Introduction to Mycobacteriology Testing

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CDC’s DTBE Laboratory Branch

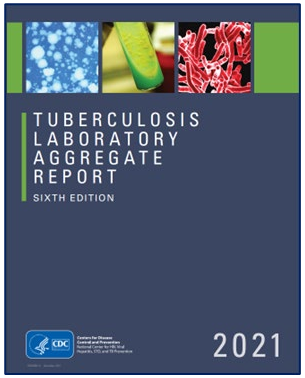
Mission of DTBE/ Laboratory Branch

Dedicated to the elimination of TB by:

1. conducting applied research on *Mycobacterium tuberculosis*
2. providing laboratory services to support TB control and surveillance
3. directly supporting U.S. public health laboratories to increase their capacity to combat TB.

CDC TB Cooperative Agreement

- **Includes focus on strengthening public health laboratory services and activities at state and local levels**
  - 58 awardees
    - 50 state public health labs, 7 large cities (San Francisco, Los Angeles, San Diego, Houston, NYC, Washington DC, and Philadelphia) and Puerto Rico



Workload Variable	Total Number 2017	Total Number 2018	Total Number 2019
Clinical specimens processed	201,374 (124–18,357)	193,534 (108–18,258)	186,849 (105–17,458)
Number of patients for whom specimen was processed	86,700 (79–9,939)	79,490 (48–9,675)	77,208 (51–9,687)

Mycobacteriology Testing in the United States

Important Definitions

- **Clinical specimen**- material taken directly from the patient (e.g., sputum, CSF, pleural fluid); may be "raw" specimen or may be "processed" specimen (e.g., sediment)

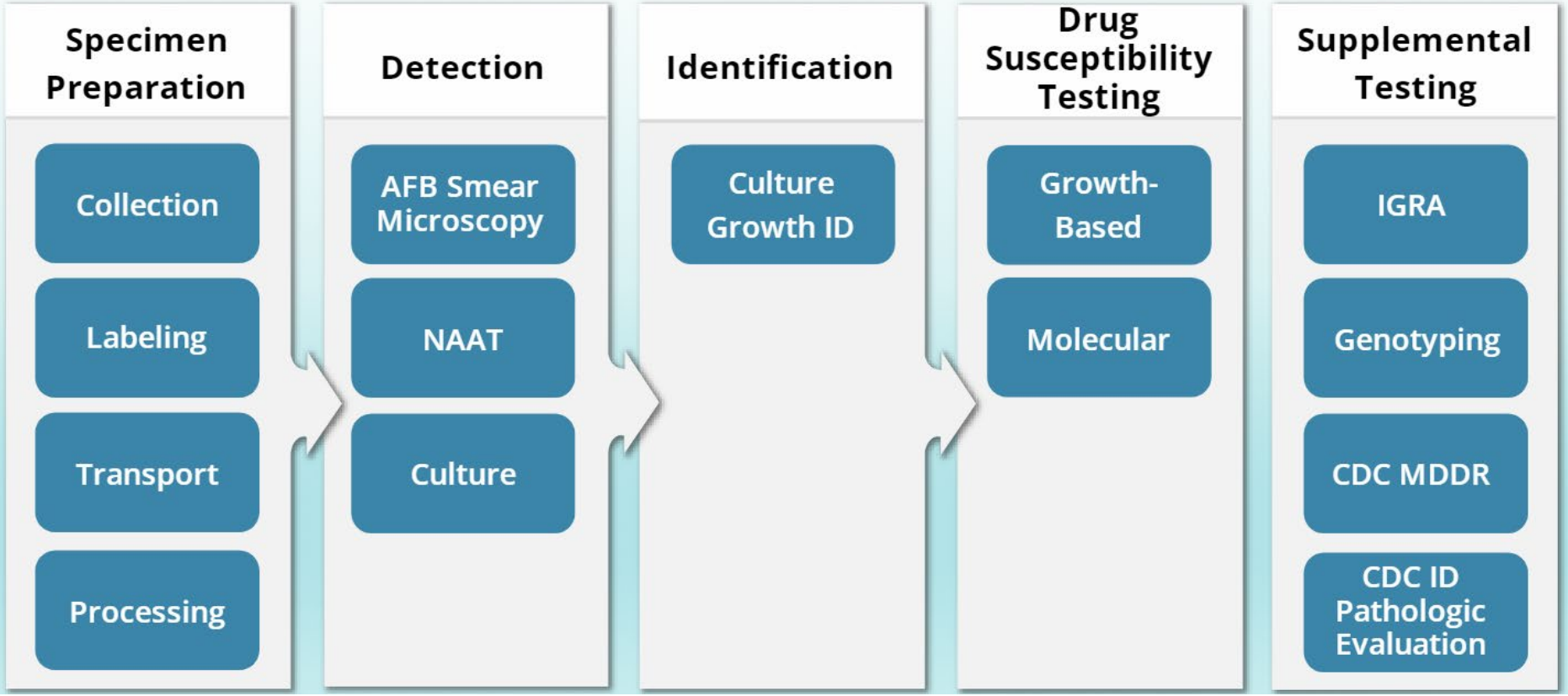
- **Isolate**- organism isolated (i.e., grown) from culture of a clinical specimen [e.g., an LJ tube with *Mycobacterium tuberculosis* complex (MTBC) growth]

- **Direct detection**- detection of RNA or DNA sequences of interest in organisms present in a clinical specimen
  - Usually referred to as NAAT, or nucleic acid amplification test

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

TB Testing Workflow



https://www.aphl.org/programs/infectious_disease/tuberculosis/Understanding_TB_Lab_Test_Nurses/story.html

Recommended Turn Around Times for Lab

- AFB microscopy ("smear")
  - Within 1 day of specimen receipt in lab
- Direct detection (NAAT)
  - Within 2 days of specimen receipt in lab
- Identification (ID) of MTBC in culture
  - Within 21 days of specimen receipt in lab
  - Specimens with low bacterial loads (e.g., smear negative, 1+ smear) generally take longer to grow than specimens with high bacterial loads (e.g., 3+, 4+ smear)
  - Specimens from patients on therapy may take longer to grow than diagnostic specimens
- First-line drug susceptibility testing (DST)
  - Within 17 days from identification of *M. tuberculosis*
  - This is if all the "pieces fit together" perfectly!

