



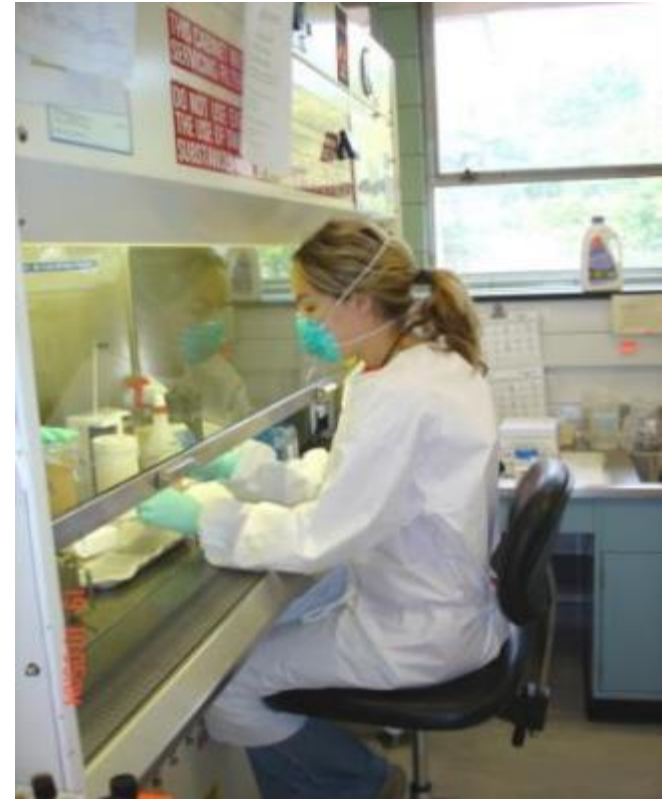
# The Role of the Laboratory in the Diagnosis & Management of Tuberculosis

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2021 NAR Post-grad Course  
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# Laboratory is Essential

- **Critical partner for clinical care and public health activities**
  - Rapid, reliable results for diagnosis
  - Drug susceptibility testing for appropriate treatment
  - Monitoring response to therapy
  - Genotyping to detect recent transmission

