

MICs in TB Susceptibility Testing: Challenges and Solutions for Implementation

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MICs in TB Susceptibility Testing: Challenges and Solutions for Implementation



Goals

- *Describe antimicrobial susceptibility testing (AST) for *M. tuberculosis* complex using the TREK Sensititre[®] MIC Microtitre Plate method*
- *List some of the challenges and solutions for implementing TREK Sensititre[®] MIC Microtitre Plate method*
- *Discuss the reporting and interpretation of MIC results*

BPHL-Jacksonville Mycobacteriology Laboratory

Staff: 19 FTEs (17 technical staff)

Yearly Workload: 21,082 (2012 total specimens)

- Testing:*
- AFB Smear Microscopy*
 - Nucleic Acid Amplification Test*
 - Molecular Detection of Drug Resistance*
 - Culture*
 - PCR Restriction Analysis*
 - GenProbe Accuprobe*
 - Conventional Antimicrobial Susceptibility Testing*

BPHL Mycobacteriology Laboratory

Antimicrobial Susceptibility Testing



Antimicrobial Susceptibility Testing (AST)

- Molecular:*
- Hain GenoType[®] MTBDRplus Assay
 - Workload: ~800/year
 - GeneXpert MTB/RIF Assay
 - Workload: ~20/year
 - DNA Sequencing (Sanger)
 - Workload: ~1000/year (*pncA*)

- Conventional:*
- TREK Sensititre[®] MIC Plate Method
 - Workload: ~1000/year

Algorithm: BPHL performs AST on all first-time MTBC-positive patients and those who are positive after 60 days of treatment

BPHL Mycobacteriology Laboratory Antimicrobial Susceptibility Testing



No more BACTEC 460TB!



Alternatives for AST from Culture

- Options:*
- *MGIT 960*
 - *Agar Proportion Method (APM)*
 - *TREK Sensititre[®] Microtitre*
 - *MYCOTB Plate Method*

BPHL: Evaluated the TREK method in 2011-2012 and compared to BACTEC 460TB and decided to move forward with this method. The TREK method was fully implemented January 2013

Antimicrobial Susceptibility Testing

TREK Sensititre[®] MIC Microtiter Plate Method



Method

Company: TREK Diagnostic Systems – Thermo Scientific

Equipment:

- Nephelometer
- Sensititre AIM[™] – Automated Inoculation Delivery System
- Sensititre Vizion System[®] (PC, Software, Plate Reader)

