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**

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

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

**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

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
# Use of Molecular Assays in the TB Laboratory

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

## 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


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 Rifamycins

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>IU</th>
<th>7H10</th>
<th>7H11</th>
<th>MGIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Rifampicin*</td>
<td>40</td>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Rifabutin*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rifapentine*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

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)
Transferring 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
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

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- California Microbial Diseases Laboratory: Zenda Berrada, PhD
- Wadsworth Center: Vincent Escuyer, PhD

For more information, contact CDC
1-800-CDC-INFO (232-4636)

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