Florida Department of Health

Phenotypic Antimicrobial Susceptibility Testing for Mycobacterium Tuberculosis
GLOSSARY

- MIC—Minimum Inhibitory Concentration
- AST—Antimicrobial Susceptibility Testing
- MTB—*Mycobacterium tuberculosis*
- MDR—Multidrug resistant
- XDR—Extensively drug resistant
OVERVIEW

- Practical Aspects of Implementing a Microdilution AST System
- Florida’s Experience with Microdilution AST
- Developing a Customized Sensititre® panel to Meet Clinicians’ Needs
Will this be your laboratory’s only antimicrobial susceptibility test?

- Provides more information to treating clinicians
- Turn-around time 14–21 days after setup from pure MTB growth on solid media
- Robust molecular-based prediction of antimicrobial resistance recommended
Which antimicrobial drugs will the laboratory be testing?

- Which drug regimens are the clinicians in your state considering?
- What will be the range of concentrations tested?
- How will the results be reported and interpreted?
Equipment—Florida’s implementation of the Sensititre® system:

- Inoculum
- Inoculation method
- Incubation
- Reading Method

Sensititre Equipment, Courtesy of Patrick Valois
Common issues to consider when interpreting Sensititre® plates:

- **Trailing**—Precipitate or media carry over that is indicated by the presence of pellets in several wells with the same size and density. Most often seen with Linezolid and Para-aminosalicylic acid.

- **Skips**—A well without growth bordered by wells showing growth. One skip may be ignored.

- **Air bubbles**—Form in broth media and may appear as growth.
Troubleshooting common issues:

- An inverted microscope can be used to differentiate growth from trailing or air bubbles.

- When two or more skipped wells are present, the culture used for setup is checked for contamination. If the culture is not found to be contaminated, repeat testing is performed.
Whenever phenotypic results do not match genotypic prediction, results are confirmed by a second technologist.

Common causes:

- Low level rifampin-resistance mutations
- Mutations outside commonly-tested molecular targets
- Heteroresistance not detected by molecular methods
- Fluoroquinolone monoresistance
DEVELOPING A CUSTOMIZED SENSITITRE® PANEL TO MEET CLINICIANS’ NEEDS

Design—Meeting with Medical Director, TB Physician’s Consultation Network

- Antimicrobial drugs requested
  - Meropenem
  - Clofazimine
  - Bedaquiline
  - Rifapentine
  - Pretomanid or Delamanid

- Antimicrobial drugs removed from current panel
  - Rifabutin
  - Amikacin
  - Streptomycin
  - Capreomycin
### Table Key
- **CPS**–Capreomycin
- **MXF**–Moxifloxacin
- **RIF**–Rifampin
- **AMI**–Amikacin
- **STR**–Streptomycin
- **RFB**–Rifabutin
- **PAS**–Para-aminosalicylic acid
- **LZD**–Linezolid
- **CYC**–Cycloserine
- **INH**–Isoniazid
- **LEVO**–Levofloxacin
- **EMB**–Ethambutol
- **CFZ**–Clofazimine
- **BDQ**–Bedaquiline
- **MR/C**–Meropenem and Clavulanic acid

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**Table 1 Current Sensititre® Custom Panel**

**Table 2 New Sensititre® Custom Panel**
Design—Antimicrobial drug specifications

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<th>Antimicrobial drug</th>
<th>Range (µg/ml)</th>
<th>Interpretive criteria</th>
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<tr>
<td></td>
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<td>Susceptible</td>
<td>Resistant</td>
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<td>Bedaquiline</td>
<td>0.015–2.0</td>
<td>≤0.25</td>
<td>≥0.5</td>
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<tr>
<td>Clofazimine</td>
<td>0.015–2.0</td>
<td>≤0.25</td>
<td>≥0.5</td>
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<td>Rifapentine</td>
<td>0.06–8.0</td>
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<td>Meropenem with Clavulanic acid</td>
<td>0.5/2.5–64/2.5</td>
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Table 3. Antimicrobial drug concentrations and interpretation
Collaboration—New York State Department of Health, Mycobacteriology Laboratory, Wadsworth Center

Testing 50 well-characterized isolates:

- 25 pan-susceptible strains, 15 MDR (including 5 low-level rifampin-resistant strains), 1 linezolid-resistant strain, 2 bedaquiline-resistant strains, 2 clofazimine-resistant strains and 5 XDR (or pre-XDR) strains

- Comparison of phenotypic results with WGS genotypic results

- Interlaboratory reproducibility

- Frozen panel versus lyophilized panel for antimicrobial drugs common to both
Goal:

Validate the use of a new customized panel which incorporates the antimicrobial drugs that may be used in new and future drug regimens for the treatment of *Mycobacterium tuberculosis*. 
Updating our antimicrobial susceptibility testing to meet the new drug regimens that are being utilized by clinicians to treat *Mycobacterium tuberculosis* is essential. As *Mycobacterium tuberculosis* resistance continues to evolve, so too must our methods detect and combat it.
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REFERENCES


Photos Courtesy of Patrick Valois and The Jacksonville Mycobacteriology Laboratory, Florida Department of Health