Mycobacteriology Testing in United States

- Types of laboratories (not mutually exclusive):
  - Public health laboratories (e.g., state, county, city)
  - Commercial laboratories (e.g., LabCorp, Quest, ARUP)
  - Reference Laboratories (Nat. Jewish, CDC, Mayo)
  - Hospital/medical center laboratories
- TB laboratory tests may be performed at several different laboratories
  - Work is often piecemeal – specimens or isolates referred from one lab to another
  - Different laboratories may only perform some test methods (e.g., AFB smear only)
- Communication is key especially when testing becomes further removed from originating laboratory

Piecemeal Nature of TB Testing

- **Referral to multiple laboratories may be needed for a complete panel of testing**
  - Lack of awareness on where to obtain testing
- **More complex cases likely involve testing at more than one laboratory**
- **Differences in methods and test performance**
- **Communication is key but can be challenging**
  - Laboratory, healthcare providers, TB Program
- **Differences in how results may be reported**
  - Can impact turnaround time for results to healthcare provider
  - Potential source of confusion with differences in format, terminology and nomenclature

TB Testing Workflow—Specimen Collection

Sputum Collection

- Most common specimen type when evaluating for tuberculosis
- For diagnosis: 3 specimens collected 8–24 hours apart; at least one early morning
- Follow-up specimens, during treatment, collected monthly for smear, culture, and repeat DST if needed (remaining culture positive after intensive phase)
- Patient must be instructed about proper collection
- Collect in sterile, leak-proof, disposable, non-breakable, appropriately labeled, lab-approved containers
- Ensure rapid transport to laboratory and proper storage conditions (2-8°C)

Suboptimal and Unacceptable Specimens

- Processing of poor or suboptimal quality specimens is a burden on both financial and personnel resources
- Results generated from processing inappropriate specimens may not be reliable
- Each laboratory must develop its own specimen rejection criteria and make these criteria readily accessible to providers
- Clinicians should be notified when a specimen is rejected and the reason for rejection should be provided
- Specimens collected by invasive procedures should not be rejected

https://learn.aphl.org/learn/course/59/play/155:16/tb3-specimen-collection-handling-transport-and-processing

Possible Rejection Criteria (1)

- Unlabled or mislabeled specimens or specimen labeling does not match identifiers on requisition form
- Insufficient volume
- Dried swabs in general are not optimal
  - Provide limited material
  - Hydrophobicity of mycobacterial cell envelope inhibits transfer to media
- Pooled sputum or urine
- Sputums left at room temperature for 24 hours

Possible Rejection Criteria (2)

- Broken or leaking specimen containers
- Excessive delay between specimen collection and receipt in the laboratory
- Blood specimens collected in EDTA, ACD, or red-top might be rejected for culture as these inhibit growth of MTBC
- Fixed tissue
  - Unable to culture
  - DNA could be extracted for nucleic acid amplification testing and potentially molecular drug resistance testing
- Gastric lavage fluid if pH not adjusted within one hour of collection

https://learn.aphl.org/learn/course/59/play/155:16/tb3-specimen-collection-handling-transport-and-processing

Specimen Types

Respiratory

- Sputum (expectorated, induced)
- Bronchoalveolar lavage (BAL)
- Bronchial wash/brush
- Transtracheal aspirate
- Endotracheal aspirate

Non-Respiratory

- Abscess
- Blood
- Body fluids
- Bone marrow aspirate
- Cerebral spinal fluid
- Gastric lavage or wash
- Stool
- Tissue or lymph node
- Urine

Processing Pulmonary Specimens—Digestion/Decontamination

- Facilitates optimal recovery of mycobacteria in respiratory specimens and other specimens from non-sterile sites
- Specimens are complex organic matrix contaminated with a variety of organisms that can rapidly outgrow mycobacteria in/on media
- 30% or more mycobacteria killed during processing

Digestion:

Mucolytic agent used to liquefy sputum specimens to release acid-fast bacilli (AFB) and expose normal flora to decontamination

Decontamination:

Toxic agent used to kill rapidly growing normal flora that would otherwise overgrow slow-growing mycobacteria

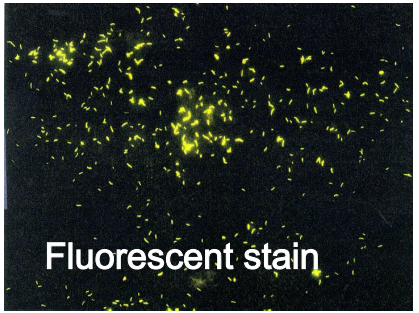
Centrifugation:

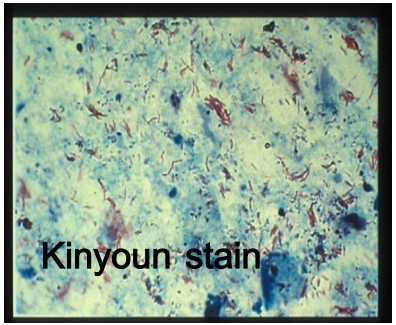
Used to sediment bacteria following digestion/ decontamination

TB Testing Workflow—AFB Smear

Acid-Fast Bacilli (AFB) Smear Microscopy

- Rapid and inexpensive method performed to detect **AFB**
- Specially stained specimen **smears** are examined under a microscope to determine if acid-fast organisms such as **MTBC** and nontuberculous mycobacteria, or **NTM**, are observed
- Low sensitivity and not specific for MTBC
- Reliability depends on the number of AFB present in the specimen





https://www.aphl.org/programs/infectious_disease/tuberculosis/Understanding_TB_Lab_Test_Nurses/story.html

AFB Smear Result Reporting Language

AFB Smear Microscopy Result Reporting
No AFB Seen *or* Negative
1-2 bacilli seen; Order repeat specimen
1+ *or* Rare
2+ *or* Few
3+ *or* Numerous
4+ *or* Many

https://www.aphl.org/programs/infectious_disease/tuberculosis/Understanding_TB_Lab_Test_Nurses/story.html

Microscopy vs. Culture

- Sensitivity
  - 5,000 to 10,000 AFB/mL for smear
  - 10 to 100 AFB/mL for culture
- In United States, about half of TB cases are originally smear-positive*
- Significance of culture
  - Confirm TB/mycobacteriosis, obtain isolate for DST, genotyping, evaluate response to therapy
  - Only 78% cases of pulmonary TB are culture-positive (others are culture-negative TB; clinical or provider diagnosis)

*Tuberculosis in the United States, surveillance reports, 1993-2012. Table 9

TB Testing Workflow—Nucleic Acid Amplification Testing

Direct Detection of MTBC in Clinical Specimens; Nucleic Acid Amplification (NAA) Tests

- Objective: rapidly detect MTBC directly in clinical specimens
  - Turnaround time 24-48 hr after specimen receipt
  - Positive result demonstrates the presence of MTBC
- Does not distinguish live and dead bacilli
  - Negative result does not necessarily mean the absence of MTBC
    - Inhibition of amplification
    - Target below the limit of detection (more likely to detect when bacillary load is higher)
- Not useful for monitoring treatment response
- Clinical judgment still needed to guide decision to treat or isolation practices
  - Sensitivity >95% for AFB smear-positive TB patients; 55–75% of AFB smear-negative, culture-positive TB patients
  - Performance improves with increased clinical suspicion of TB

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
  - Isolate needed for DST and genotyping
- 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

NAA Tests for Direct Detection of MTBC

- Only one FDA-approved/authorized test for use with respiratory specimens
  - Cepheid Xpert® MTB/RIF
    - Should only be requested if patient has been on treatment for <3 days
- Non-FDA approved tests (Research Use Only [RUO])
  - Bruker Lifescience Genotype® MTBDRplus and MTBDRsl
- Laboratory developed tests or LDT (e.g., real-time PCR or DNA sequencing assays)

Which Patients, Specimens Should be Tested by NAA?