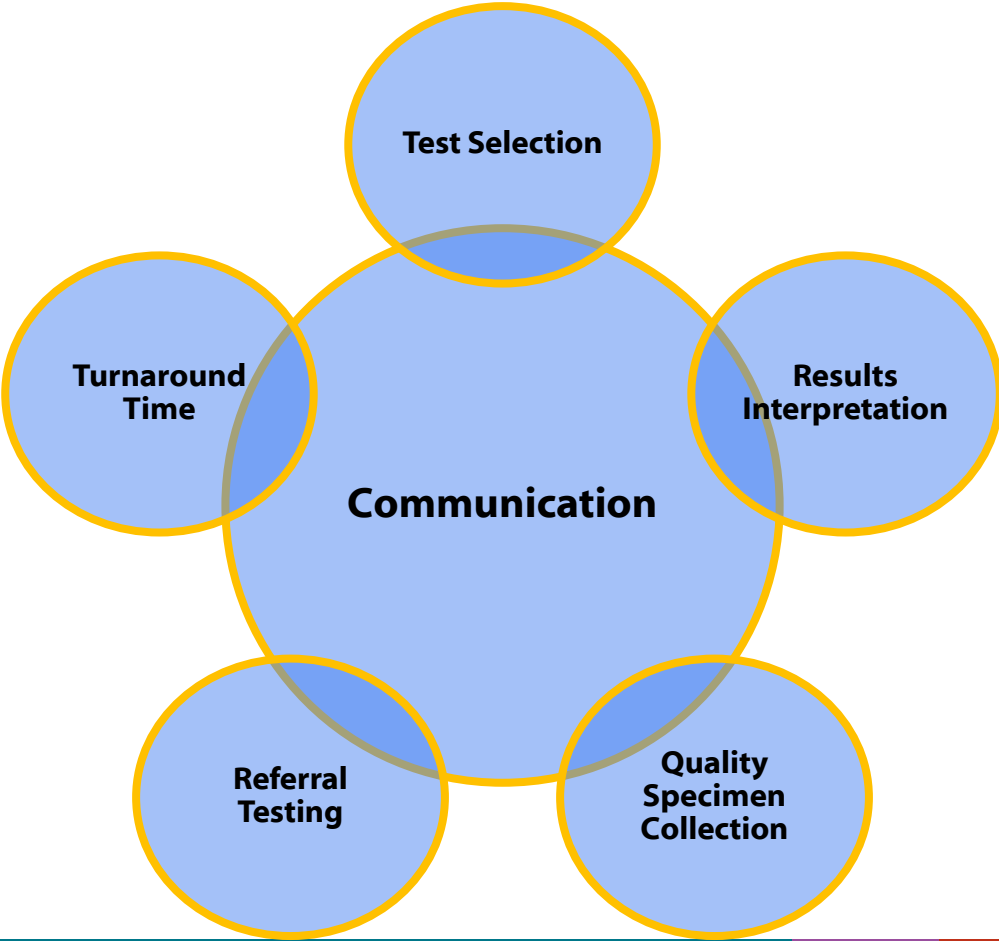


# Common Goals

- Ensuring patients get best care
- Using latest tools to guide decision making
- Ultimately, contributing to decreased transmission of TB



# Strong Relationships are Important for Patient Care



# Practical Laboratory Perspectives

# General Considerations from the Laboratory

- **Not all tests are equal**
- **The more test types performed within or between labs, the higher the likelihood of discordant results**
- **Laboratories are subject to regulatory compliance and constrained by resources**
  - Always want to help but may be limited in what services can be provided
  - Understanding access through referral important
- **We all wish *M. tuberculosis* grew faster!**
  - Growth-based results take time especially if repeat testing is needed
  - Contact lab if results pending beyond expected turnaround times

# Expected Turnaround Times

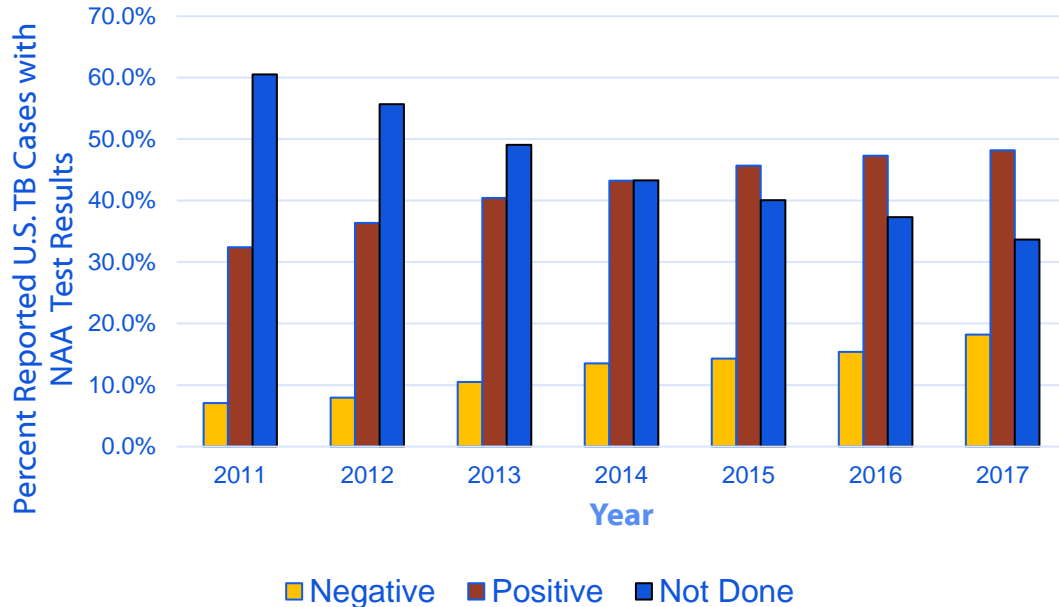
Result	Recommended Turnaround Time
Acid Fast Bacilli Smear	Within 24 hours of specimen receipt
Nucleic Acid Amplification Test	Within 48 hours of specimen receipt
Culture positive for <i>M. tuberculosis</i> (MTB)	≤ 21 days of specimen receipt
First-line drug susceptibility test results	≤28 days from specimen receipt (Tenover et. al, 1993)  ≤17 days from identification of MTB from culture (revised 2016)
Molecular detection of drug resistance	TBD

