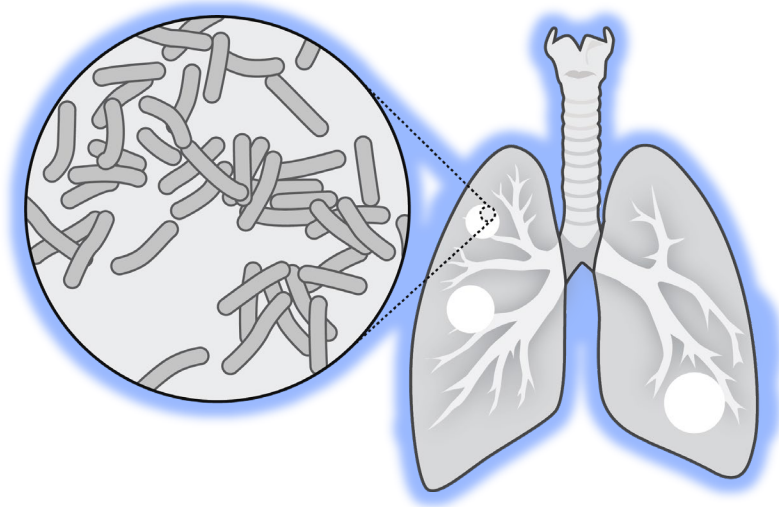




The Evolving Landscape of Laboratory Testing for Tuberculosis



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Division of Tuberculosis Elimination

National Center for HIV, Viral Hepatitis,

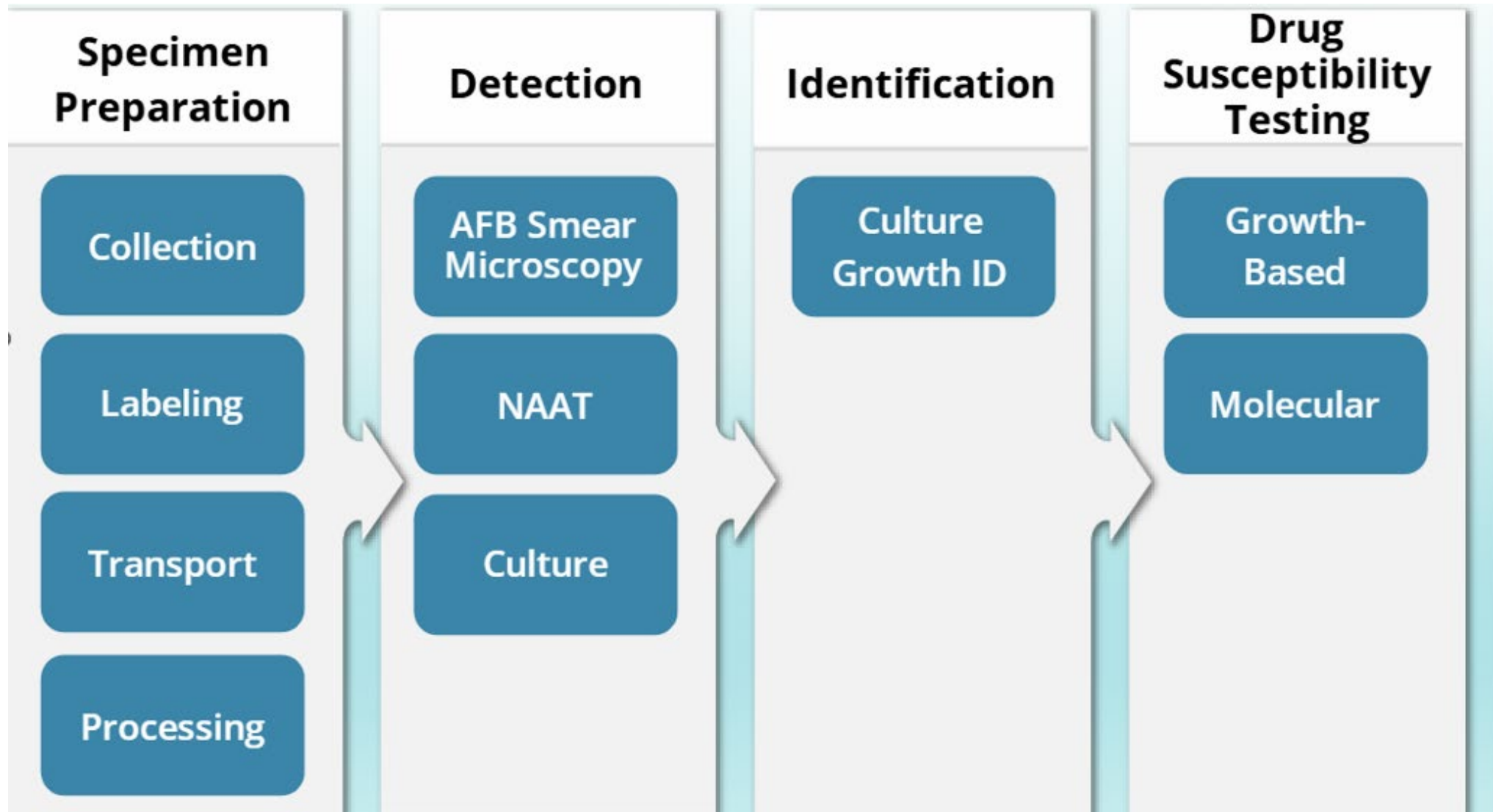
STD, and TB Prevention

February 12, 2026

OBJECTIVES

- Provide an overview of the current and emerging global TB diagnostics landscape to understand the types, availability, and intended use of TB diagnostic tools worldwide
- Highlight innovations in testing approaches to prepare learners with knowledge for potential future applications
- Examine U.S. public health laboratory capacity and CDC-supported services to support appropriate and timely provider utilization for TB care
- Discuss future needs, challenges, and opportunities in TB diagnostics to optimize rapid diagnosis of TB and reduce disease transmission

