National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention
Division of TB Elimination

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

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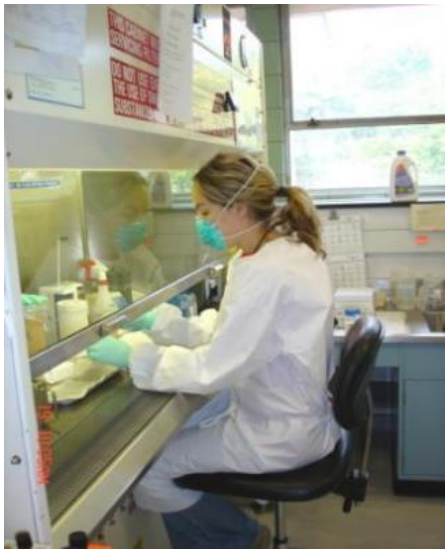
## Laboratory is Essential

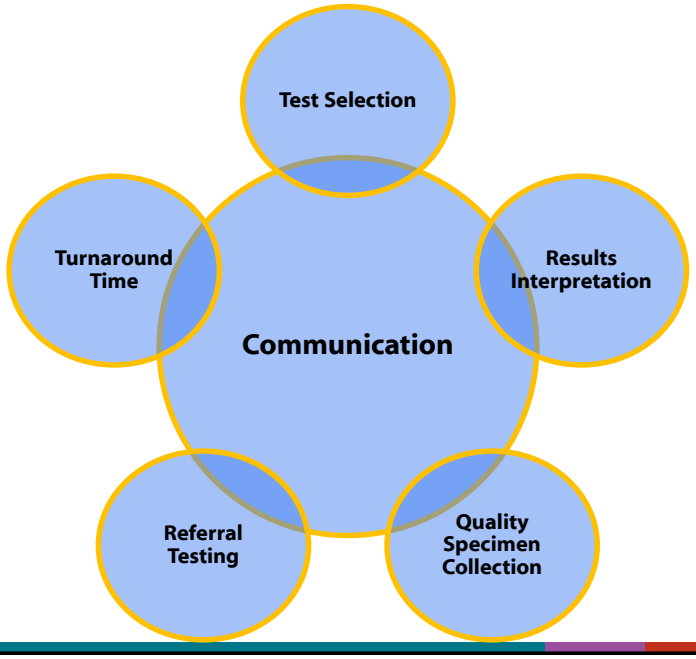
- **Critical partner for clinical care and public health activities**
  - Rapid, reliable results for diagnosis
  - Drug susceptibility testing for appropriate treatment
  - Monitoring response to therapy
  - Genotyping to detect recent transmission



## 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

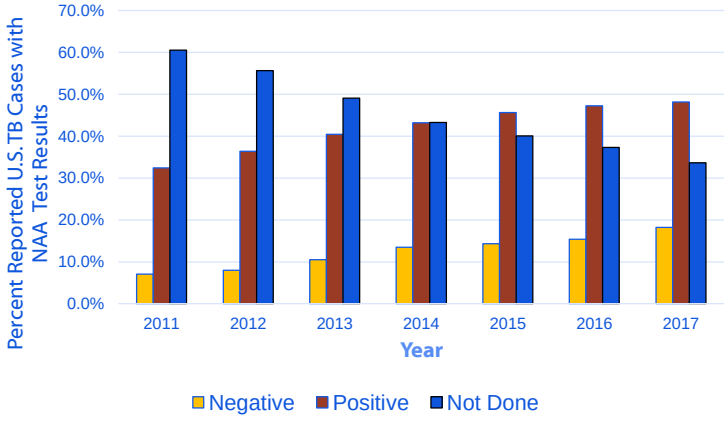
| Result | Recommended Turnaround Time |
|---|---|
| Acid Fast Bacilli Smear | Within 24 hours of specimen receipt |
| Nucleic Acid Amplification Test | Within 48 hours of specimen receipt |
| Culture positive for M. tuberculosis (MTB) | ≤ 21 days of specimen receipt |
| First-line drug susceptibility test results | ≤28 days from specimen receipt (Tenover et. al, 1993)<br><br>≤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

## 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**



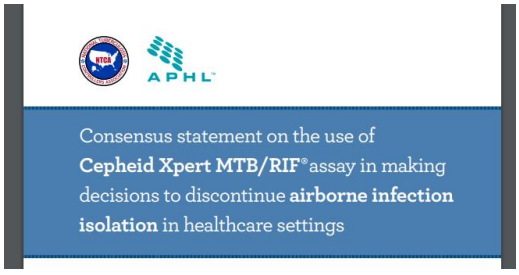

https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm

## 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




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

## 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

https://www.cdc.gov/mmwr/pdf/wk/mm6241.pdf
https://jcm.asm.org/content/jcm/early/2015/02/26/JCM.03433-14.full.pdf

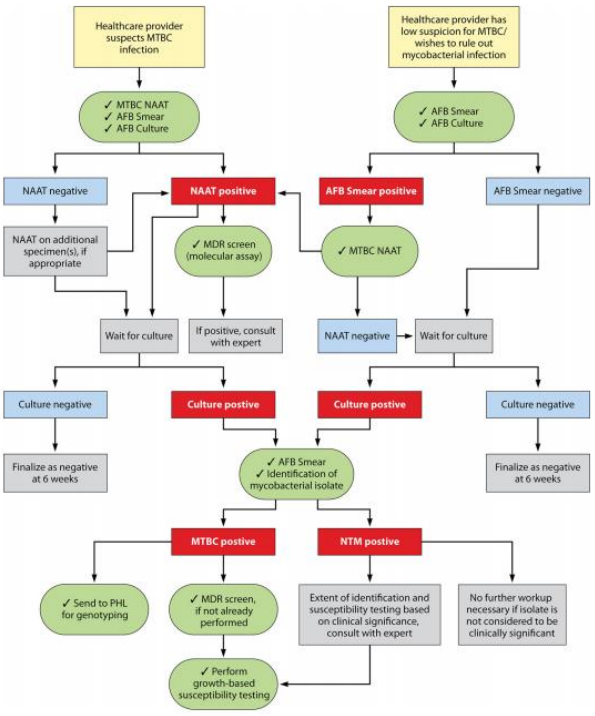

## Lack of standardized reporting language

- **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)




