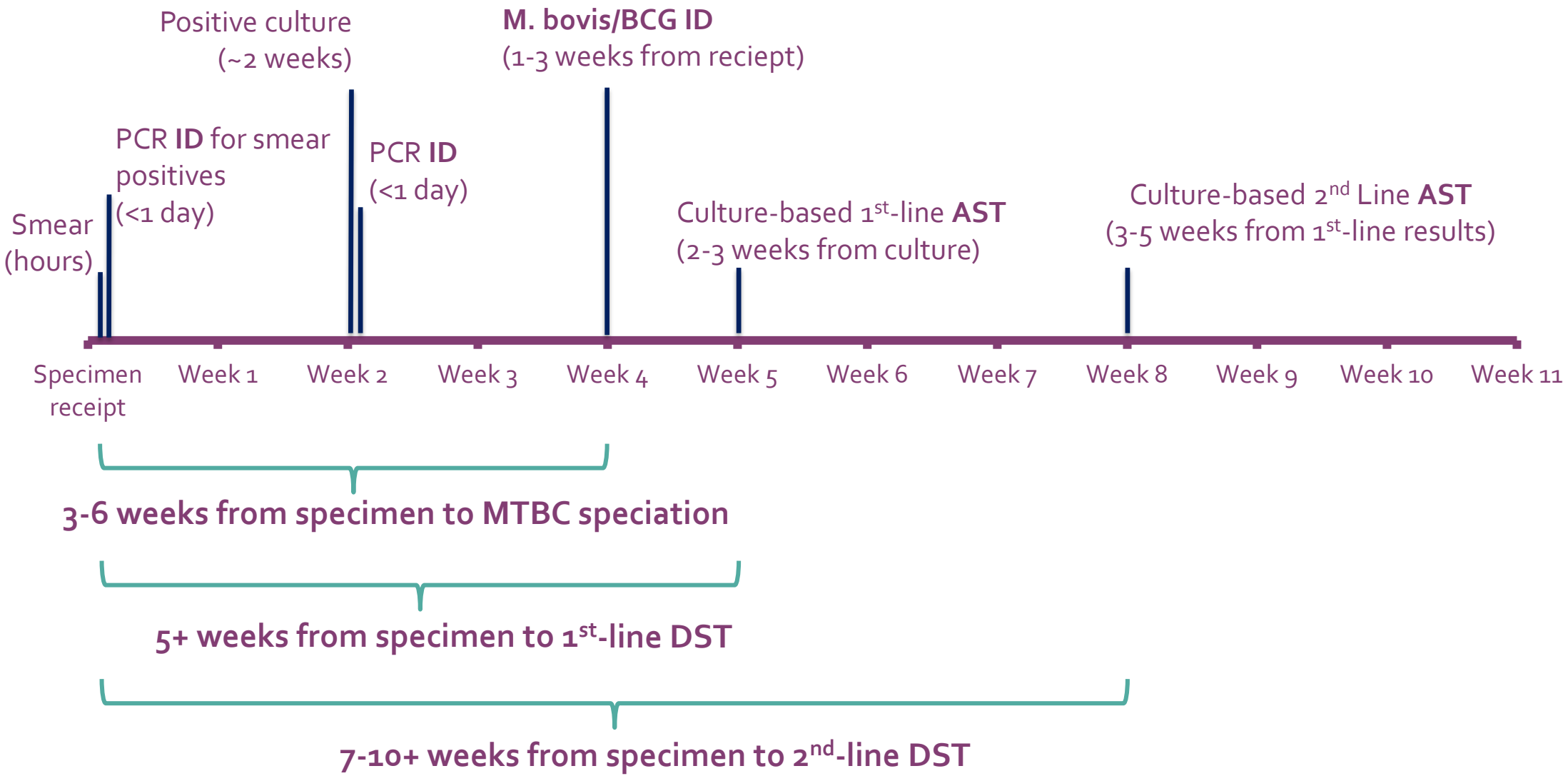
MTBC Agar Proportion Susceptibility*	% Resistant	Interpretation
Isoniazid 0.2 µg/mL	100 %	Resistant
Isoniazid 1.0 µg/mL	100 %	Resistant
Isoniazid 5.0 µg/mL	67 %	Resistant
Rifampin 1.0 µg/mL	0 %	Susceptible
Ethambutol 5.0 µg/mL	33 %	Resistant
Streptomycin 2.0 µg/mL	100 %	Resistant
Streptomycin 10.0 µg/mL	33 %	Resistant
Rifabutin 2.0 µg/mL	0 %	Susceptible
Ciprofloxacin 2.0 µg/mL	0 %	Susceptible
Kanamycin 5.0 µg/mL	0 %	Susceptible
Ethionamide 10.0 µg/mL	0 %	Susceptible
Capreomycin 10.0 µg/mL	0 %	Susceptible
PAS 2.0 µg/mL	0 %	Susceptible
Ofloxacin 2.0 µg/mL	0 %	Susceptible
Amikacin 4.0 µg/mL	0 %	Susceptible