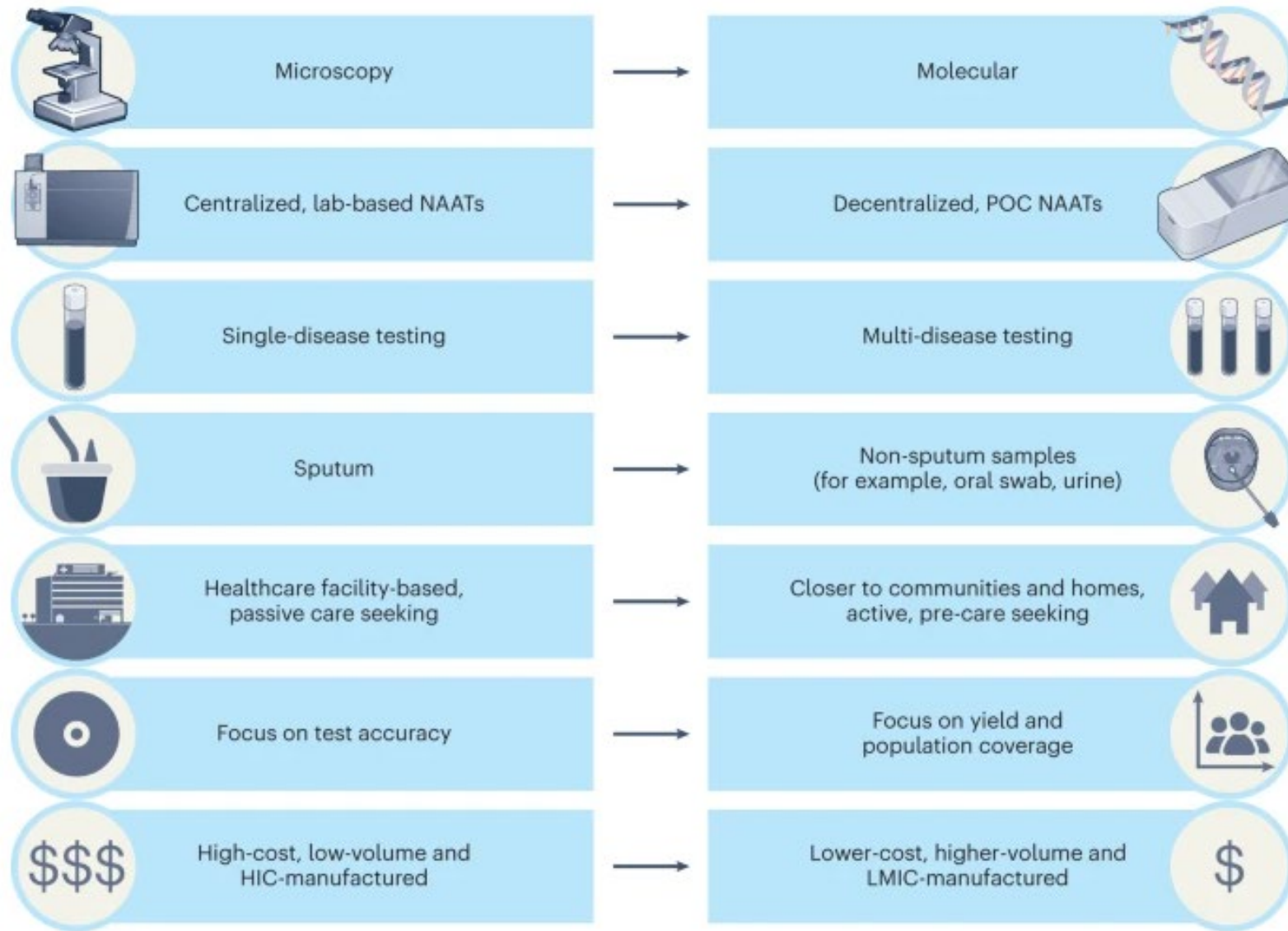
TB Testing Workflow



TB Testing Continues to Evolve

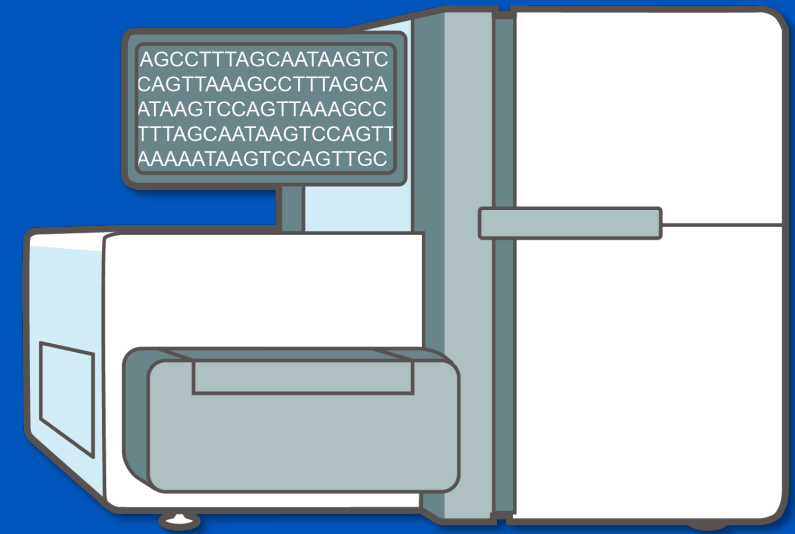
- Technological advancements
- Improvements in scientific understanding
- New drugs and treatment regimens
- Interest in alternative specimen types
- Needs for testing access nearer to patients
- Interest in multi-disease platforms
- Drive for shorter turnaround times to reduce diagnostic delay
- Workforce and infrastructure needs

Critical Transitions Proposed to Close the Diagnostic Gap



NAATs, nucleic acid amplification tests; HIC, high-income country.

Global TB Diagnostics Landscape



Antigen Detection Test: Urine-LAM

- Lipoarabinomannan (LAM) is a mycobacterial cell wall component
- Degradation of mycobacteria leads to shedding of free LAM
- LAM shed in the kidneys (or elsewhere in the body) circulates in host blood and is filtered by the kidneys into urine

Tool (Developer)	Level	Stage of Development	Sample	Accuracy	Time to Result	Price (USD)
Fujifilm SILVAMP TB LAM II (Fujifilm, Japan)	Primary care center	Commercially available	Urine	PLWHIV SE: 70.7%, SP: 90.9%; HIV-negative individuals SE: 53.2%, SP: 98.9%	1 hour	NA
ichroma LAM Ag (Boditech, South Korea)	Primary care center	Early	Urine	SE: 93%, SP: 78%	<20 minutes	NA
PF-LAM (Brightest Bio, USA)	Primary care center	Late	Urine	Adults SE: 54%, SP: 100%; Adults with HIV SE: 71%, SP: 92%	60 minutes	Test: <\$4, Instrument: <\$250
Biopromic TB LAM assay (Biopromic AB, Sweden)	Primary care center	Late	Urine	SE: 63%, SP: 93%	NA	NA
FLOW TB Urine-LAM Assay (Salus Discovery, USA)	Primary care center	Late	Urine	NA	NA	NA

Benefits and Limitations of Urine LAM Tests

BENEFITS	PRACTICAL LIMITATIONS
✓ Urine easy to collect ✓ Used for bacteriologic confirmation of TB among PLHIV that are unable to produce sputum ✓ Simple test; requires minimal infrastructure and supplies ✓ Point-of-care assay that can be performed by trained lab- & non-lab testers ✓ Fastest diagnostic test time for same-day result reporting for patient impact & clinical action	✗ Variable sensitivity ✗ Small proportion of population recommended for use of this testing ✗ Cannot distinguish tuberculous and non-tuberculous mycobacteria ✗ Cannot detect drug resistance

Molecular-based Diagnostic Tests for Active TB

- Nucleic acid amplification tests (NAATs)
 - Molecular test used to detect the nucleic acid (typically DNA) of *Mycobacterium tuberculosis* complex (MTBC) in a clinical sample (e.g., sputum)
 - Involves amplification of MTBC specific targets to improve sensitivity
 - Intended to provide rapid results more quickly than culture