<https://jcm.asm.org/content/31/4/767.long>

[https://www.aphl.org/programs/infectious\\_disease/tuberculosis/Documents/TB\\_CoAg\\_Toolkit\\_2016.pdf](https://www.aphl.org/programs/infectious_disease/tuberculosis/Documents/TB_CoAg_Toolkit_2016.pdf)

# Nucleic Acid Amplification (NAA) Tests

- Use of rapid NAA testing should be standard of care for those presumed to have TB (CDC guidelines) but continued progress needed





# Considerations for Culture vs. Rapid NAA Tests

- **Rapid detection key for patient care and public health**
- **Not yet able to replace culture; culture remains most sensitive method**
- **NAA tests do not differentiate live from dead organism**
- **Some TB patients will have both a negative culture and a negative NAA test**
- **Laboratory may not have validated multiple matrices for molecular testing, especially extrapulmonary sources (e.g., off-label use of FDA approved assay)**
- **Testing for pathology samples, when sample not viable for culture, may be an option**

# Use of NAA testing results to guide decision making in use of airborne infection isolation (A.I.I.)

- February 2015, U.S. FDA approved expanded claims for Xpert MTB/RIF related to A.I.I.
- National TB Controllers Association and Association of Public Health Laboratories issued guidance in 2016
- Based on negative results from 1 or 2 sputum specimens predictive of results of 2 or 3 AFB smears being negative
  - Sputum test results alone should NOT be only criteria for decision making



Consensus statement on the use of  
Cepheid Xpert MTB/RIF<sup>®</sup> assay in making  
decisions to discontinue **airborne infection  
isolation** in healthcare settings

# Considerations for Growth-based Drug Susceptibility Testing and Molecular Detection of Drug Resistance

- **Assays for molecular detection of drug resistance are not necessarily equal**
  - Performance characteristics, loci examined, sample tested, output/results
- **Important to understand the information provided by tests, limitations, and expected turnaround time**
  - Communication between laboratory and healthcare provider is key
- **Heteroresistant populations (mix of susceptible and resistant organisms) can cause discordant results**
- **Whole genome sequencing will help but not solve everything**
- **What is true for one drug may not be true for another**
  - Silent mutations in *rpoB* do not cause rifampin resistance
  - Silent mutation (Leu203Leu) in *fabG1(mabA)* results in isoniazid resistance

# Confirmation of Detection of Rifampin Resistance by Probe-based Methods Such as Xpert MTB/RIF

## ■ MMWR 2013

- To confirm Xpert rifampin resistance detected, genetic loci associated with rifampin and isoniazid resistance should be sequenced
- If rifampin resistance confirmed, rapid molecular testing for mutations associated with resistance to other first- and second-line drugs should be performed
- All molecular testing should prompt growth-based drug-susceptibility testing

## ■ CDC's Molecular Detection of Drug Resistance Service

- 19% of samples tested for confirmation of RIF resistance detected by Xpert had silent mutations (i.e., not resistant) and 14% had mutations associated with low-level resistance
  - Low level rifampin-resistance associated mutations may be missed by growth-based methods but clinically relevant

