Drug Susceptibility Testing
Growth-Based Assays
Drug Susceptibility Testing (DST)

Molecular-based Assays
Definitions

- **Discordant**
  - Being at variance; disagreeing; incongruous

- **Discrepant**
  - Differing; disagreeing; inconsistent

- **Inconsistent**
  - Lacking agreement, as one thing with another or two or more things in relation to each other
Examples of Discordance

- **Within One laboratory**
  - Same test
    - Pyrazinamide (PZA) is resistant (R) by MGIT first time and susceptible (S) on repeat
  - Different tests
    - Ethambutol (EMB) is S by MGIT but R on agar proportion (AP)
    - Rifampin (RIF) R by molecular method but S by growth-based DST

- **Between laboratories**
  - Same test
    - PZA is R by MGIT at lab #1 and S at a reference lab
  - Different tests
    - EMB is S by MGIT at lab #1 but R by molecular method at reference laboratory
    - Levofloxacin is R by molecular and growth-based methods at reference lab #1 but S by growth-based method at reference lab #2
Reasons for Discordant Growth-Based DST Results

- "Human error/lab error"
  - Transcription, labeling errors, cross-contamination
- Bacterial population (original isolate vs. subculture)
  - Different “inoculum” – e.g., isolates from different specimens; sampling from same specimen – “culture bias”
  - Heteroresistance – presence of 2 or more mycobacterial populations with different susceptibilities (emergence of resistance)
  - Different growth kinetics
- Different inoculation methods (size, clumps)
- Different method (“equivalent” critical concentrations)
- Different media components
- The “bug” – the minimum inhibitory concentration (MIC) is close to the critical concentration
- The drug – the MIC ≈ critical concentration
Reasons for Discordance Between Molecular and Growth-based DST Results

- “Human error/lab error”
- Transcription, labeling errors, cross-contamination
- Bacterial population (original isolate vs. subculture)
  - Different “inoculum” – e.g., isolates from different specimens; sampling from same specimen
  - Heteroresistance – presence of 2 or more mycobacterial populations with different susceptibilities (emergence of resistance)
- Limited genes and sites may be targeted
- Lack of a mutation ≠ susceptibility
- Not all mutations are associated with phenotypic resistance
  - Silent (synonymous) mutations – no change in protein
  - Neutral polymorphisms
  - Output is “platform dependent”
- “Gold-standard” DST may not be perfect
  - Mutations resulting in elevated MICs but are S at critical concentration
Reasons for Discordance Between Different Molecular Platform Results

- “Human error/lab error”
  - Transcription, labeling errors, cross-contamination
- Bacterial population (original isolate vs. subculture)
  - Different “inoculum” – e.g., isolates from different specimens; sampling from same specimen
  - Heteroresistance – presence of 2 or more mycobacterial populations with different susceptibilities (emergence of resistance)
    - Assays may have different limits of detection
- Not necessarily evaluating the same segment of DNA
  - Assay for a particular single nucleotide polymorphisms (SNP) in one codon versus an assay looking at 30 codons
  - Limited genes and sites targeted – \( \text{katG} \) only versus \( \text{katG}+\text{inhA}+\text{fabG1} \) (\text{mabA})
Inconsistencies for RIF

- Usually resistance levels of RIF-R strains are high and clearly separated from wild-type strains
- “Disputed mutations” in *rpoB*
  - Also referred to as non-canonical mutations, mutations associated with borderline resistance
  - Associate with treatment failure / relapse
  - Examples:
    - Leu511Pro, Leu533Pro, Asp516Tyr, His526Cys, His526Leu, His 526Asn, His526Ser, Ile572Phe
  - Test S, especially in broth-based systems
  - MICs are below critical concentration but higher than wild-type strains
**RIF—CDC Model Performance Evaluation Program (MPEP) Strain H (2008) and Strain T (2010)**

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<tbody>
<tr>
<td></td>
<td>No. RIF-R/ No. Results</td>
<td>%</td>
<td>No. RIF-R/ No. Results</td>
<td>%</td>
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<tr>
<td>LJ Proportion</td>
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<td>41</td>
<td>7/17</td>
<td>37</td>
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<td>MGIT</td>
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<td>18</td>
<td>9/61</td>
<td>15</td>
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<td>0</td>
<td>0/5</td>
<td>0</td>
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<tr>
<td>Total</td>
<td>54/139</td>
<td>38</td>
<td>31/108</td>
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*His526Leu mutation in *rpoB*
Isoniazid (INH)—Are there inconsistencies?