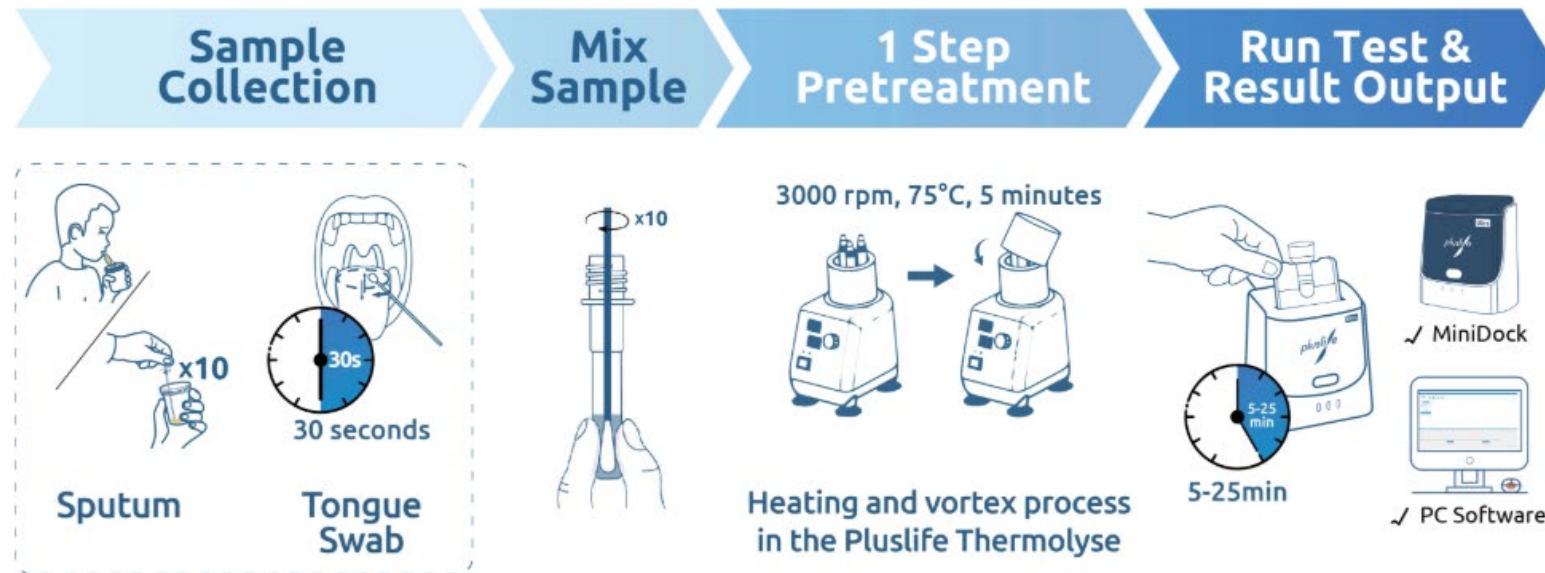


Example: Near Point of Care (nPOC) Molecular Test for TB Diagnosis

- **MiniDock MTB Test—Pluslife**

- Detects *Mycobacterium tuberculosis* from sputum or tongue swabs
- Fast results (≤ 30 minutes)
- Cost*: \$3.60/ test, \$155 for MiniDock device, \$180 for Pluslife Thermolyse device
- Performance: **Sputum** - 85.7% sensitivity, 97.6% specificity / **Tongue swab** - 79.6% sensitivity, 99.5% specificity

Simple Workflow



Example: nPOC Molecular Test for TB Diagnosis (2)

- **Truenat MTB Ultima**

- Detects *Mycobacterium tuberculosis* from sputum and oral swab
- Fast results (≤ 1 hour)
- Cost[^]: \$TBD/ test, \$10,000–\$18,000 for devices
- Portable, battery operated, chip-based test
- Performance: **Sputum** - 93.6% sensitivity, $\geq 95\%$ specificity[^] / **Tongue swab** - 77.5% sensitivity, 98% specificity[^]
- Availability of TrueNat MTB RIF Dx for rifampin resistance (targeting *rpoB*)

Collect Sample

Mix Sample

Lyse Sample

Transfer and Incubate

Transfer and Run Test



Tongue swab



Sputum swab

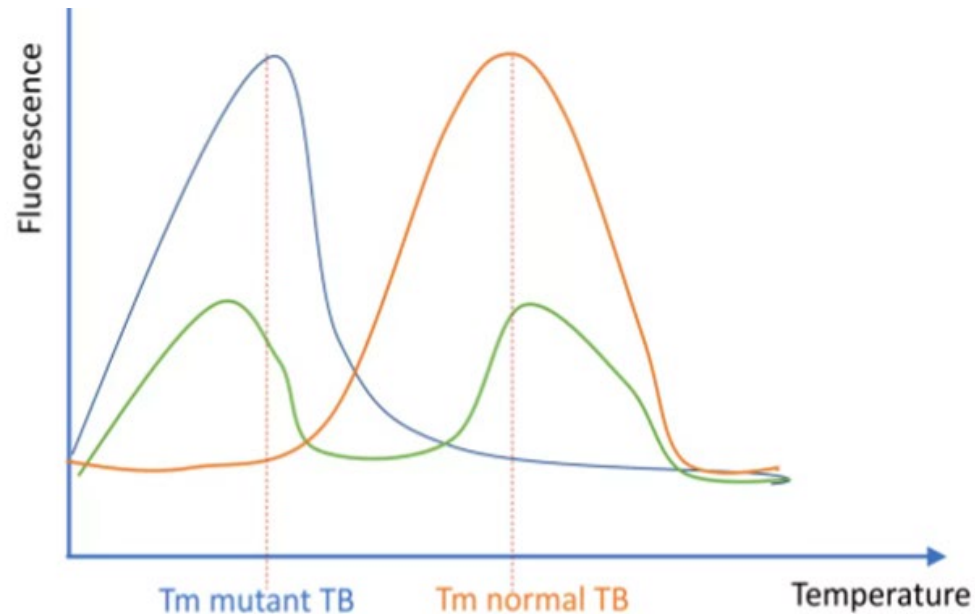


Other Nucleic Acid Amplification Tests in the Global Pipeline

- Treatment Action Group 2024 and 2025 TB Diagnostics Pipeline Reports
- Numerous NAATs in early, late, or commercialized phase
- Some tests also include detection of drug resistance
- NAATs used globally
 - Xpert MTB/RIF and Xpert MTB/RIF Ultra
 - TrueNat MTB Plus
 - TB-LAMP
 - Abbott Realtime MTB and MTB RIF/INH
 - BD MAX MDR-TB
 - Bruker-Hain FluroType MTBDR
 - Roche Cobas MTB and MTB-RIF/INH

Xpert MTB/RIF Ultra Not Available in United States

- Utilizes melt curve technology to detect MTBC and RIF resistance
 - Targets include *rpoB*, IS6110, and IS1081
- Focused on rifampin resistance determining region (RRDR)
- Some benefits for reducing resistance calls for silent mutations (not associated with rifampin resistance)
- Limit of detection (16 cfu/ml) lower than Xpert MTB/RIF (US market)



Tests for Detection of Drug Resistance

- **Molecular tests**

- Numerous tests in early, late, or commercialized phase
- Xpert MTB/XDR (isoniazid, ethionamide, fluoroquinolones, and second-line injectables)
- Line Probe Assays: Nipro Genoscholar PZA-TB II, Bruker GenoType MTBDRplus ver 2.0, & Nipro NTM+MDRTB II
- DNA sequencing
 - Targeted Next Generation Sequencing (TNGS): Specific areas of the genome
 - Whole Genome Sequencing (WGS): Examining most of the genome

Globally Recommended tNGS Assays

- **Deeplex® Myc-TB (Genoscreen, France):**
 - Detects resistance to rifampicin, isoniazid, pyrazinamide, ethambutol, fluoroquinolones, bedaquiline, linezolid, clofazimine, amikacin and streptomycin
 - Assay relies on deep sequencing of a 24-plex amplicon
 - Probes 18 drug-resistance targets
- **AmPORE-TB® (Oxford Nanopore Diagnostics, UK):**
 - Detects resistance to rifampicin, isoniazid, fluoroquinolone linezolid, amikacin and streptomycin
 - Assay relies on sequencing of a 27-plex amplicon
 - Probes 24 drug-resistance targets
- **Both available in United States as Research Use Only**