codon  
synonymous  
resistance  
Xpertmolecular  
genome  
DNA  
genotypic  
mutation  
protein  
eis  
target gene  
tlyA  
SNP  
rpoB  
katG  
RNA  
deletion  
sequencing  
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phenotypic  
locus  
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frameshift  
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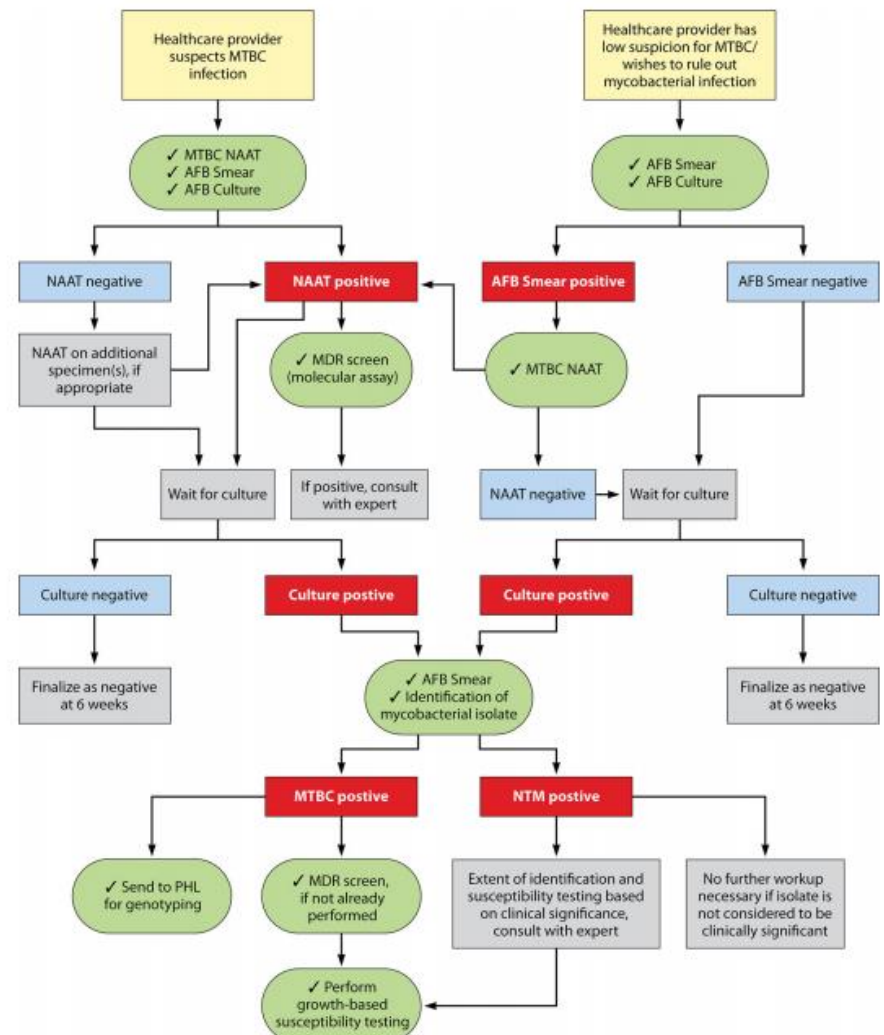
# Lack of standardized reporting language

- **No single standard for terminology or nomenclature for reporting of molecular results**
  - Different labs may report similar results using different language
    - Silent mutation, synonymous mutation, mutation detected not clinically significant
    - Point mutation, nonsynonymous mutation, mutation detected clinically significant
    - Use of abbreviations: S450L or Ser450Leu
- **Can lead to confusion potentially impacting interpretation**
- **Underscores need for good communication and focused tools and resources to aid understanding of results**



# Proposed Ideal Algorithm for Mycobacteriology Testing

- Algorithm proposed in Clinical Microbiology Reviews (2018)



# Piecemeal Nature of TB Testing

- Referral to multiple laboratories may be needed for a complete panel of testing
- More complex cases likely involve testing at more than one laboratory
- **Again—Communication is key**
  - Laboratory, healthcare providers, TB Program
- **CDC sponsors TB Centers of Excellence for Training, Education, and Medical Consultation for strengthening clinical practice and patient care**
  - [https://www.cdc.gov/tb/education/tb\\_coe/default.htm](https://www.cdc.gov/tb/education/tb_coe/default.htm)



# Use of Molecular Assays in the TB Laboratory

Purpose	Specimen Type	Importance	Platforms
Direct detection of MTBC by NAA testing	<ul style="list-style-type: none"><li>• AFB smear positive and smear negative clinical specimens</li></ul>	<ul style="list-style-type: none"><li>• Patient isolation and initiation of therapy</li></ul>	<ul style="list-style-type: none"><li>• Cepheid GeneXpert</li><li>• Hologic MTD</li><li>• Line probe assays (LPA)</li><li>• Lab developed tests (LDTs)</li></ul>
Identification of mycobacteria	<ul style="list-style-type: none"><li>• AFB positive cultures</li><li>• Clinical specimens (dependent on assay)</li></ul>	<ul style="list-style-type: none"><li>• Rapid, accurate identification of MTB</li><li>• Initiation of therapy</li></ul>	<ul style="list-style-type: none"><li>• LPAs</li><li>• DNA sequencing (LDT)</li><li>• MALDI-TOF</li><li>• Other LDTs</li></ul>
Detection of drug resistance mutations	<ul style="list-style-type: none"><li>• Clinical specimens</li><li>• MTB-positive cultures</li></ul>	<ul style="list-style-type: none"><li>• Patient therapy decisions</li><li>• Surveillance</li></ul>	<ul style="list-style-type: none"><li>• Cepheid GeneXpert</li><li>• LPAs</li><li>• DNA sequencing (LDT)</li><li>• Other LDTs</li></ul>

