Florida Department of Health

Phenotypic Antimicrobial Susceptibility Testing for *Mycobacterium tuberculosis*

Presentation by

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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

Practical Aspects of Implementing a Microdilution AST System

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

Practical Aspects of Implementing a Microdilution AST System (continued)

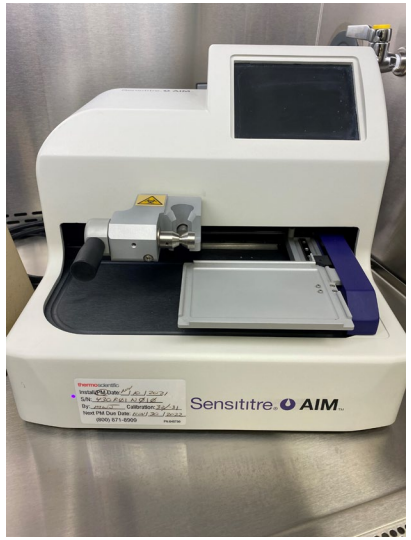
Which antimicrobial drugs will the laboratory be testing?

- 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?

Practical Aspects of Implementing a Microdilution AST System (continued)

Equipment—Florida's implementation of the Sensititre® system:

- Inoculum
- Inoculation method
- Incubation
- Reading Method



Sensititre Equipment, Courtesy of Patrick Valois

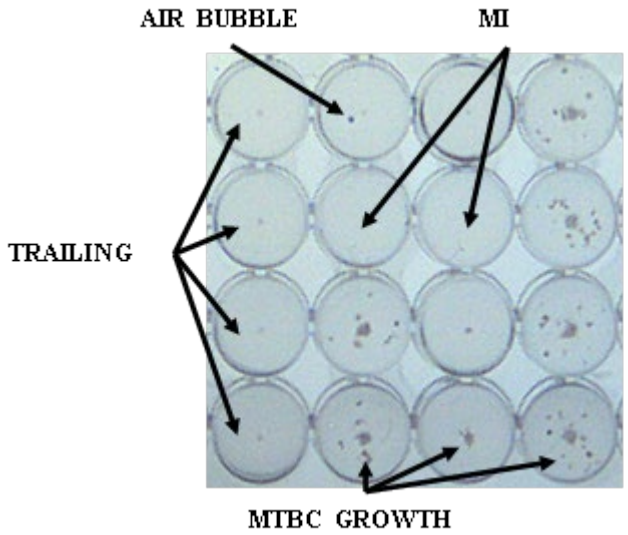
FLORIDA'S EXPERIENCE WITH MICRODILUTION AST

Common issues to consider when interpreting Sensititre® plates:

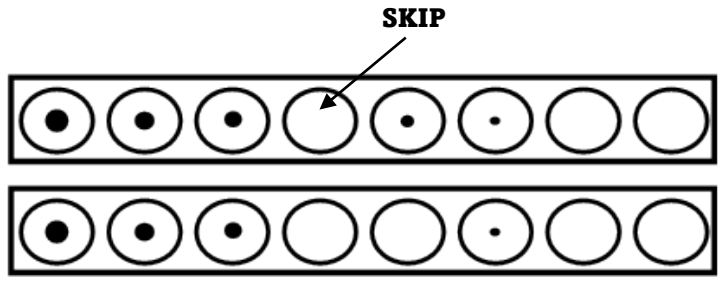
- Trailing—Precipitate or media carry over that is indicated by the presence of pellets in several wells with the same size and density.
  Most often seen with Linezolid and Para-aminosalicylic acid.

- 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.



Sensititre plate, Courtesy of Mycobacteriology Laboratory



Skip Example, Courtesy of Mycobacteriology Laboratory

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Florida's Experience with Microdilution AST (continued)

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.