Deeplex® Myc-TB
From clinical samples to drug resistance profile

1 st line antibiotics		2 nd line antibiotics	
<i>rpoB</i>	Rifampicin	Fluoroquinolones	<i>gyrA, gyrB</i>
<i>ahpC, fabG1, katG, inhA</i>	Isoniazid	Amikacin	<i>rrs</i> *
<i>pncA</i> *	Pyrazinamide	Kanamycin	<i>eis, rrs</i> *
<i>embB</i>	Ethambutol	Capreomycin	<i>tlyA</i> *, <i>rrs</i> *
		Streptomycin	<i>gidB</i> *, <i>rrs</i> *, <i>rpsL</i> *
		Ethionamide	<i>ethA</i> *, <i>inhA</i> , <i>fabG1</i>
<i>hsp65</i>	Species ID	Bedaquiline, Clofazimine	<i>rv0678</i> *
CRISPR/DR	Spoligotyping	Linezolid	<i>rrl, rplC</i>
phyloSNPS	Genotyping		

Mycobacterium tuberculosis Drug Resistance Test
Rapid testing of AMR resistance with Oxford Nanopore Technologies sequencing



AmPORE-TB
6h from sample to report
2h sequencing
16 anti-TB drugs screened

Alternative Specimen Types

Alternative Specimen Types for TB Testing

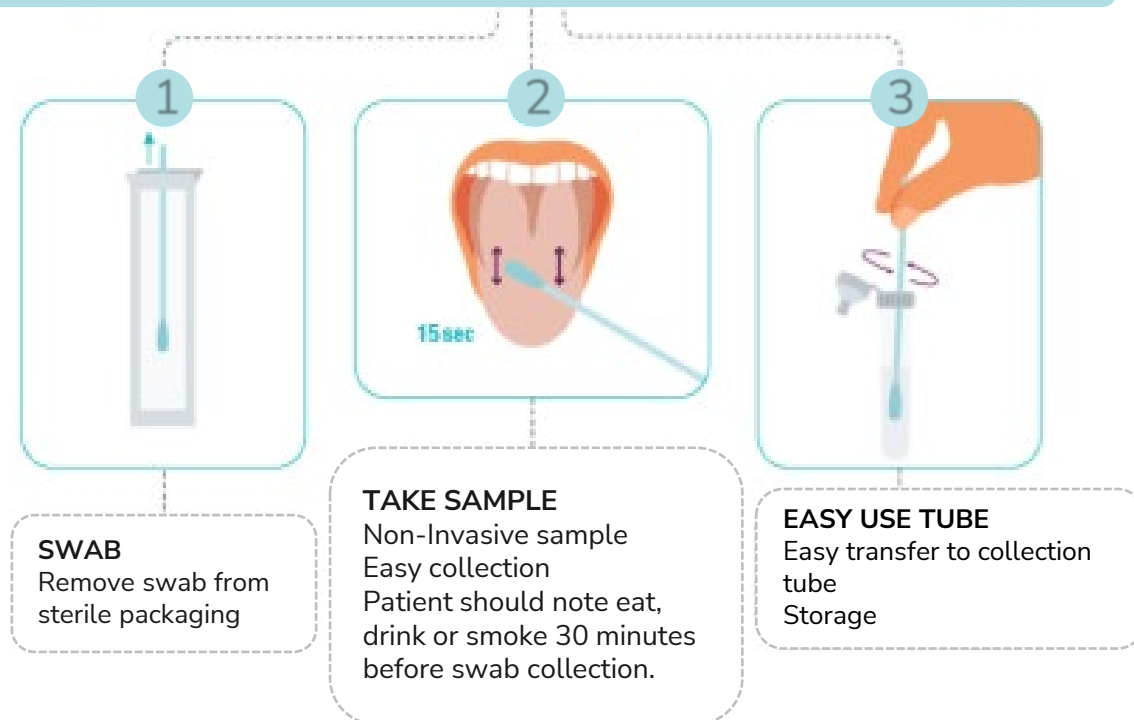
- Sputum can be difficult to obtain
- Evaluation of alternative specimen types
 - Easier and non-invasive collection
 - Potential to increase access to testing
 - Possibly improved time to diagnosis
 - Reduced safety concerns during collection

Types of Alternative Specimens

- Oral swabs
- Saliva
- Stool
- Urine
- Blood
- Breath/aerosol sample

TONGUE SWAB COLLECTION

To provide a tongue swab sample you would need to follow the instructions as below:



Performance of Alternative Specimen Types

- Performance can vary depending on specimen type examined, collection method, test method used, and other factors (e.g., population tested and bacterial burden)
 - Systematic review by Church et al. (2024) of buccal mucosa and tongue swabs found sensitivity in adults ranged from 36 to 91% and in children from 5% to 42%. Specificity was generally high across all 15 studies examined.
 - Study by Olson et. al. (2024)
 - Concentration of eluate from tongue using foam swab had overall sensitivity of 83% and 100% specificity with use of quantitative PCR
 - › Better sensitivity in those without HIV coinfection
 - Sediments from tongue swabs from patients exhibiting higher semiquantitative values for sputum tested by Xpert, had better sensitivity
 - Study highlighted how differences in swab material, sample processing, and test method (especially those designed for sputum testing) can impact results

Testing of Cell-free RNA or DNA

- Specimen types: primarily urine or blood
- Reports in literature exploring potential to detect TB disease, latent infection, and possibly monitoring treatment response
- Metagenomic and CRISPR-based diagnostic approaches
- Studies continuing to assess performance and potential for earlier diagnosis and usefulness for monitoring treatment response

Article

<https://doi.org/10.1038/s41467-024-49245-6>

Circulating cell-free RNA in blood as a host response biomarker for detection of tuberculosis

Received: 8 March 2023

Accepted: 29 May 2024

Published online: 10 June 2024

 Check for updates

Adrienne Chang^{1,9}, Conor J. Loy^{1,9}, Daniel Eweis-LaBolle^{1,9}, Joan S. Lenz¹, Amy Steadman², Alfred Andgras³, Nguyen Viet Nhung⁴, Charles Yu⁵, William Worodria³, Claudia M. Denking⁶, Payam Nahid⁷, Adithya Cattamanchi^{7,8} & Iwijn De Vlaminc¹✉

Tuberculosis (TB) remains a leading cause of death from an infectious disease worldwide, partly due to a lack of effective strategies to screen and triage individuals with potential TB. Whole blood RNA signatures have been tested as

<https://www.nature.com/articles/s41467-024-49245-6>

RESEARCH

Open Access



Cell-free DNA next-generation sequencing for *Mycobacterium tuberculosis* obtained from plasma of children with active tuberculosis