BPAL/BPAL(M) TESTING OPTIONS

Laboratory	Bedaquiline
NY State Department of Health – Wadsworth Center Mycobacteriology Laboratory	**Molecular testing for BDQ and LZD** **Phenotypic testing for BDQ, Pa, LZD**
CDC Department of TB Elimination	**Molecular testing for BDQ and LZD**
Johns Hopkins Mycobacteriology	Molecular testing for BDQ and LZD Phenotypic testing for BDQ, Pa, LZD
Florida Department of Health State Lab	Molecular testing for BDQ and LZD Phenotypic testing for BDQ, LZD

**Not all testing is CLIA-validated or has interpretable breakpoints

TESTING FOR TB DRUG RESISTANCE IS SLOOOOOOW



WSLH VALIDATION OF WHOLE GENOME SEQUENCING: MTBC IDENTIFICATION AND SUSCEPTIBILITY PREDICTION

- WSLH has sequenced close to 400 MTBC isolates over the past 5 years
- Validation focused on MTBC species ID, 1st-line drug susceptibility prediction (INH, RIF, EMB, PZA)
- Validation set included:
  - *M. tuberculosis*, *M. bovis*, *M. bovis*-BCG, and various common non-tuberculous mycobacteria
  - Isolates mono-resistant to INH, RIF, PZA, MDR-TB, pan-resistant strains, pan-susceptible strains

MTBC WGS VALIDATION SUMMARY

MTBC Identification: Accuracy

M. tb Identification	*M. bovis* Identification	*M. bovis-BCG* Identification	Non-MTBC identification
100%	100%	100%	100%

Did WGS correctly predict species?

Phenotypic DST: Accuracy

	Rifampin	Isoniazid	Ethambutol	Pyrazinamide
Positive Prediction	100%	88.2%†	100%	84.2%‡
Negative Prediction	95.6%	100%	100%	97.22%

Did WGS correctly predict resistance?

Did WGS correctly predict susceptibility?

Genotypic DST: Accuracy

	Rifampin	Isoniazid	Ethambutol	Pyrazinamide
Positive Prediction	100%	100%	100%	100%

Did WGS correctly identify known mutation?

†: 2 isolates expected to be INH resistant had *katG*(D94N) mutation of unknown significance
‡: 3 isolates expected to be PZA resistant with *ClpC1*(V63A) disputed mutation

WSLH TESTING UPDATES

- Uncertainty around FDA LDT rule is impacting ability of all clinical labs to bring new tests online
- Phenotypic PZA testing at WSLH has resumed
  - Isolates displaying phenotypic resistance will be sent to CDC for *pncA* sequencing to confirm
- Phenotypic moxifloxacin susceptibility testing validation is under final review
  - Hope to offer as part of standard TB 1st-line DST
- MTBC whole-genome sequencing for MTBC speciation and 1st-line susceptibility prediction validation is complete
  - Training staff and wrapping up final workflow and clerical details before going live!

QUESTIONS & ANSWERS

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