https://cmr.asm.org/content/cmr/31/2/e00038-17.full.pdf

## 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

## Use of Molecular Assays in the TB Laboratory

| Purpose | Specimen Type | Importance | Platforms |
|---|---|---|---|
| Direct detection of MTBC by NAA testing | • AFB smear positive and smear negative clinical specimens | • Patient isolation and initiation of therapy | • Cepheid GeneXpert<br>• Hologic MTD<br>• Line probe assays (LPA)<br>• Lab developed tests (LDTs) |
| Identification of mycobacteria | • AFB positive cultures<br>• Clinical specimens (dependent on assay) | • Rapid, accurate identification of MTB<br>• Initiation of therapy | • LPAs<br>• DNA sequencing (LDT)<br>• MALDI-TOF<br>• Other LDTs |
| Detection of drug resistance mutations | • Clinical specimens<br>• MTB-positive cultures | • Patient therapy decisions<br>• Surveillance | • Cepheid GeneXpert<br>• LPAs<br>• DNA sequencing (LDT)<br>• Other LDTs |

## 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

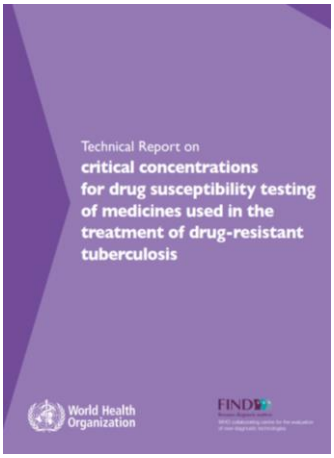
## Whole Genome and Targeted Next Generation Sequencing

- **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
  - Delaminid




https://www.who.int/tb/publications/2018/WHO_technical_report_concentrations_TB_drug_susceptibility/en/

## Clinical and Laboratory Standards Institute M24 (3rd 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

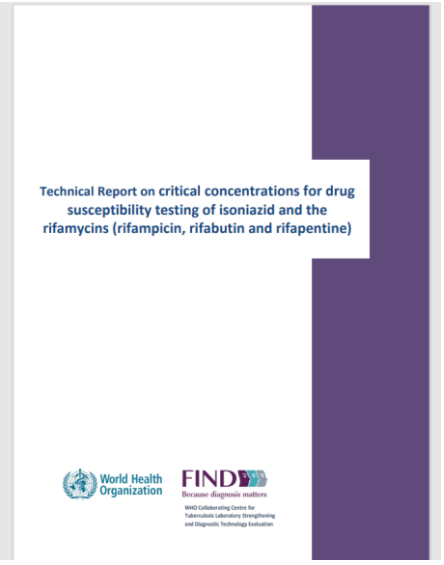
# Revised Critical Concentration For Rifamycin

- 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[a] | 40 | 0.5 | 1.0 | 0.5 |
| Rifabutin[b] | – | – | – | – |
| Rifapentine[c] | – | – | – | – |

Changes indicated in red




9789240017283-eng.pdf (who.int)

# 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 Consoritum 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

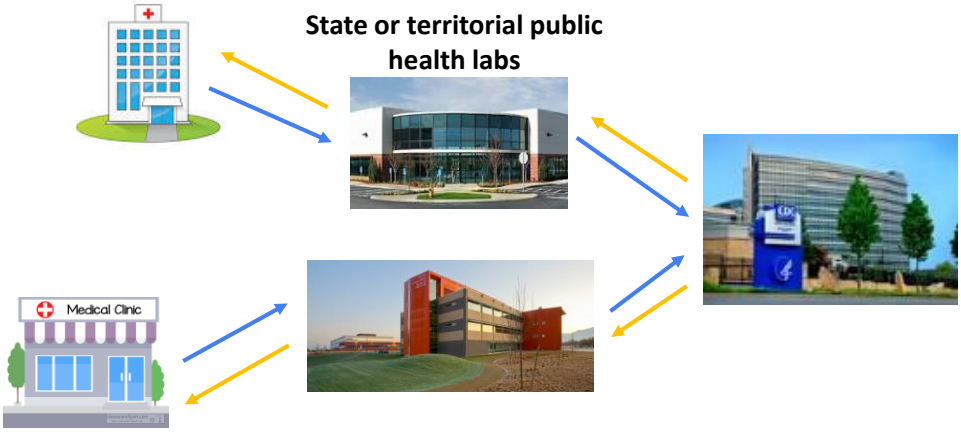
## CDC's Molecular Detection of Drug Resistance (MDDR)

- **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-002-00059e03 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: *M. tuberculosis* 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 | **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.**

Reviewed by: Beverly Metchock

CLIA ID # 11D2030855

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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, rrl*
- Bedaquiline: *atpE*, *rv0678* (*mmpR*), *pepQ*
- *tlyA* dropped

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

**BEFORE**

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

# Acknowledgements


- CDC/DTBE: Tracy Dalton, PhD
  Jamie Posey, PhD
  Calin Chiribau, PhD
- California Microbial Diseases Laboratory: Zenda Berrada, PhD
- Wadsworth Center: Vincent Escuyer, PhD


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