<https://pmc.ncbi.nlm.nih.gov/articles/PMC11874688/>

Case Report

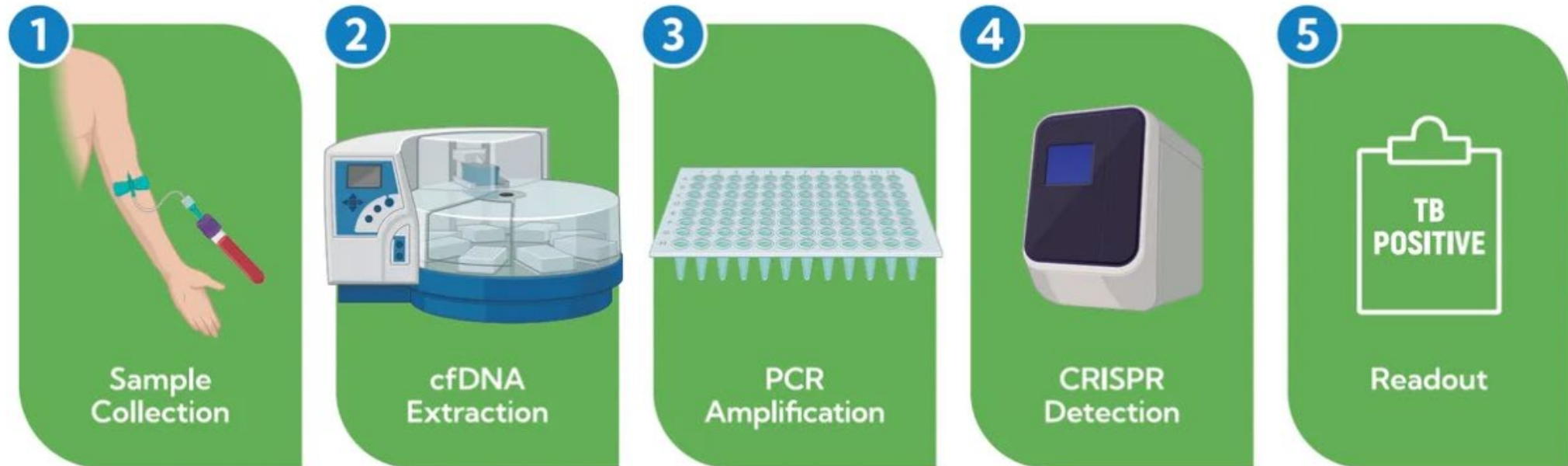
Cell-free DNA blood test for the diagnosis of pediatric tuberculous meningitis

Guyu Li^a✉, Kendall Cannon^b, Carlos Sisniega^c, Jaime Fergie^d

<https://www.sciencedirect.com/science/article/pii/S2405579424000081>

IntelliGenome Receives FDA Breakthrough Device Designation for CRISPR-TB Blood Test

January 21, 2025 | 1 min read



<https://intelligenome-us.com/crispr-tb-benchtop-assay>

Challenges and Opportunities Across the US Diagnostics Landscape

What is the status of NAAT for TB in the United States?

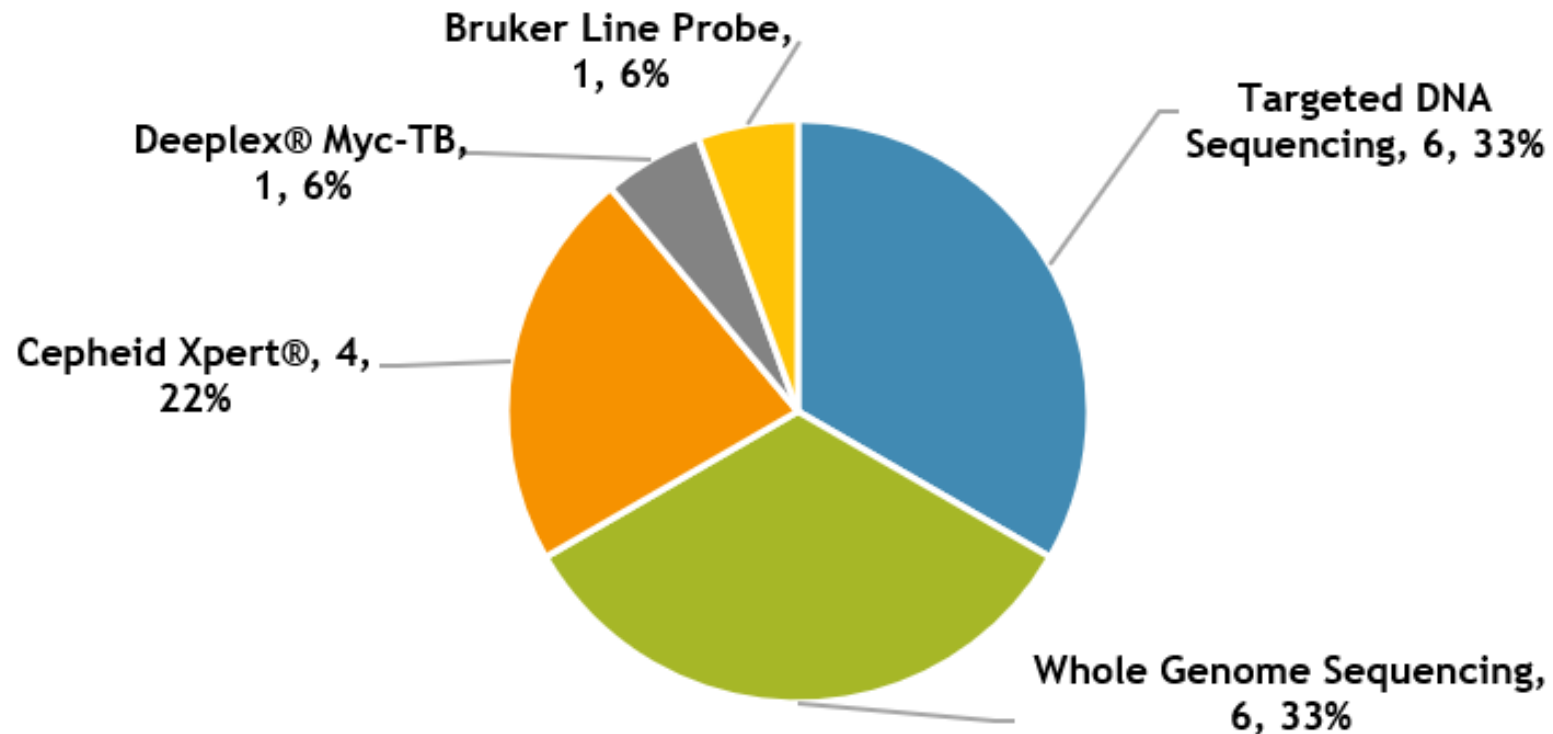
- Xpert MTB/RIF only FDA market authorized NAAT in United States
 - Rapid results for TB and rifampin resistance
 - Platform used for other pathogens
 - Older generation test with lower sensitivity and some issues with false positive calls for rifampin resistance
 - Limited market as Xpert MTB/RIF Ultra available and used globally

	Xpert MTB/RIF	Xpert MTB/RIF Ultra*
Limit of Detection	114 cfu/ ml	16 cfu/ ml “MTB Detected, trace”
Sensitivity		
All TB	83%	88%
HIV-Infected	76%	88%
Pediatric	47%	71%
Specificity		
All TB	98%	95%
No History of TB	98%	96%
Any History of TB	97%	92%
Test Time	< 2 hours	65-77 minutes



Status of U.S. Molecular Susceptibility Testing

- Data from CDC's Model Performance Evaluation Program (MPEP) March 2025 panel
 - 18/56 participating laboratories (public health, clinical, commercial, and federal) reported use of molecular methods