- CDC Guidelines: "NAAT should be performed on at least one specimen from each patient with signs and symptoms…"
  - Lab doesn't always know this information
  - Most common algorithm is routinely on new smear-positive and smear-negative on request
- JCM Publication on maximizing yield of NAA tests in TB diagnosis
  - "…application of nonclinical criteria for TB NAA testing substantially increased number of TB cases identified without the prohibitive expense of universal testing. "

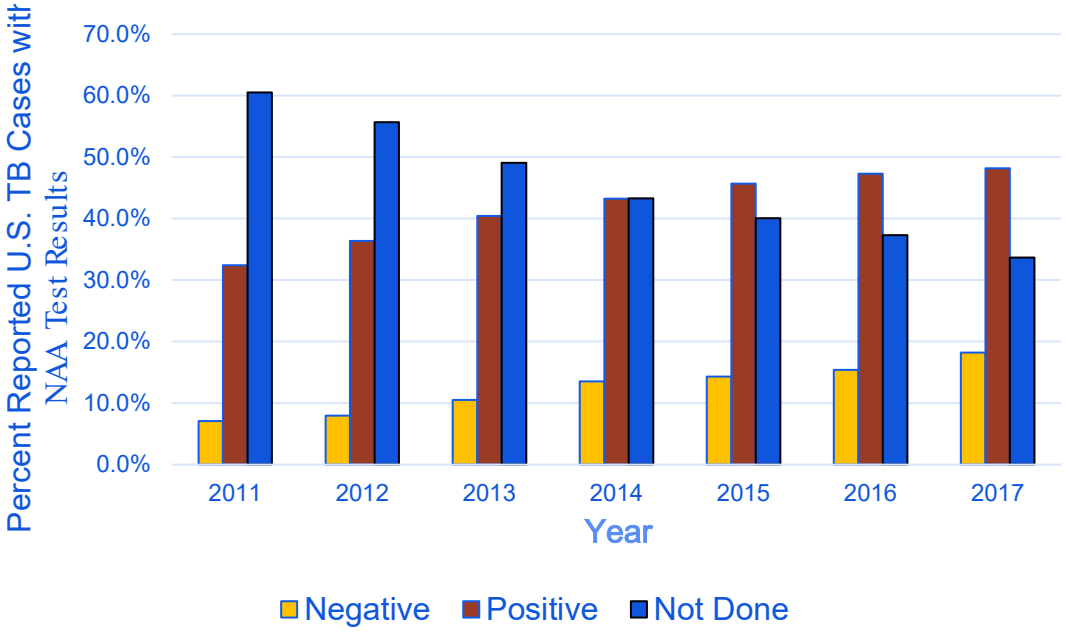
Non-clinical selection criteria for Maximizing Yield of Nucleic Acid Amplification Tests in Tuberculosis Diagnosis

Linda L. Han, Paul Elvin and John Bernardo Published Ahead of Print 23 May 2012.

10.1128/JCM.00871-12.

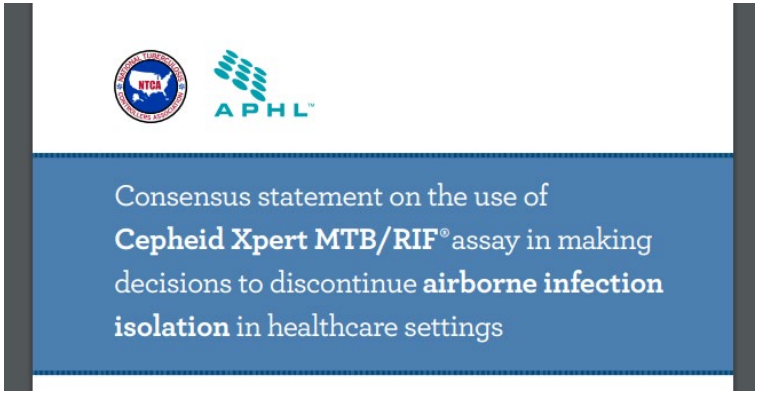
Nucleic Acid Amplification (NAA) Tests

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



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



http://www.tbcontrollers.org/docs/resources/NTCA_APHL_GeneXpert_Consensus_Statement_Final.pdf

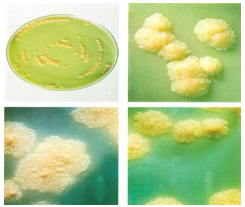
TB Testing Workflow—Culture

Culture Methods

- Solid Media
- Middlebrook agar
- Lowenstein-Jensen
- Advantage –colonies visible on media surface
- Incubate 6 to 8 weeks

- Liquid (broth) Media systems
- MGIT
- TREK
- MB/BacT ALert
- Faster recovery than solid
- Incubate 6 weeks

Current recommendations are to use at least one piece of solid media with broth (mixed culture detection; increased sensitivity)



Considerations for Culture

- MTBC is slow-growing
  - Most diagnostic specimens, if positive, will grow within 4–5 weeks
  - Follow-up specimens (if positive) may take a little longer if patient is on effective treatment

- Specimens with higher bacillary load will have cultures turn positive faster than those with lower burden

- If a culture is positive for AFB growth, identification, or ID, method performed

Common methods of ID from culture growth:

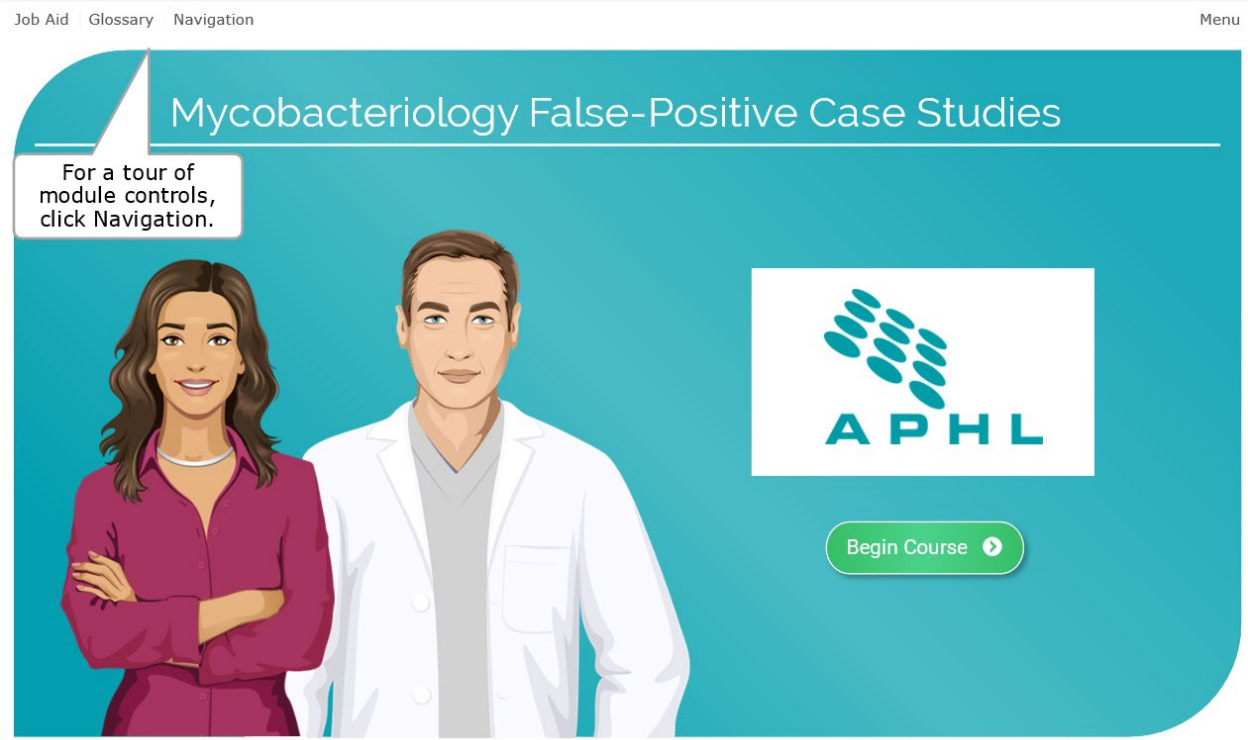
- Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF)
- DNA Sequencing
- Real-time Polymerase Chain Reaction (PCR)
- Line Probe Assay (LPA)
- Cepheid Xpert® MTB/RIF