# Whole Genome Sequencing

- **DNA sequencing method using next generation sequencing (i.e., high throughput) technology**
- **Data useful for**
  - Determining genetic relatedness of strains to detect possible recent transmission
  - Identifying mutations in genetic loci known to be associated with drug resistance
  - Detecting novel associations with drug resistance
- **For clinical care, WGS is another laboratory tool**
  - Can replace other tests as data can be used for multiple purposes
  - For drug susceptibility, primarily examining genetic loci known to be associated with resistance

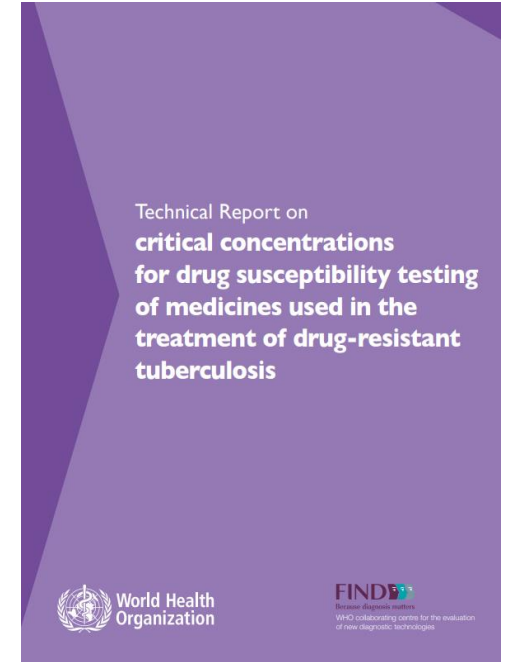
# Whole Genome and Targeted Next Generation Sequencing

- **Still limited to primarily reference laboratories for TB**
- **Adaptable to provide rapid, accurate, and clinically actionable results and provide large amounts of data**
- **Whole genome sequencing**
  - Sequencing most of the genome
  - Still difficult to perform directly from specimen so culture isolate needed
- **Targeted next-generation sequencing (NGS)**
  - Sequencing specific areas of the genome
  - Can be performed from patient samples in addition to culture

# Recent Proposed Changes to Growth-based Drug Susceptibility Testing

# Updated Critical Concentration Values

- Released 2018
- Based on systematic review of MIC and sequencing data for phenotypically wild type and phenotypically non-wild type strains
- Anti-TB drugs evaluated
  - Fluoroquinolones
  - Amikacin
  - Kanamycin
  - Linezolid
  - Clofazimine
  - Bedaquiline
  - Delamanid



# Clinical and Laboratory Standards Institute M24 (3<sup>rd</sup> edition) and M62

- Additional information on molecular testing, challenges with low-level resistance, and pharmacodynamics/ pharmacokinetics
- Breakpoints and interpretive criteria for MIC testing in Sensititre
  - EMB, RIF, and INH
- Critical concentrations for second line drugs in MGIT
- Minimum inhibitory concentration quality control ranges for H37Rv

# Revised Critical Concentration For Rifamycin

- Released February 2021
- Based on systematic review of critical concentrations and consensus from WHO Technical Expert Group meeting 2/24/2020

Table 1. Critical concentrations for INH and the rifamycins.