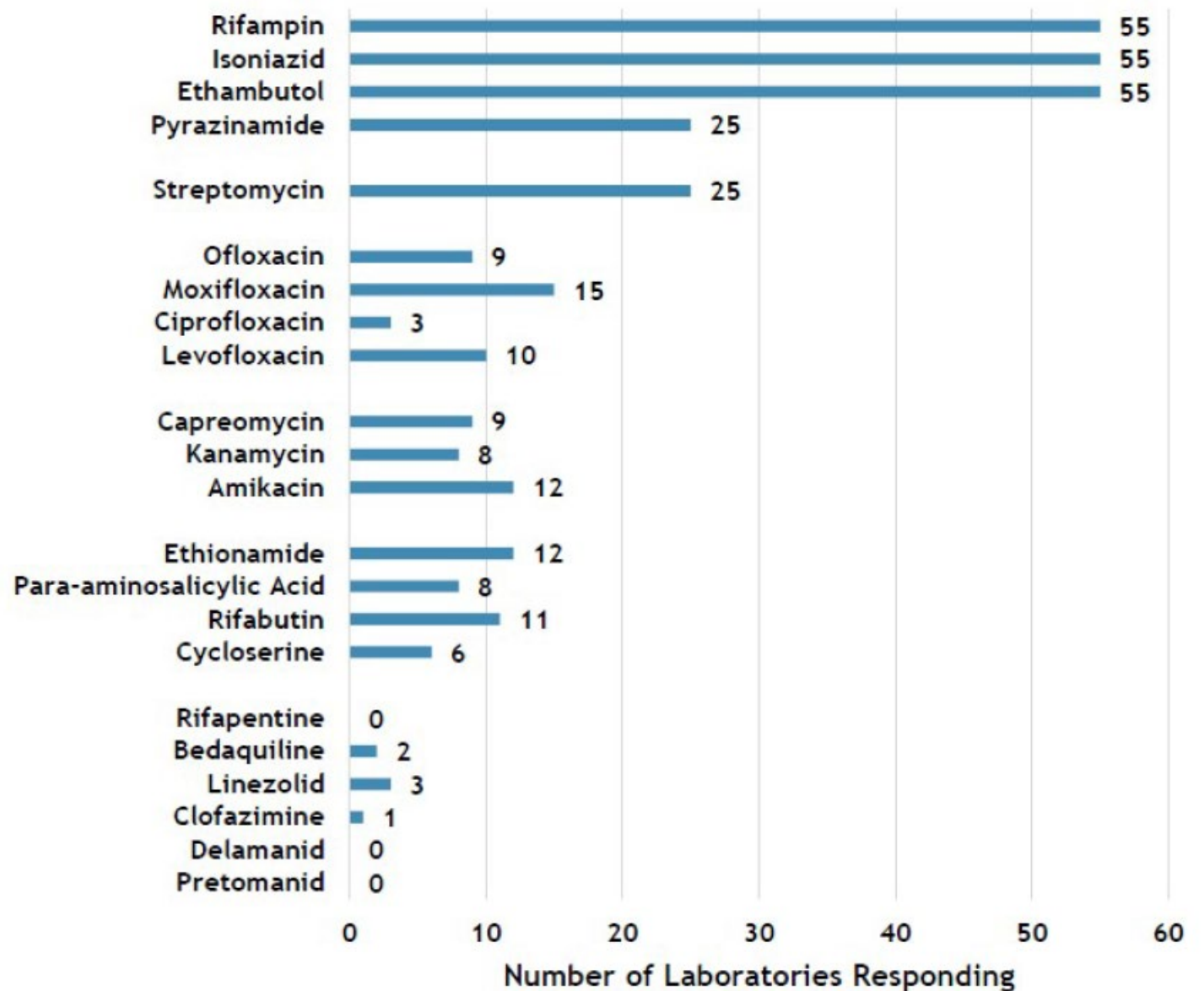


Note: Cepheid Xpert MTB/RIF reported here for molecular susceptibility only.

Status of TB Susceptibility Testing in United States

- Data from CDC's
March 2025
Model
Performance
Evaluation
Program (MPEP)
Report

Figure 5. Antituberculosis Drugs Tested by Growth-based Method by Participating Laboratories



Limited Access to Susceptibility Testing for New and Repurposed Drugs

- No FDA approved, commercial assay for this purpose - requires validation as a laboratory developed test
- Knowledge base for optimal growth-based and molecular testing continuing to grow
- Testing recommended before initiating use of BPaL/BPaLM
- Currently limited to 3 U.S. laboratories

Other Challenges/Opportunities for U.S. TB Testing: Rifampin

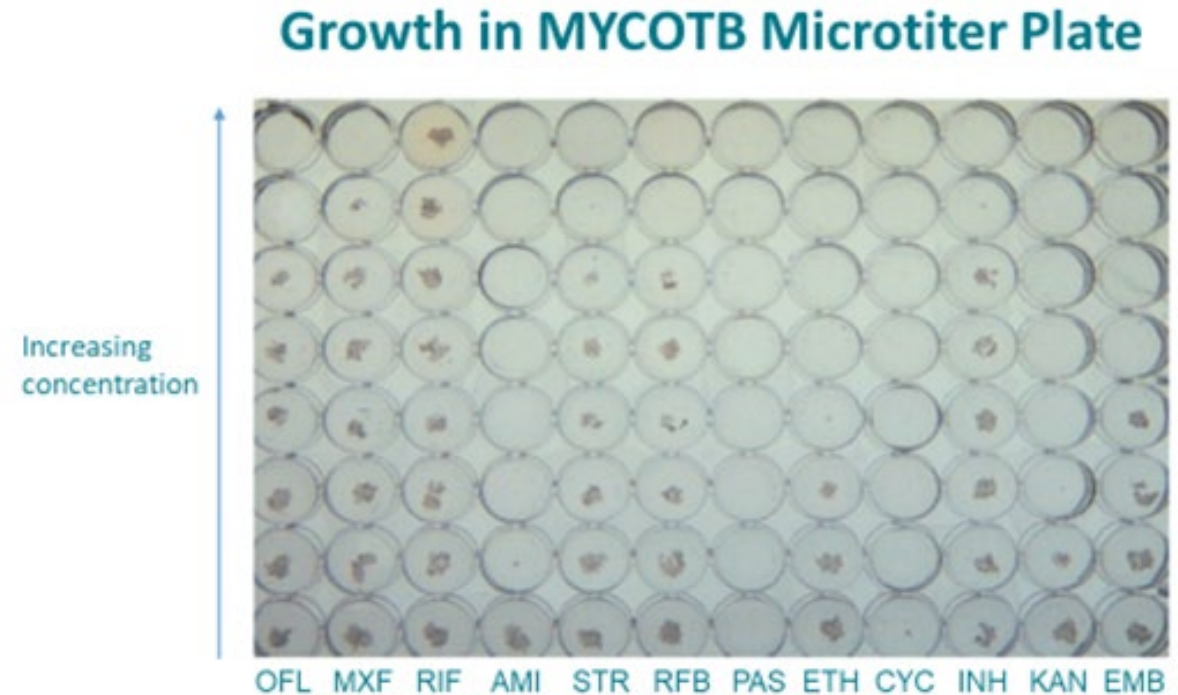
- **Global recommendation for use of reduced critical concentration for rifampin**
 - Lowered to address classification of strains with certain *rpoB* mutations
- **CDC worked with the Association of Public Health Laboratories on funding opportunity to support evaluation and provided panel of isolates**
 - 11 public health laboratories awarded funding
 - 2/11 implemented testing at lower critical concentration, others did not due to mix of performance and concern about regulatory requirements
- **The challenge is that modified testing requires validation as a laboratory developed test**
 - No FDA approved, commercially available product readily available at lower concentration

Other Challenges/Opportunities for U.S. TB Testing: Fluoroquinolones

- Fluoroquinolone (FQ) susceptibility testing not routinely performed as part of first-line panel
- Recommended before initiating 4-month rifapentine-moxifloxacin regimen and when first-line resistance is detected
- No FDA approved, commercial assay for this purpose—requires validation as laboratory developed test
- CDC worked with Association of Public Health Laboratories to fund evaluation at 8 public health laboratories
 - 5 either have or will be implementing routine molecular or growth-based FQ testing

Minimum Inhibitory Concentration (MIC) Testing

- Increasing interest in use of broth microdilution for growth-based susceptibility testing of *Mycobacterium tuberculosis* complex
- Allows evaluation of different drugs at different concentrations to provide quantitative levels of resistance
- MIC is the lowest concentration of drug that inhibits visible growth—provides more granular results
 - Challenge in that formal breakpoints not established for all drugs so categorical result (R or S) not always possible
- Global recommendation for optimized plate design and standardized methodology