Additional methods:

- High Performance Liquid Chromatography (HPLC)
- Hologic AccuProbe®
  (Discontinued in late 2022)

https://www.aphl.org/programs/infectious_disease/tuberculosis/Understanding_TB_Lab_Test_Nurses/story.html

Interactive Web Module for Learning about False-Positives



https://www.aphl.org/programs/infectious_disease/tuberculosis/TBFalse_Positive_Case_Studies/story_html5.html

TB Testing Workflow—Drug Susceptibility Testing

Growth-based DST

- Some **DST** methods test at a **critical concentration**, which is the lowest concentration of an anti-tuberculosis drug that will inhibit growth of most strains of MTBC.

For these methods, typically a categorical result of **"resistant"** or **"susceptible"** is provided.

- Other DST methods use a series of different drug concentrations that result in determination of the **minimum inhibitory concentration (MIC)**, which is the lowest concentration of an antimicrobial drug that prevents growth of the microorganism.

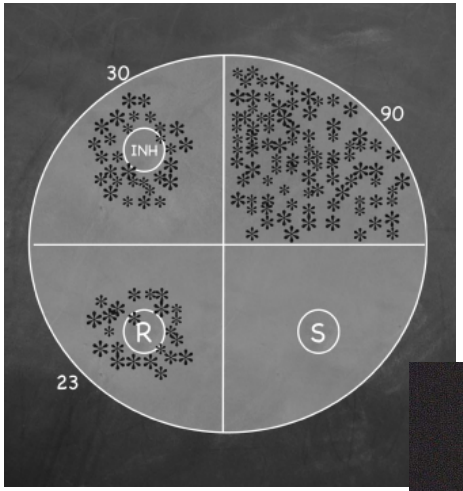
For these methods, a categorical result of "resistant" or "susceptible" may not be provided for all drugs tested, but instead the **MIC value is reported.**

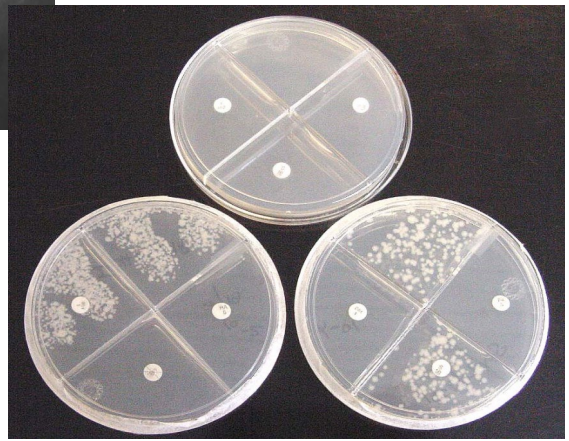
https://www.aphl.org/programs/infectious_disease/tuberculosis/Understanding_TB_Lab_Test_Nurses/story.html

Growth-based Drug Susceptibility Testing (DST) of MTBC

- Current recommendations Clinical and Laboratory Standards Institute and WHO
- Initial isolate should be tested against primary or first-line drugs (FLD)
  - INH, RMP, PZA, EMB
  - FLD panel may change as newer regimens are used (test for fluoroquinolones?)
- For RMP-resistant isolates, or resistance to any 2 FLD, test second-line drugs (SLD)
  - Fluoroquinolones (FQs): levofloxacin or moxifloxacin
  - Second-line injectables (AMK/CAP)—less frequently used for treatment and changing definition of XDR TB
  - Newer and repurposed drugs: bedaquiline, clofazimine, linezolid, and pretomanid

Agar Proportion





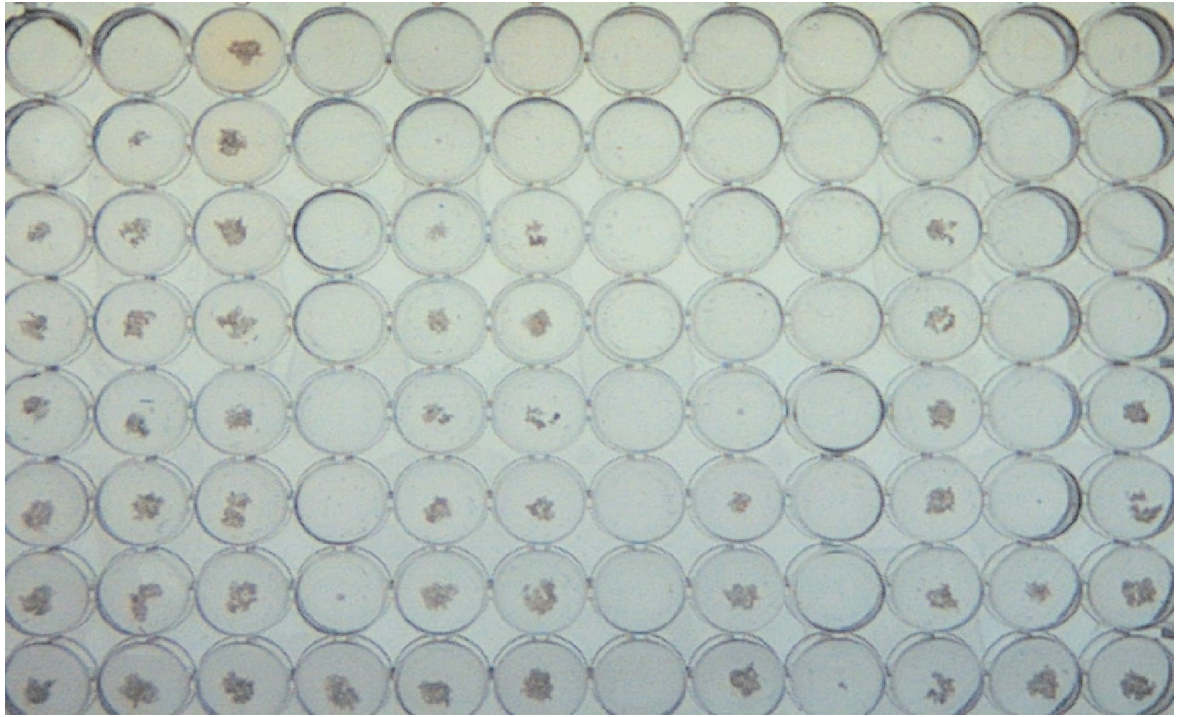
https://www.currytbcenter.ucsf.edu/sites/default/files/2022-05/tb_sg3_chap3_laboratory.pdf