- **katG mutation:** Ser315Thr
  
<table>
<thead>
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<th>MPEP Isolate</th>
<th>Agar Proportion</th>
<th>MGIT</th>
<th>Sensititre</th>
<th>Versatrek</th>
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<td>2016J</td>
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<td>73/73 (100)</td>
<td>4/4 (100)</td>
<td>2/2 (100)</td>
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<tr>
<td>2017B</td>
<td>22/22 (100)</td>
<td>73/73 (100)</td>
<td>4/4 (100)</td>
<td>2/2 (100)</td>
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</table>

- **inhA mutation:** C-15T
  
<table>
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<th>MPEP Isolate</th>
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<th>MGIT</th>
<th>Sensititre</th>
<th>Versatrek</th>
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</thead>
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<tr>
<td>2015E</td>
<td>16/24 (67)</td>
<td>68/72 (94)</td>
<td>3/5 (60)</td>
<td>2/2 (100)</td>
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- **fabG1 (mabA) mutation:** Leu203Leu*
  
<table>
<thead>
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<th>MPEP Isolate</th>
<th>Agar Proportion</th>
<th>MGIT</th>
<th>Sensititre</th>
<th>Versatrek</th>
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<td>35/70 (50)</td>
<td>1/5 (20)</td>
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<td>2017D</td>
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<td>12/72 (17)</td>
<td>1/4 (25)</td>
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* Silent mutation; up-regulates inhA
Molecular Detection of INH Resistance Update

- Whole genome sequencing (WGS) can detect uncommon INH resistance mutations that are otherwise missed by current targeted molecular testing methods:
  - Mutations are outside commonly targeted hot-spots
  - Sensitivity increased to >90%

EMB—Problems with False-susceptibility by Growth-based Tests?

- EMB results reported by MPEP participants for six isolates of EMB-resistant *M. tuberculosis* complex, 2009–2010

<table>
<thead>
<tr>
<th>Isolate</th>
<th>embB mutation</th>
<th>Agar Proportion</th>
<th>BACTEC 460</th>
<th>MGIT</th>
<th>VersaTrek</th>
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</thead>
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^a same strain
Inconsistencies for EMB

- Factors in inconsistencies:
  - Critical concentration may not be optimal
  - EMB resistance is near the critical concentration (MICs of S strains not clearly separated from MICs of R strains)
- Resistance mechanisms not completely understood
- EMB mono-resistance is almost unknown
### PZA—Known Issues with False-resistance

#### November 2010 MPEP

<table>
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<tr>
<th>Isolate</th>
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<td>1/64 (2)</td>
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<td>2010B</td>
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<td>7/62 (11)</td>
<td>0/3 (0)</td>
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<tr>
<td>2010C&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>20/62 (32)</td>
<td>3/3 (100)</td>
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<tr>
<td>2010D&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/17 (0)</td>
<td>21/63 (33)</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>2010E&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/17 (0)</td>
<td>0/64 (0)</td>
<td>(0)</td>
</tr>
</tbody>
</table>

<sup>a</sup> same strain; <sup>b</sup> same strain

70-90% of PZA-R isolates have a *pncA* mutation; however, not all *pncA* mutations = PZA-R
What is more desirable?

- An assay with the problem of false-R, or an assay with the problem of false-S?

- Will MIC testing “solve” some of the issues with discordance?

- Will WGS provide the solution?
“Rules” of Molecular Detection of Drug Resistance

- Provide a rational basis to support immediate therapeutic choices
- We don’t always test for, or more importantly, even necessarily know all the mechanisms of resistance to look for.
- Association of clinical outcomes is difficult due to multidrug therapy used to treat TB

- End-users should not be lulled into a false sense of complacency that the “new” technologies are infallible. As with any laboratory test, clinical assessment of patient response, (i.e., the end result) is mandatory.
Acknowledgements

- DTBE Reference Laboratory Team
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- Dr. James Posey

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

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