Drug	LJ	7H10	7H11	MGIT
Isoniazid	0.2	0.2	0.2	0.1
Rifampicin <sup>a</sup>	40	0.5	1.0	0.5
Rifabutin <sup>b</sup>	–	–	–	–
Rifapentine <sup>c</sup>	–	–	–	–

Changes indicated in red

Technical Report on critical concentrations for drug susceptibility testing of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine)



World Health Organization



Because diagnosis matters  
WHO Collaborating Centre for  
Tuberculosis Laboratory Strengthening  
and Diagnostic Technology Evaluation

# Low-level rifampin-resistance associated *rpoB* mutations

- Also referred to as disputed, discordant, low-level, or mutations associated with borderline resistance
- Associated with a high degree of treatment failure/relapse\*
- Examples:
  - Leu430Pro (Leu511Pro), Asp435Tyr (Asp516Tyr), His445Asn (His526Asn), His445leu (His526Leu), Leu452Pro (Leu533Pro), Ile491Phe (Ile572Phe)
- Often test susceptible by growth-based DST

\*Van Deun A, et al. 2009, Rigouts L et al. 2013, Van Deun A, et al, 2013, Shah NS, et al. 2016



**Questions Received by the Laboratory**

# Questions—Molecular Detection of Drug Resistance

- **When is DNA sequencing needed?**
  - Some areas universally performed
  - Others, primarily a clinical decision based on patient history, known laboratory results, and clinical indications

# Questions—Molecular Detection of Drug Resistance (2)

- **If sequencing shows no mutations, can I confidently use those drugs for treatment?**
  - Clinical decision but would want to know more about testing performed (sequencing vs. probe based) (If sequencing, what loci?)
  - CRyPTIC Consortium and the 100,000 Genomes Project correlated WGS data with growth-based DST and found good correlation between molecular prediction of susceptibility to first-line drugs with growth-based susceptibility (NEJM 2018; 379:1403-1415)
  - Wadsworth Center with use of WGS found susceptible-predictive value of 96% with improved turnaround time (JCM 2017; 55(6):1971-1882)

# Questions—Molecular Detection of Drug Resistance (3)

- **What does it mean if there's an unknown mutation? How should I proceed with patient treatment?**
  - An unknown or novel mutation is one that the laboratory has not detected previously or has limited data supporting association with resistance
  - How to proceed with treatment is a clinical decision but should ensure growth-based testing proceeds, when possible

# Questions—Molecular Detection of Drug Resistance (4)

- **How often do you see discrepancies between molecular and growth-based drug susceptibility methods?**
  - It does happen due to several different reasons (not all inclusive)
    - Assay limit of detection
    - Specific genetic loci examined and mutations outside those areas
    - Molecular testing of specimen and growth-based testing of isolate (some difference in bacterial populations)
    - Unknown mechanisms of resistance

# Questions—Molecular Detection of Drug Resistance (5)

- **If results from multiple tests (same or different labs) are different, which one is right/wrong?**
  - Advise not to consider right or wrong, results depend on sample tested and assay performed
  - Understanding the performance characteristics of each test is important