BD MGIT

https://legacy.bd.com/ds/technicalCenter/promotionalFlyers/ss-MGIT_PZA.pdf

Growth in MYCOTB Microtiter Plate

Increasing concentration

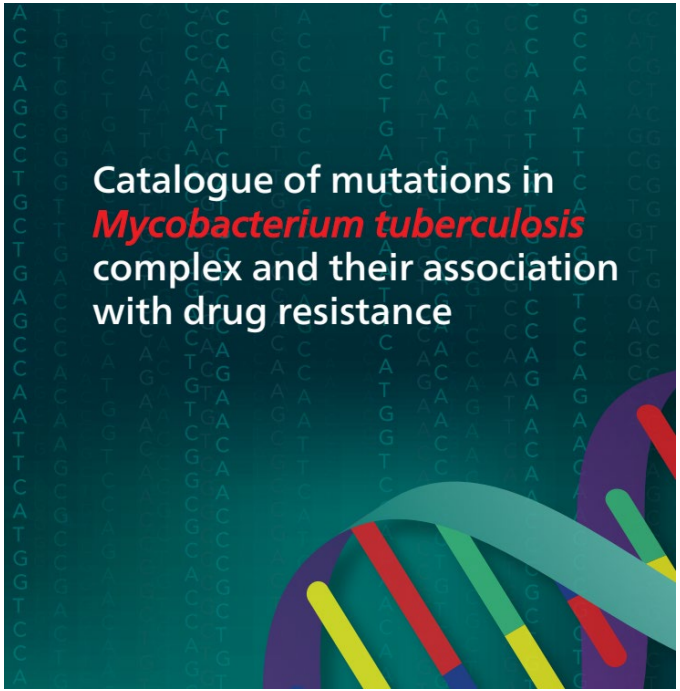


OFL MXF RIF AMI STR RFB PAS ETH CYC INH KAN EMB

TB Testing Workflow— Molecular Detection of Drug Resistance

Molecular Detection of Drug Resistance (Molecular DST)

- Examining DNA of specific genes for mutations known to be associated with phenotypic resistance
  - Mutations in what genes are associated with resistance?
  - Where are the mutations within the gene?
  - Some areas are “hot spots”—resistance determining regions (e.g., *rpoB* rifampin resistance determining region or RRDR)
- Newer methods becoming standard methodology
  - Wadsworth (NYS) using WGS as primary method replacing most growth-based DST
- Patients are being treated based on molecular DST results



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

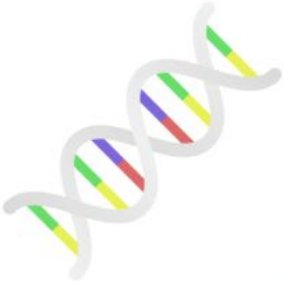
Potential Effect of Mutations

- Could have no effect on drug resistance
- Change in protein structure inhibiting drug activity (e.g., no activation of prodrug like isoniazid)
- Change in protein structure such that drug cannot bind to target
- Change in regulatory region leading to changes in expression level to overcome the effects of drug
- Change in affinity for drug
- Can result in different levels of resistance depending on the particular mutation

Molecular DST Methods

CEPHEID XPERT®
MTB/RIF ASSAY

LINE PROBE ASSAY
(LPA)



DNA SEQUENCING

https://www.aphl.org/programs/infectious_disease/tuberculosis/Understanding_TB_Lab_Test_Nurses/story.html

Genes Associated with Anti-TB Resistance

Drug name	Abbreviation	Region where mutations associated with resistance (Gene name)*
First-line drugs		
Isoniazid	INH	*katG*,*inhA* promoter, *fabG1*-*ahpC*-*oxyR*
Rifampin	RIF	*rpoB*
Pyrazinamide	PZA	*pncA*
Ethambutol	EMB	*embB*
Second-line drugs		
Ethionamide	ETH	*inhA*,*ethA*
Rifabutin	RBT	*rpoB*
Streptomycin	SM	*rrs*, *rpsl*

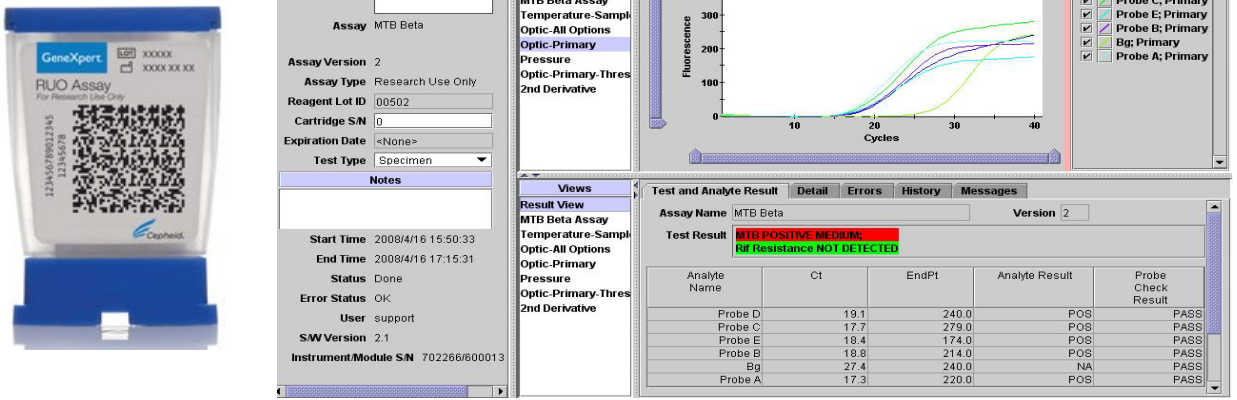
*List includes most common regions examined

Genes Associated with Anti-TB Resistance (2)

Drug name		Abbreviation	Region where mutations associated with resistance (Gene name)
Fluoroquinolones (FQ): Moxifloxacin (MOX), Levofloxacin (LVX)			*gyrA, gyrB*
Second-line injectable drugs	Amikacin	AMK	*rrs*
	Kanamycin	KAN	*rrs, eis*
	Capreomycin	CAP	*rrs,tlyA*
New and repurposed drugs	Bedaquiline	BDQ	*Rv0678,atpE, pepQ*
	Pretomanid/ Delamanid	DLM	*fbiA, fbiB, fbiC,fbiD, ddn, fgd1*
	Linezolid	LZD	*rplC, rrl*
	Clofazimine	CFZ	*Rv0678,pepQ*

Cepheid® Xpert MTB/RIF

- Automated commercial system for identification of MTBC and mutations in *rpoB* for rifampin resistance
- Uses real-time PCR with molecular beacons
- Decontamination, digestion, DNA extraction, amplification, and detection in same cartridge; limited biosafety requirements
- Results in ~2 hours
- Minimal hands on manipulation- technically simple



CDC's Molecular Detection of Drug Resistance (MDDR)