Status of PZA Susceptibility Testing

- BD BACTEC™ MGIT™ 960 PZA test kits were unavailable from **July 18, 2024 through late September 2024**
- BD identified a corrective action and released a new lot of kits that they deemed suitable for use
- BD issued another medical device correction (May 2025) as implemented corrective action may not have fully resolved the issue and further investigation needed
- Communication issued November 2025 indicating resumption of production of a modified version of the test kit
 - Change to days post-positivity that inoculum can be prepared (3–5 days only)
 - Adjustment of shelf life from 18 to 13 months

Availability of *pncA* Sequencing (Interim Solution)

- Submitters to the National PHL DST Reference Center at California Microbial Diseases Laboratory (MDL) will continue to receive services through the reference center
- CDC's Molecular Detection of Drug Resistance (MDDR) service available to all jurisdictions for testing when drug resistance (other than PZA) is suspected or known

Laboratory	States	POC
CA MDL	California, Hawaii, Minnesota, and Nevada + All states currently submitting to National PHL DST Reference Center	cdphtbdst@cdph.ca.gov
DTBE/LB	Alabama, Connecticut, Georgia, Illinois, Indiana, New Jersey, North Dakota, Ohio, Oregon, and Wisconsin	tblab@cdc.gov
FL BPHL	Florida and North Carolina	Patrick.valois@flhealth.gov
TX DSHS	Arizona, Arkansas, Louisiana, Missouri, Tennessee, Texas, Virginia, and West Virginia	Jan.owen@dshs.texas.gov
Wadsworth	Delaware, Maryland, Massachusetts, Michigan, Pennsylvania, New York, Washington (state) and Washington DC	tb_wgs@health.ny.gov

Access to TB Testing Services

CDC's Molecular Detection of Drug Resistance (MDDR) Service

- **CLIA-compliant testing service**
- **Rapid detection of drug-resistant TB by DNA sequencing**
 - Phenotypic drug susceptibility testing (DST) in parallel using agar proportion for first and second-line drugs
 - No longer performing growth-based testing for pyrazinamide
 - *pncA* sequencing only
- **Available to all 50 states, U.S. territories, and U.S. Affiliated Pacific Islands**
- **Testing service is free of charge**
- **Clinical consultation regarding test results available**

What sample types are accepted for CDC's MDDR?

- Nucleic acid amplification test positive (NAAT+) sediments
- Isolates of *Mycobacterium tuberculosis* complex

How do I learn more about submitting samples to CDC for MDDR testing?

- **MDDR User Guide:**

- <https://www.cdc.gov/tb/php/laboratory-information/mddr-user-guide.html>



MDDR Service Updates

(Tentatively Planned for March/April)

- Reimplementing MDDR testing for DNA extracts from CDC's Infectious Diseases Pathology Branch
 - DNA extracts from formalin-fixed paraffin-embedded (FFPE) tissue samples positive for *M. tuberculosis*
 - Transferred to the Division of TB Elimination/Laboratory Branch
 - Evaluated by DNA sequencing for rifampin, isoniazid, and fluoroquinolone resistance
 - Also targeted region of *pncA*, primarily for *M. bovis* His57Asp
 - Enhancement from previous pyrosequencing assay - only rifampin and isoniazid resistance previously evaluated

MDDR Service Updates (2)

(Tentatively Planned for March/April)

- **Implementation of minimum inhibitory concentration (MIC) testing**
 - Includes moxifloxacin, levofloxacin, linezolid, clofazimine, and bedaquiline by broth microdilution
 - MIC for pretomanid evaluated by MGIT (0.06 µg/ml–16 µg/ml)

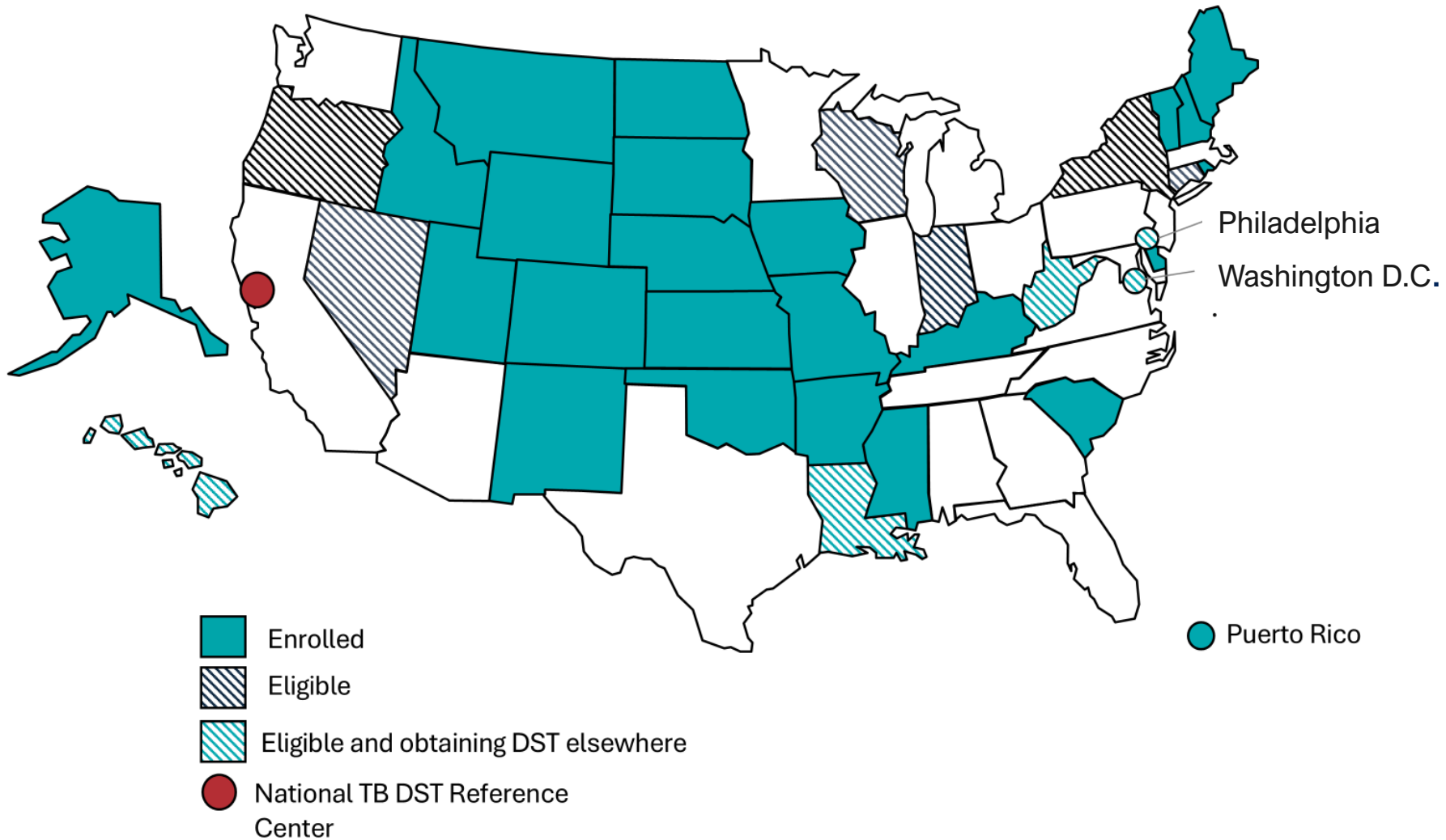


- Reporting MIC value—no consensus-based breakpoints for many drugs with method used
- Established QC range for each drug