# **CDC's Molecular Detection of Drug Resistance Service**

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

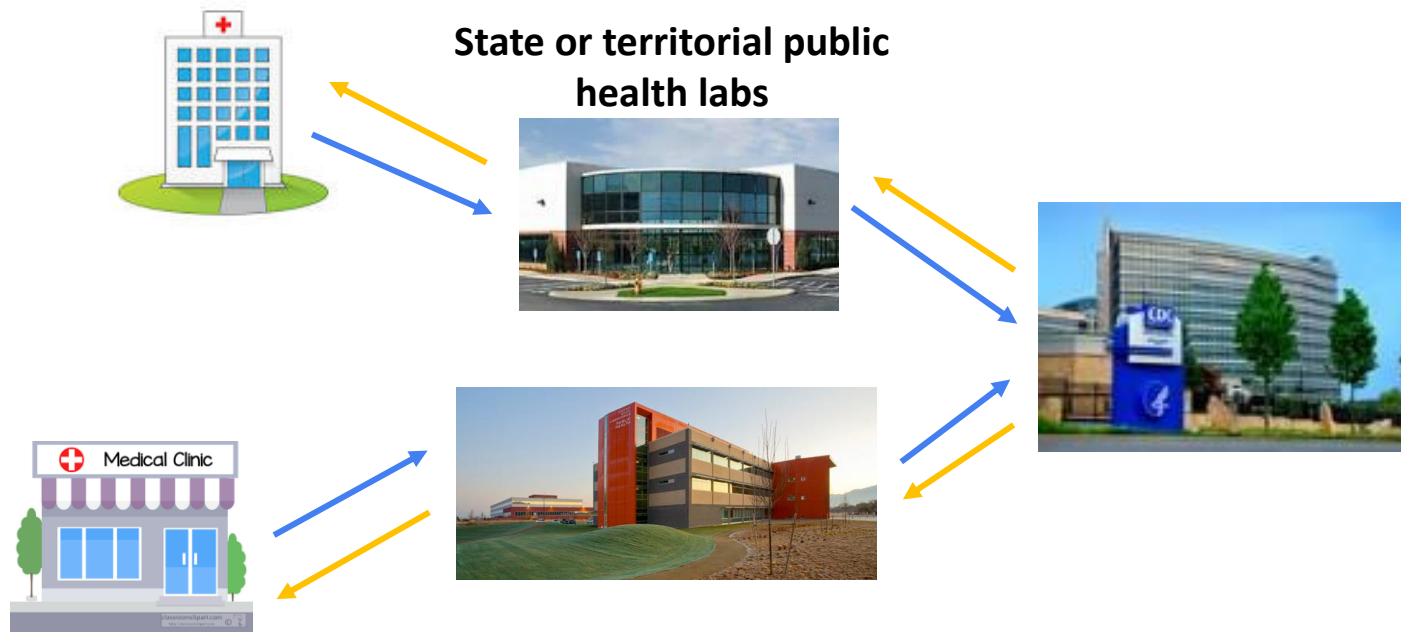
- **CLIA compliant service implemented in 2009**
  - Clinical testing service for MTBC
    - Rapid detection of drug resistant TB by DNA sequencing
    - Provide additional information second-line drugs, when applicable
    - Growth-based drug susceptibility testing also performed
  - Available to all 50 states, U.S. territories, and U.S. Affiliated Pacific Islands
  - Testing service is free and shipping costs are covered by FedEx account managed by Association of Public Health Laboratories (APHL)
  - Clinical consultation regarding test results available
- **Turnaround time (TAT) from sample receipt: ≤ 4 days (most cases)**



# Acceptable Testing Criteria

- **Isolate, nucleic acid amplification test (+) sediment (not raw specimen), and DNA extracts from fixed tissue samples**
  - Patients at higher-risk for RIF-R/MDR TB
    - From population with high rates of drug resistance
    - Exposed to drug resistant case
    - Lack of clinical response to therapy
    - Previously treated for TB
  - Cases of public health importance (e.g., day care/long-term care facility)
  - Known rifampin resistance (molecular or growth-based)
  - Inability to tolerate first-line drugs
  - DNA extract from CDC Infectious Disease Pathology Branch (IDPB)
  - Mixed culture or non-viable (growth-based DST not possible)
  - Other reasons

# Sample Submission and Results Reporting



- Results sent back to submitting PHL (typically state) by either fax (MDDR) or encrypted email (growth-based DST)

# MDDR reports-Sanger DNA sequencing

CDC-002-00059-03 Effective: 12/16/2020



Centers for Disease Control & Prevention  
National Tuberculosis Reference Laboratory  
Mailstop H17-4, 1600 Clifton Rd NE, Atlanta, GA 30329, United States

Report Status: Interim

Patient Name:	Specimen ID:
Sex: Birthdate: Age:	
CDC Specimen ID:	Public Health Submitter:
Material Submitted: <i>M. tuberculosis</i> complex isolate Specimen Source: MGIT broth Medium:	Texas Department of State Health Services Laboratory Services Section MC 1947 1100 W. 49th Street /P.O. Box 149347 Austin, TX 78714-9347
Date Collected: Date Received: Date Reported:	

## Results for Molecular Detection of Drug Resistance (Complete Panel); Conventional Drug Susceptibility Test in progress.