- CDC service implemented in 2009
  - Clinical testing service for *Mycobacterium tuberculosis*
    - Rapid detection of DR TB and confirmation of MDR
    - Provide additional information quickly for second-line drugs
  - Available to all 50 states and U.S. territories
  - Testing service is free and shipping costs covered
  - Consultation available and provided on request
- Transitioned to new targeted next generation sequencing (tNGS) assay (February 2023)

https://www.cdc.gov/tb/topic/laboratory/mddrusersguide.pdf

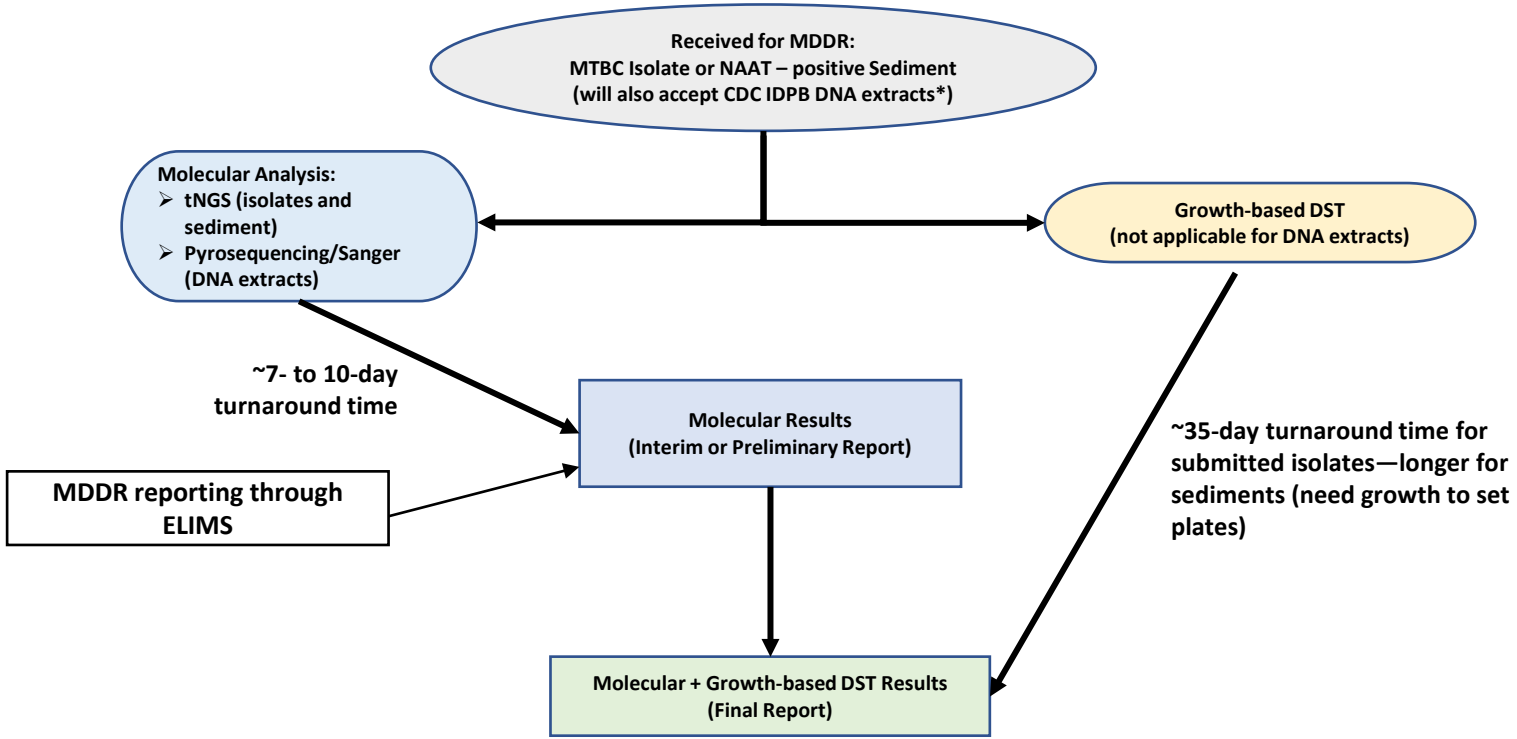
Acceptable Specimen Types

- **Confirmed *Mycobacterium tuberculosis* complex (MTBC) isolates or mixed and non-viable MTBC cultures**
- **MTBC nucleic acid amplification test positive (NAAT+) processed sediments**
- **Fixed-tissue DNA extracts (through the CDC Infectious Diseases Pathology Branch)**
- **Acceptable collection, storage and transport—** https://www.cdc.gov/tb/topic/laboratory/mddrusersguide.pdf

New primary testing panel for tNGS

tNGS Panel	
Drug	**Genetic loci tested**
Rifampin	*rpoB* RRDR, *rpoB*-170, and *rpoB*-491
Isoniazid	*inhA* promoter, *katG* gene, *fabG1*-203
Ethambutol	*embB*
Pyrazinamide	*pncA*
Fluroquinolones	*gyrA* and *gyrB*
Amikacin, Kanamycin, and Capreomycin	*rrs*
Kanamycin	*eis*
Bedaquiline	*atpE*, *rv0678*, and *pepQ*
Clofazimine	*rv0678* and *pepQ*
Linezolid	*rplC* and *rrl*

MDDR Algorithm



*DNA extracts only accepted from CDC IDPB and will be tested by conventional sequencing methods (not tNGS)

MDDR report comparison

Previous format (manually reported)

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

Drug	Locus *	Result	Interpretation
Rifampin	rpoB	**Mutation:** 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	**Mutation:** AGC>ACC, Ser315Thr	
	fabG1	No mutation	
Ethambutol	embB	**Mutation:** 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: • 91% of AMK-R isolates have a mutation in the rrs locus; • 87% of KAN-R isolates have a mutation in either the rrs locus or the eis locus; • 55% of CAP-R isolates have a mutation in either the rrs locus or the tlyA locus.)
	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.

New simplified format (ELIMS)

Centers for Disease Control & Prevention
National Tuberculosis Reference Laboratory

Patient Name: **None Provided**
Sex: Birthdate: Age: Date of Onset:

Public Health / International Submitter IDs
Patient ID: Alt. Patient ID:
Specimen ID: Alt. Specimen ID:

CDC Specimen ID: **3015909757** CDC Unique ID: **ZZYGOA1B** CDC Local Aliquot ID: **23-2902**

Rifampin (RIF)	**Result**	**Interpretation**
RIF interpretation		RIF resistant
rpoB	Thr444Ala, Gln429Asp	
Isoniazid (INH)	**Result**	**Interpretation**
INH interpretation		INH resistant
inhA	G-48A	
fabG1	No mutation	
katG	Ser315Thr	
Ethambutol (EMB)	**Result**	**Interpretation**
EMB interpretation		EMB resistant
embB	Met306Val	
Pyrazinamide (PZA)	**Result**	**Interpretation**
PZA interpretation		Cannot rule out PZA resistance.
pncA	No mutation	
Fluoroquinolones (FQ)	**Result**	**Interpretation**
FQ interpretation		Cannot rule out FQ resistance.
gyrA	No mutation	
gyrB	No mutation	

Changes in report format (3)

- **MDDR report is labeled as “preliminary” instead of “interim” and is now 4 pages long**
  - Page 1: Patient, submitter, and sample information
  - Page 2: MDDR results (first-line drugs + fluoroquinolones)
  - Page 3: MDDR results (injectables, bedaquiline, clofazimine, and linezolid)
  - Page 4: Final page with report comments and contact information of the approver
    - Indication that conventional drug susceptibility testing in progress with the exception of bedaquiline, clofazimine, and linezolid
    - Links for state TB programs and TB Centers of Excellence
    - Disclaimer that MDDR assay not FDA approved (i.e., laboratory developed test)

Changes in report format (4)