National Public Health Laboratory Drug Susceptibility Testing (DST) Reference Center

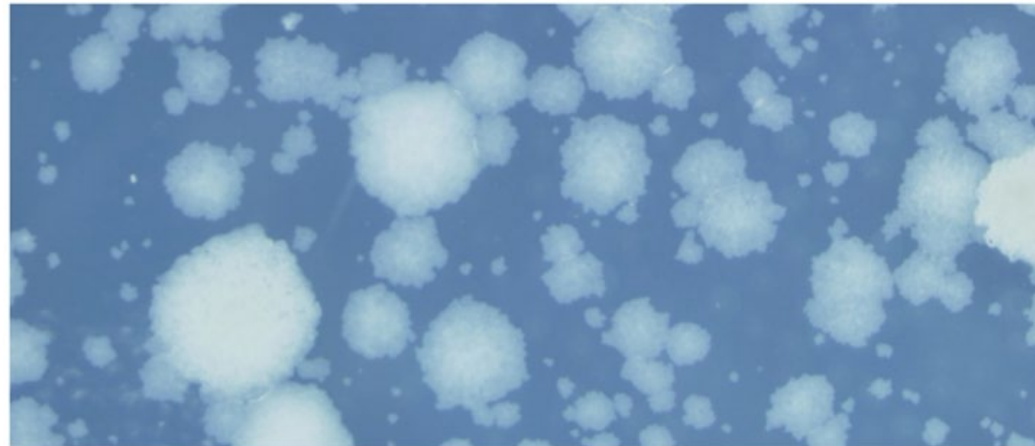
- CDC collaboration with Association of Public Health Laboratories
- Currently awarded to the California Microbial Diseases Laboratory in Richmond, California
- Provides quality-assured DST for eligible US public health laboratories with low testing volumes (<50 isolates per year)
- Services include whole genome sequencing and phenotypic DST for first and second-line antituberculosis drugs

National DST Reference Center Serving Low Volume PHLs



Learning More About Services Available at State and Local Public Health Laboratories

- Public health laboratories (PHLs) ensure core TB laboratory testing services
- Many PHLs perform phenotypic and some perform molecular susceptibility testing in-house
- All PHLs can assist with obtaining these services through referral
- PHL contact information: <https://web.aphl.org/atb/Public-Health-Laboratories>



M. tuberculosis on agar media. Picture courtesy of APHL.

National Molecular Surveillance for TB

- **National TB Molecular Surveillance Center (NTMSC) currently at Michigan Bureau of Laboratories**
 - Supports whole genome sequencing (WGS) prospectively on new *M. tuberculosis* isolates
 - WGS data generated at NTMSC available to states for other surveillance or research purposes (POC: Lauren Cowan los4@cdc.gov)
- **More public health laboratories implementing WGS in-house**
 - Public health laboratories performing WGS in-house (meeting quality criteria) may submit data files in lieu of referral to Michigan
- **CDC deposited deidentified whole genome sequencing data for 28,850 samples in an NCBI BioProject (PRJNA1237251)**

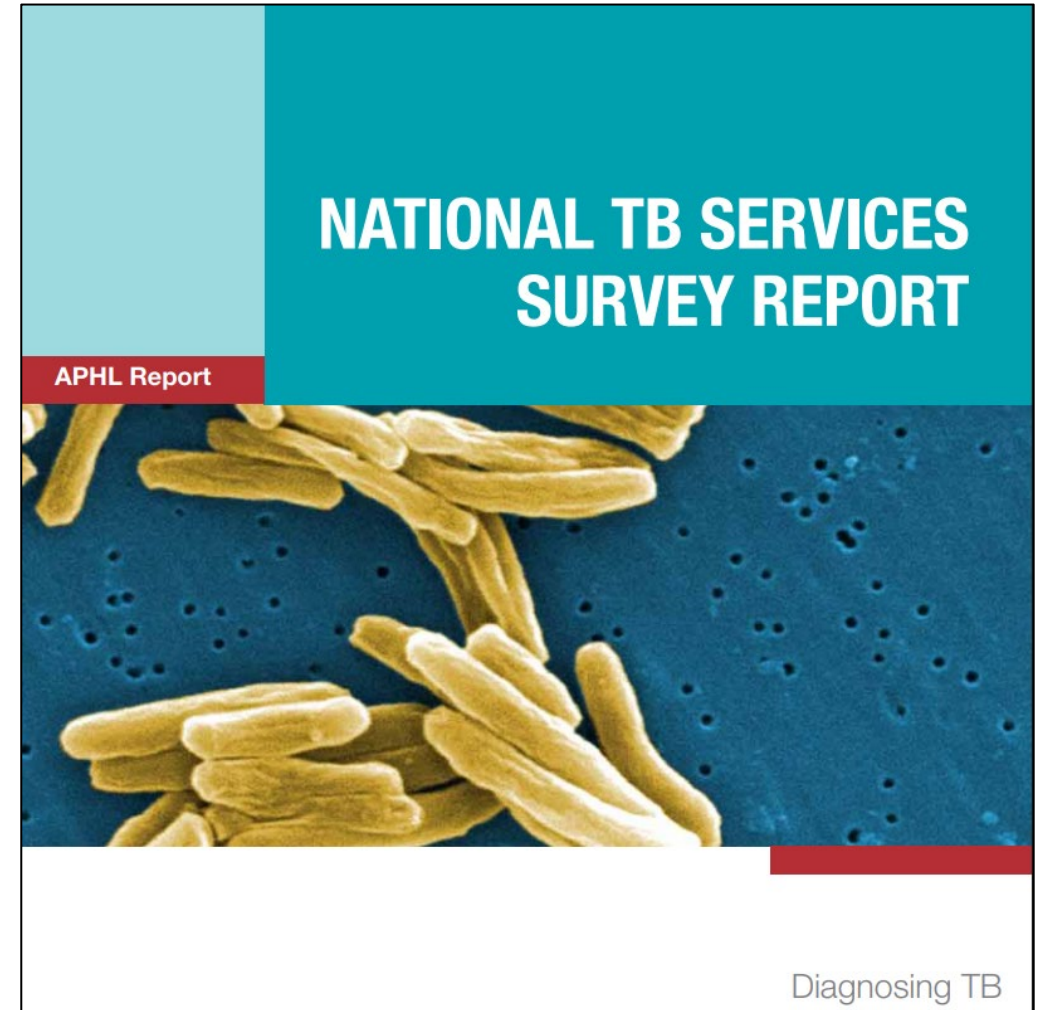
Final Thoughts

Other Considerations for the Laboratory

- Procurement and supply chain issues
- Decreasing/increasing cost of some supplies and reagents
- Changes in workforce
- Changes in technology and testing algorithms
- Regulatory landscape
- New drugs or treatment regimens
- Artificial Intelligence

Understanding Current Capacities: APHL National TB Laboratory Services Survey

- Last completed in 2010
- Purpose:
 - Assess the overall ability of U.S. laboratories to provide quality TB diagnostic services
- Results:
 - Useful to identify gaps in capabilities and capacities and identify opportunities to strengthen laboratory systems
- Release date anticipated mid/late 2026



Future Needs and Opportunities in TB Diagnostics

- Accelerate the availability of innovative, FDA-approved diagnostic tests
- Evaluate use of nPOC and performance with use of alternative specimen types (i.e., non-sputum)
- Establish a test to assess risk for progression to active TB disease among individuals with latent infection
- Advance and evaluate faster, more comprehensive tests for susceptibility testing
- Integrate new diagnostics in parallel with the introduction of new TB drug regimens
- Sustain laboratory capacity and expertise

Thank you

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 <https://www.cdc.gov/>
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