Drug	Locus *	Result	Interpretation
Rifampin	rpoB	<b>Mutation:</b> CAC>GAC, His526Asp	Rifampin resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are RMP-R.)
Isoniazid	inhA	No mutation	Isoniazid resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are INH-R.)
	katG	<b>Mutation:</b> AGC>ACC, Ser315Thr	
	fabG1	No mutation	
Ethambutol	embB	<b>Mutation:</b> GGC>GAC, Gly406Asp	Likely ethambutol resistant (88% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are EMB-R.)
Pyrazinamide	pncA	No mutation	Cannot rule out PZA resistance. (86% of PZA-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)
Fluoroquinolones	gyrA	No mutation	Cannot rule out fluoroquinolone resistance. (80% of FQ-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at locus gyrA.)
	gyrB	No mutation	
Second Line Injectables	rrs	No mutation	Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 550 clinical isolates: <ul style="list-style-type: none"> <li>91% of AMK-R isolates have a mutation in the rrs locus;</li> <li>87% of KAN-R isolates have a mutation in either the rrs locus or the eis locus;</li> <li>55% of CAP-R isolates have a mutation in either the rrs locus or the tlyA locus.)</li> </ul>
	eis	No mutation	
	tlyA	No mutation	

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

MDDR 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.

Reviewed by: Beverly Metchock

CLIA ID # 11D2030855

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	katG	<b>Mutation:</b> AGC>ACC, Ser315Thr	
	fabG1	No mutation	
Ethambutol	embB	<b>Mutation:</b> GGC>GAC, Gly406Asp	Likely ethambutol resistant (88% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are EMB-R.)
Pyrazinamide	pncA	No mutation	Cannot rule out PZA resistance. (86% of PZA-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)
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# Transitioning to Targeted Next Generation Sequencing Assay

- Panel expanded to 24 amplicons
- Isoniazid: expanded to sequence the entire *katG* gene
- Linezolid: *rplC*, *rri*
- Bedaquiline: *atpE*, *rv0678* (*mmpR*), *pepQ*
- tlyA* dropped

	SANGER
1	<i>rpoB</i> -RRDR
2	<i>inhA</i>
3	<i>katG</i>
4	<i>gyrA</i>
5	<i>rrs</i>
6	<i>pncA</i>
7	<i>embB</i>
8	<i>eis</i>
9	<i>tlyA</i> -1
10	<i>tlyA</i> -2
11	<i>rpoB</i> -170
12	<i>gyrB</i>
13	<i>ahpC</i>
14	<i>fabG</i> -609

BEFORE



	tNGS
1	<i>rpoB</i> -RRDR
2	<i>rpoB</i> -170
3	<i>katG</i> -1
4	<i>katG</i> -2
5	<i>katG</i> -3
6	<i>katG</i> -4
7	<i>inhA</i>
8	<i>fabG</i> -609
9	<i>pncA</i>
10	<i>embB</i>
11	<i>gyrA</i>
12	<i>gyrB</i>
13	<i>rrs</i>
14	<i>eis</i>
15	<i>rv0678</i>
16	<i>atpE</i>
17	<i>pepQ</i> -1
18	<i>pepQ</i> -2
19	<i>pepQ</i> -3
20	<i>ahpC</i>
21	<i>rplC</i> -1
22	<i>rplC</i> -2
23	<i>rri</i> -1
24	<i>rri</i> -2

AFTER

Added  
Discontinued

# CDC Infectious Diseases Pathology Branch and MDDR

- Testing for possible Mycobacterial infections using formalin fixed samples (< 2wks or paraffin embedded)
- Requestor first contacts state health department and then IDPB for consult and approval
  - [Pathology@cdc.gov](mailto:Pathology@cdc.gov)
  - <https://www.cdc.gov/ncezid/dhcpp/idpb/specimen-submission/mycobacterium.html>
- Requestor ships fixed sample to IDPB for testing
- If MTBC detected and submitter requests MDDR, DNA transferred
- MDDR performed and results reported to requestor and IDPB

# Summary

- **Laboratory plays an essential role in patient-centered care**
- **Regular communication is key for test selection and results interpretation**
- **Increasing use of molecular assays for both diagnostic purposes and molecular detection of drug resistance**
  - Culture still needed
  - Genetic prediction of drug resistance has good correlation with phenotypic results for first-line drugs and increasingly second-line drugs
- **Discordant results from within and among labs can occur**
  - Speaking with lab, understanding assay performance characteristics and review of sample tested key to working through issues

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- Wadsworth Center: Vincent Escuyer, PhD

For more information, contact CDC  
1-800-CDC-INFO (232-4636)  
TTY: 1-888-232-6348 [www.cdc.gov](http://www.cdc.gov)

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