Florida's Experience with Microdilution AST (continued)

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

Developing a Customized Sensititre Panel to Meet Clinicians' Needs (continued)

1	2	3	4	5	6	7	8	9	10	11	12
CPS 20	MXF 8	RIF 16	AMI 16	STR 32	RFB 16	PAS 64	LZD 8	CYC 256	INH 4	LEVO 8	EMB 32
CPS 10	MXF 4	RIF 8	AMI 8	STR 16	RFB 8	PAS 32	LZD 4	CYC 128	INH 2	LEVO 4	EMB 16
CPS 5	MXF 2	RIF 4	AMI 4	STR 8	RFB 4	PAS 16	LZD 2	CYC 64	INH 1	LEVO 2	EMB 8
CPS 2.5	MXF 1	RIF 2	AMI 2	STR 4	RFB 2	PAS 8	LZD 1	CYC 32	INH 0.5	LEVO 1	EMB 4
CPS 1.2	MXF 0.5	RIF 1	AMI 1	STR 2	RFB 1	PAS 4	LZD 0.5	CYC 16	INH 0.25	LEVO 0.5	EMB 2
CPS 0.6	MXF 0.25	RIF 0.5	AMI 0.5	STR 1	RFB 0.5	PAS 2	LZD 0.25	CYC 8	INH 0.12	LEVO 0.25	EMB 1
CPS 0.3	MXF 0.12	RIF 0.25	AMI 0.25	STR 0.5	RFB 0.25	PAS 1	LZD 0.12	CYC 4	INH 0.06	LEVO 0.12	EMB 0.5
CPS 0.15	MXF 0.06	RIF 0.12	AMI 0.12	STR 0.25	RFB 0.12	PAS 0.5	LZD 0.06	CYC 2	INH 0.03	POS	POS

Table 1 Current Sensititre® Custom Panel

1	2	3	4	5	6	7	8	9	10	11	12
CFZ 2	RFP 8	RIF 16	MXF 8	BDQ 2	MR/C 64/2.5	PAS 64	LZD 8	CYC 256	INH 4	LEVO 8	EMB 32
CFZ 1	RFP 4	RIF 8	MXF 4	BDQ 1	MR/C 32/2.5	PAS 32	LZD 4	CYC 128	INH 2	LEVO 4	EMB 16
CFZ 0.5	RFP 2	RIF 4	MXF 2	BDQ 0.5	MR/C 16/2.5	PAS 16	LZD 2	CYC 64	INH 1	LEVO 2	EMB 8
CFZ 0.25	RFP 1	RIF 2	MXF 1	BDQ 0.25	MR/C 8/2.5	PAS 8	LZD 1	CYC 32	INH 0.5	LEVO 1	EMB 4
CFZ 0.12	RFP 0.5	RIF 1	MXF 0.5	BDQ 0.12	MR/C 4/2.5	PAS 4	LZD 0.5	CYC 16	INH 0.25	LEVO 0.5	EMB 2
CFZ 0.06	RFP 0.25	RIF 0.5	MXF 0.25	BDQ 0.06	MR/C 2/2.5	PAS 2	LZD 0.25	CYC 8	INH 0.12	LEVO 0.25	EMB 1
CFZ 0.03	RFP 0.12	RIF 0.25	MXF 0.12	BDQ 0.03	MR/C 1/2.5	PAS 1	LZD 0.12	CYC 4	INH 0.06	LEVO 0.12	EMB 0.5
CFZ 0.015	RFP 0.06	RIF 0.12	MXF 0.06	BDQ 0.015	MR/C 0.5/2.5	PAS 0.5	LZD 0.06	CYC 2	INH 0.03	POS	POS

Table 2 New Sensititre® Custom Panel

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
RFP–Rifapentine
BDQ–Bedaquiline
MR/C–Meropenem and Clavulanic acid

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Developing a Customized Sensititre Panel to Meet Clinicians' Needs (continued)

Design—Antimicrobial drug specifications

Antimicrobial drug	Range (µg/ml)	Interpretive criteria	
		Susceptible	Resistant
Bedaquiline	0.015–2.0	≤0.25	≥0.5
Clofazimine	0.015–2.0	≤0.25	≥0.5
Rifapentine	0.06–8.0	-	-
Meropenem with Clavulanic acid	0.5/2.5–64/2.5	-	-

Table 3. Antimicrobial drug concentrations and interpretation

Developing a Customized Sensititre Panel to Meet Clinicians' Needs (continued)

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

Developing a Customized Sensititre Panel to Meet Clinicians' Needs (continued)

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.*

Conclusion

Updating our antimicrobial susceptibility testing to meet the new drug regimens that are being utilized by clinicans to treat *Mycobacterium tuberculosis* is essential. As *Mycobacterium tuberculosis* resistance continues to evolve, so too must our methods detect and combat it.

CONTACT

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