- **MDDR report is labeled as “preliminary”**
- **Interpretations simplified**
  - Updated based on combination of historic MDDR data and WHO Mutation Catalogue
- **Conventional drug susceptibility test results will still be issued via LIMS**
  - Indicated as “final” report
  - Will not yet include bedaquiline, clofazimine, or linezolid
    - Working to implement as part of testing menu (MIC testing)
  - Will include comments if discrepancies between molecular and conventional results, as applicable

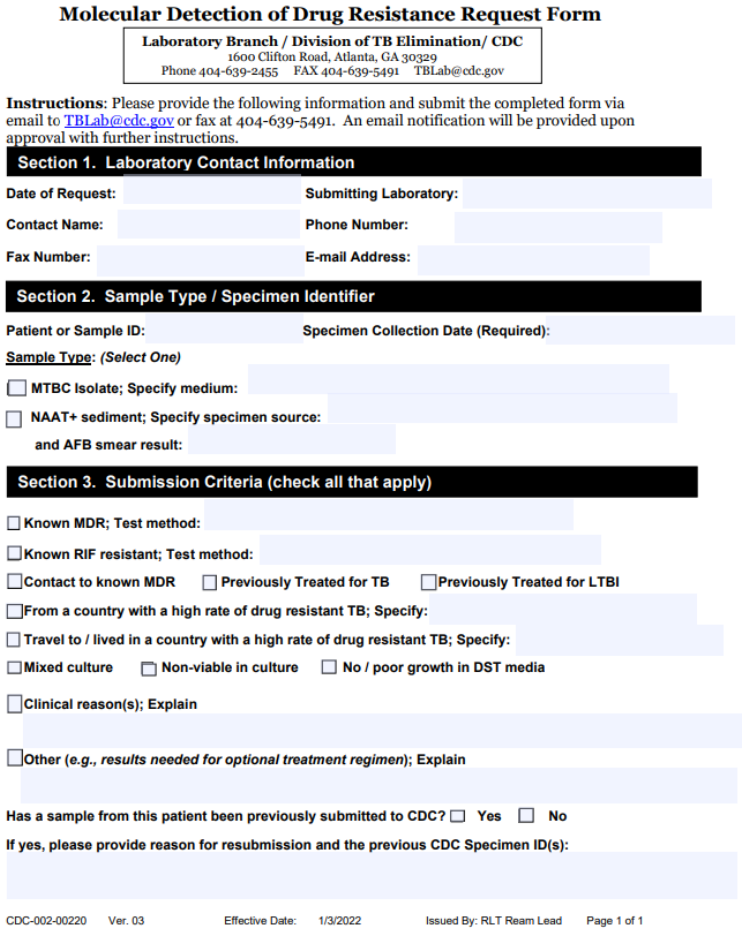
Sample Submission and Results Reporting

- Submissions come from PHLs and reports go back to primarily state PHLs
- Turnaround time (TAT) for receipt of results impacted by timeframes along this path
- Once received at CDC, anticipated initial TAT for tNGS is 7–10 days (results release to PHL)

How do I submit a sample for MDDR

- Submissions should come through public health laboratory and meet specimen acceptance criteria

- Complete the MDDR request form and submit to TBLab@cdc.gov or through CSTOR portal
  - https://www.cdc.gov/tb/topic/laboratory/mddrsubmissionform.pdf



Molecular Detection of Drug Resistance Request Form

Laboratory Branch / Division of TB Elimination/ CDC
1600 Clifton Road, Atlanta, GA 30329
Phone 404-639-2455 FAX 404-639-5491 TBLab@cdc.gov

Instructions: Please provide the following information and submit the completed form via email to TBLab@cdc.gov or fax at 404-639-5491. An email notification will be provided upon approval with further instructions.

Section 1. Laboratory Contact Information

Date of Request: Submitting Laboratory:
Contact Name: Phone Number:
Fax Number: E-mail Address:

Section 2. Sample Type / Specimen Identifier

Patient or Sample ID: Specimen Collection Date (Required):
Sample Type: *(Select One)*
☐ MTBC Isolate; Specify medium:
☐ NAAT+ sediment; Specify specimen source:
and AFB smear result:

Section 3. Submission Criteria (check all that apply)

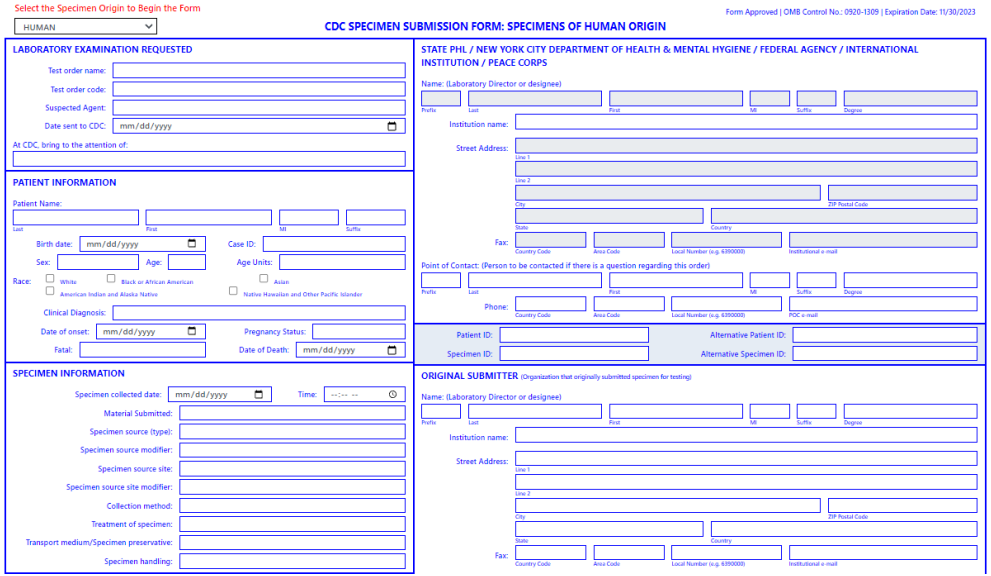
☐ Known MDR; Test method:
☐ Known RIF resistant; Test method:
☐ Contact to known MDR ☐ Previously Treated for TB ☐ Previously Treated for LTBI
☐ From a country with a high rate of drug resistant TB; Specify:
☐ Travel to / lived in a country with a high rate of drug resistant TB; Specify:
☐ Mixed culture ☐ Non-viable in culture ☐ No / poor growth in DST media
☐ Clinical reason(s); Explain
☐ Other (*e.g., results needed for optional treatment regimen*); Explain

Has a sample from this patient been previously submitted to CDC? ☐ Yes ☐ No
If yes, please provide reason for resubmission and the previous CDC Specimen ID(s):

CDC-002-00220 Ver. 03 Effective Date: 1/3/2022 Issued By: RLT Ream Lead Page 1 of 1

How do I submit a sample for MDDR? (2)

- Once request is approved, additional instructions provided via email
- Attach MDDR request form to CDC specimen submission/50.34 form



Select the Specimen Origin to Begin the Form

HUMAN

Form Approved | OMB Control No.: 0920-1309 | Expiration Date: 11/30/2023

CDC SPECIMEN SUBMISSION FORM: SPECIMENS OF HUMAN ORIGIN

LABORATORY EXAMINATION REQUESTED

Test order name:
Test order code:
Suspected Agent:
Date sent to CDC: mm/dd/yyyy
At CDC, bring to the attention of:

PATIENT INFORMATION

Patient Name: Last, First, MI, Suffix
Birth date: mm/dd/yyyy
Case ID:
Sex:
Age:
Age Units:
Race: White, Black or African American, Asian, American Indian and Alaska Native, Native Hawaiian and Other Pacific Islander
Clinical Diagnosis:
Date of onset: mm/dd/yyyy
Pregnancy Status:
Fatal:
Date of Death: mm/dd/yyyy

SPECIMEN INFORMATION

Specimen collected date: mm/dd/yyyy
Time:
Material Submitted:
Specimen source (type):
Specimen source modifier:
Specimen source site:
Specimen source site modifier:
Collection method:
Treatment of specimen:
Transport medium/Specimen preservative:
Specimen handling:

STATE PHL / NEW YORK CITY DEPARTMENT OF HEALTH & MENTAL HYGIENE / FEDERAL AGENCY / INTERNATIONAL INSTITUTION / PEACE CORPS

Name: (Laboratory Director or designee) Prefix, Last, First, MI, Suffix, Degree
Institution name:
Street Address: Line 1, Line 2, City, ZIP Postal Code, State, Country
Fax: Country Code, Area Code, Local Number (e.g. 6390000), Institutional e-mail
Point of Contact: (Person to be contacted if there is a question regarding this order) Prefix, Last, First, MI, Suffix, Degree
Phone: Country Code, Area Code, Local Number (e.g. 6390000), POC e-mail

Patient ID:
Alternative Patient ID:
Specimen ID:
Alternative Specimen ID:

ORIGINAL SUBMITTER (Organization that originally submitted specimen for testing)

Name: (Laboratory Director or designee) Prefix, Last, First, MI, Suffix, Degree
Institution name:
Street Address: Line 1, Line 2, City, ZIP Postal Code, State, Country
Fax: Country Code, Area Code, Local Number (e.g. 6390000), Institutional e-mail

Rapid Testing for Fluoroquinolone (FQ) Resistance at CDC

- **Implemented in response to drug shortages and programmatic implementation of 4-month rifapentine-moxifloxacin regimen**
- **Dear Colleague Letter sent 5/21/23 provides additional details on program and sample submission**
  - Posted on CDC internet page
    - https://www.cdc.gov/tb/publications/letters//2023/Rapid-FQ-Testing.html
- **Routine MDDR testing using full panel of genetic loci with tNGS assay remains available for cases where drug resistance is suspected or known**

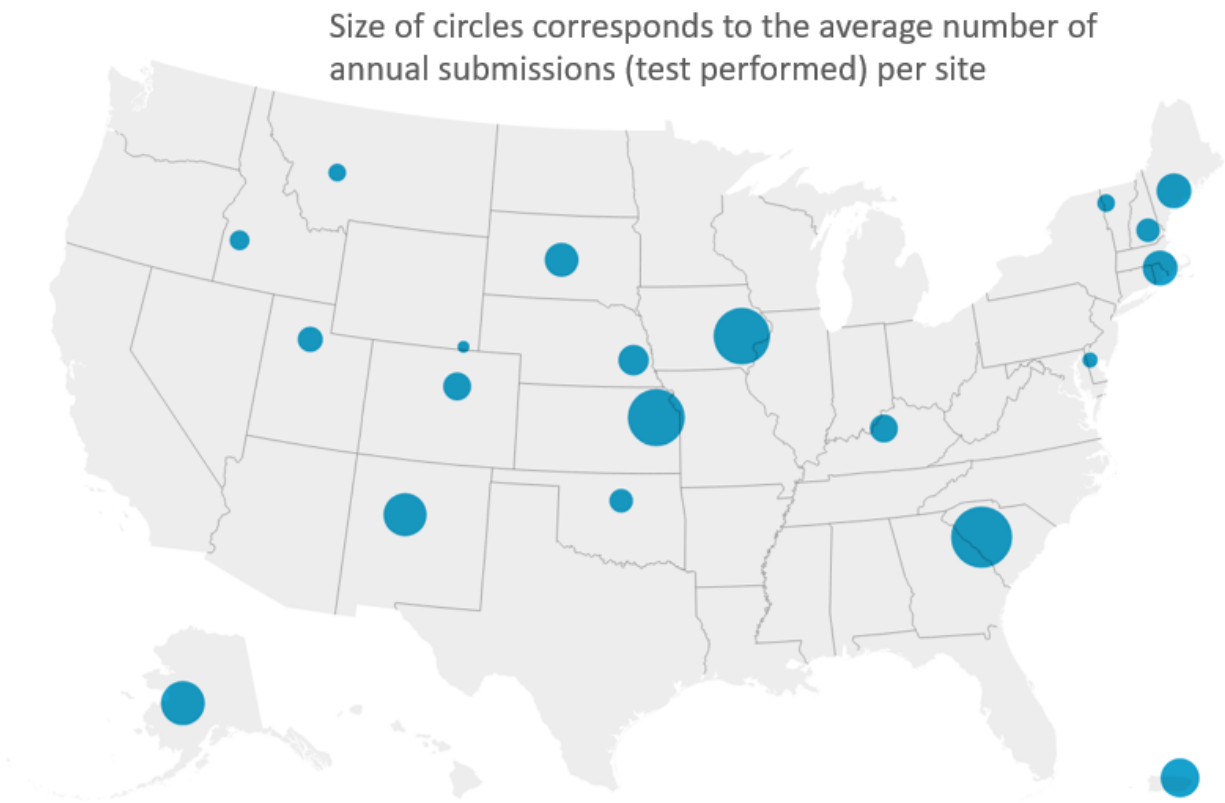
DST Reference Center

- CDC/APHL collaboration to establish center at CA Microbial Diseases Laboratory in March 2015
  - Primarily to support DST for **low volume** PHLs
  - Provides access to first-line and second-line DST as well as rapid molecular detection of drug resistance
  - https://www.aphl.org/programs/infectious_disease/tuberculosis/Pages/TB-DST.aspx

- Services complementary to those performed by DTBE Reference Laboratory and offered free-of-charge
  - Shipping charges responsibility of submitting PHL

- CDC services remain available to all programs

Active submitters to National DST Reference Center 2019-2022



State Submitter	Avg tested per year 2019-2022
South Carolina	51
Kansas	44
Iowa	43
Alaska	25
New Mexico	24
Puerto Rico	19
Maine	15
Rhode Island	15
South Dakota	14
Nebraska	11
Colorado	9
Kentucky	9
Utah	7
New Hampshire	6
Oklahoma	6
Idaho	4
Montana	3
Vermont	3
Delaware	2
Wyoming	1

20 submitters

+ occasional submitters: TX, MO, WA, VA, OH, MA

Source: Varvara Kozyreva, CDPH MDL • Created with Datawrapper

https://datawrapper.dwcdn.net/v4bJ6/1/

Web Module

https://www.aphl.org/programs/infectious_disease/tuberculosis/Understanding_TB_Lab_Test_Nurses/story.html

Angela Starks
eog0@cdc.gov
(404) 639-3205

Atanaska Marinova-Petkova
lxy8@cdc.gov
(404) 718-5254

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