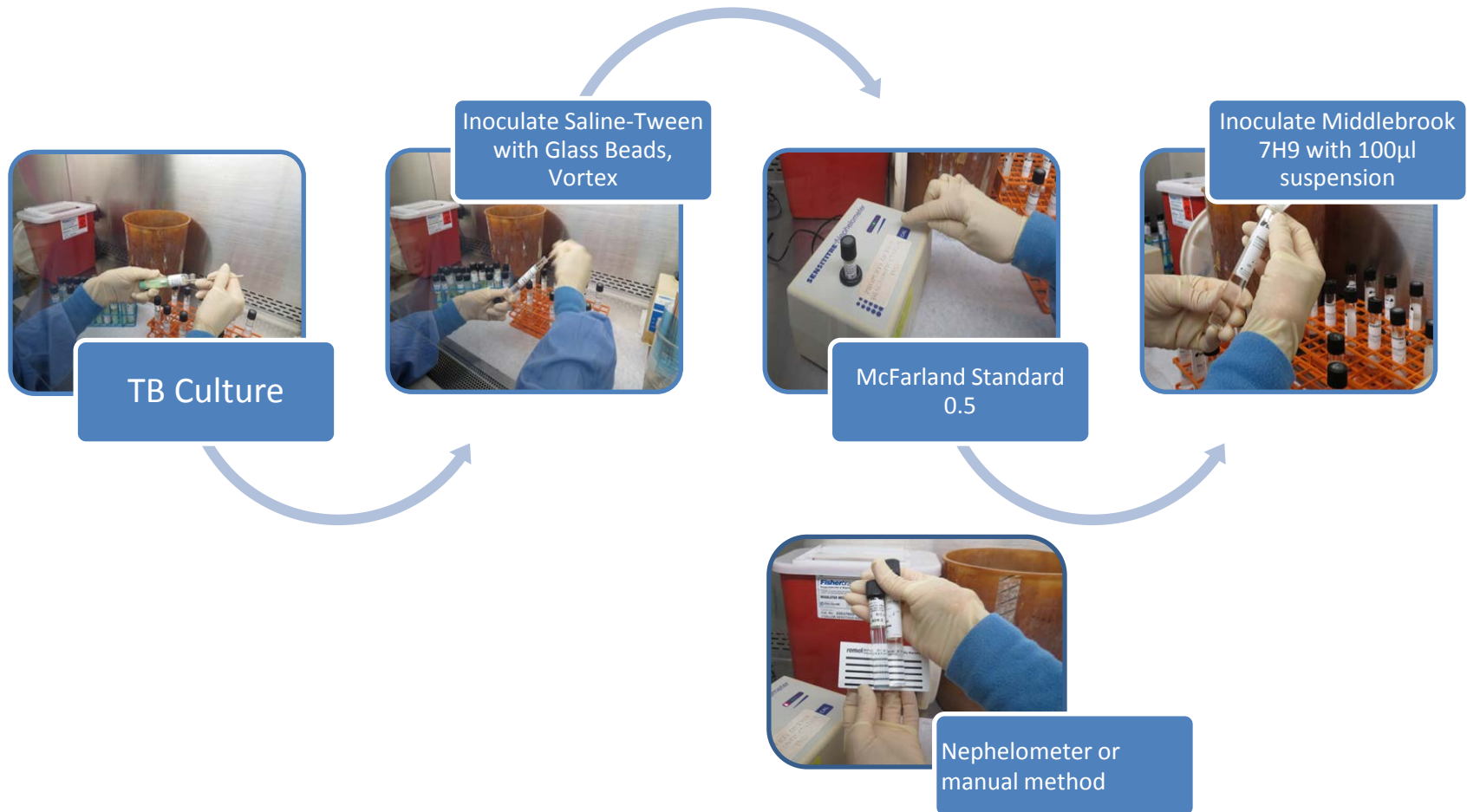
Supplies: MYCOTB 96-well plates, Dosing Heads, Media, Plate Seal, ATCC Control Strain

Workflow: We batch test and set up approx. 2x/week

Antimicrobial Susceptibility Testing

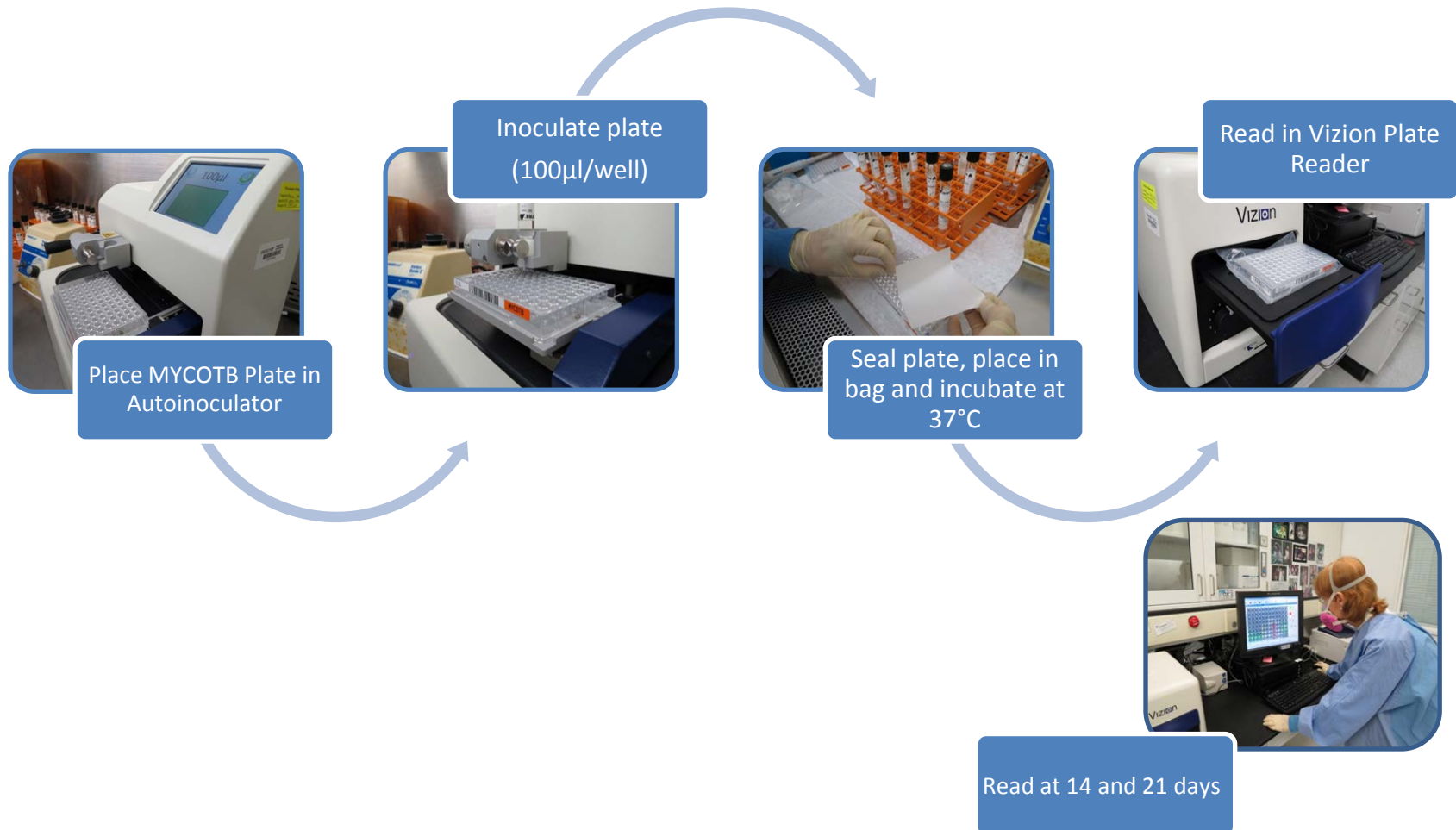
TREK Sensititre[®] MIC Microtiter Plate Method

Workflow



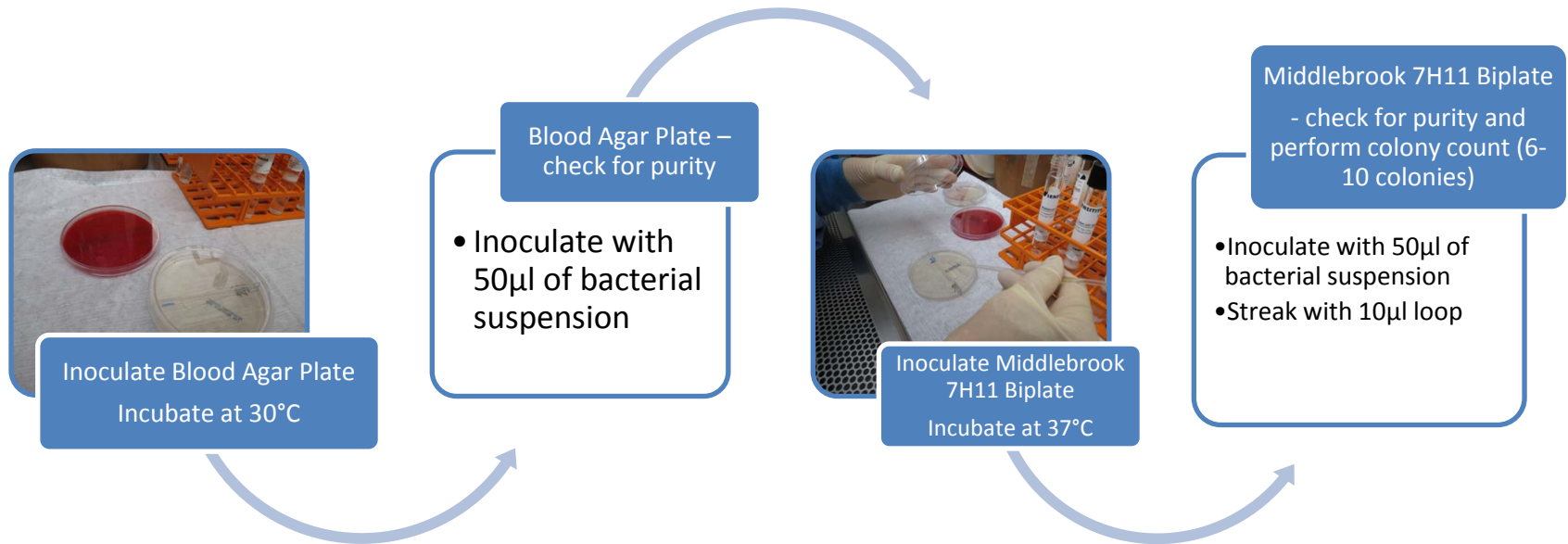
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TREK Sensititre[®] MIC Microtiter Plate Method



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TREK Sensititre[®] MIC Microtiter Plate Method



Antimicrobial Susceptibility Testing

TREK Sensititre[®] MIC Microtiter Plate Method



Results

OFL 32	MXF 8	RIF 16	AMI 16	STR 32	RFB 16	PAS 64	ETH 40	CYC 256	INH 4	KAN 40	EMB 32
OFL 16	MXF 4	RIF 8	AMI 8	STR 16	RFB 8	PAS 32	ETH 20	CYC 128	INH 2	KAN 20	EMB 16
OFL 8	MXF 2	RIF 4	AMI 4	STR 8	RFB 4	PAS 16	ETH 10	CYC 64	INH 1	KAN 10	EMB 8
OFL 4	MXF 1	RIF 2	AMI 2	STR 4	RFB 2	PAS 8	ETH 5	CYC 32	INH 0.5	KAN 5	EMB 4
OFL 2	MXF 0.5	RIF 1	AMI 1	STR 2	RFB 1	PAS 4	ETH 2.5	CYC 16	INH 0.25	KAN 2.5	EMB 2
OFL 1	MXF 0.25	RIF 0.5	AMI 0.5	STR 1	RFB 0.5	PAS 2	ETH 1.2	CYC 8	INH 0.12	KAN 1.2	EMB 1
OFL 0.5	MXF 0.12	RIF 0.25	AMI 0.25	STR 0.5	RFB 0.25	PAS 1	ETH 0.6	CYC 4	INH 0.06	KAN 0.6	EMB 0.5
OFL 0.25	MXF 0.06	RIF 0.12	AMI 0.12	STR 0.25	RFB 0.12	PAS 0.5	ETH 0.3	CYC 2	INH 0.03	POS	POS

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TREK Sensititre[®] MIC Microtiter Plate Method



Result Report

Drug	MIC ($\mu\text{g/ml}$)	Interpretation (Tentative breakpoint)
Rifampin	16	Resistant (Susceptible ≤ 1 Resistant ≥ 2)

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Validation

- *Performed validation 2011-2012*
- *230 clinical specimens*
- *Compared TREK to BACTEC 460TB method*
- *Evaluated discrepant samples with analysis of the rpoB and inhA and katG genes, when possible (Hain GenoType MTBDRplus assay or DNA sequencing)*

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TREK Sensititre[®] MIC Microtiter Plate Method



Validation - Results

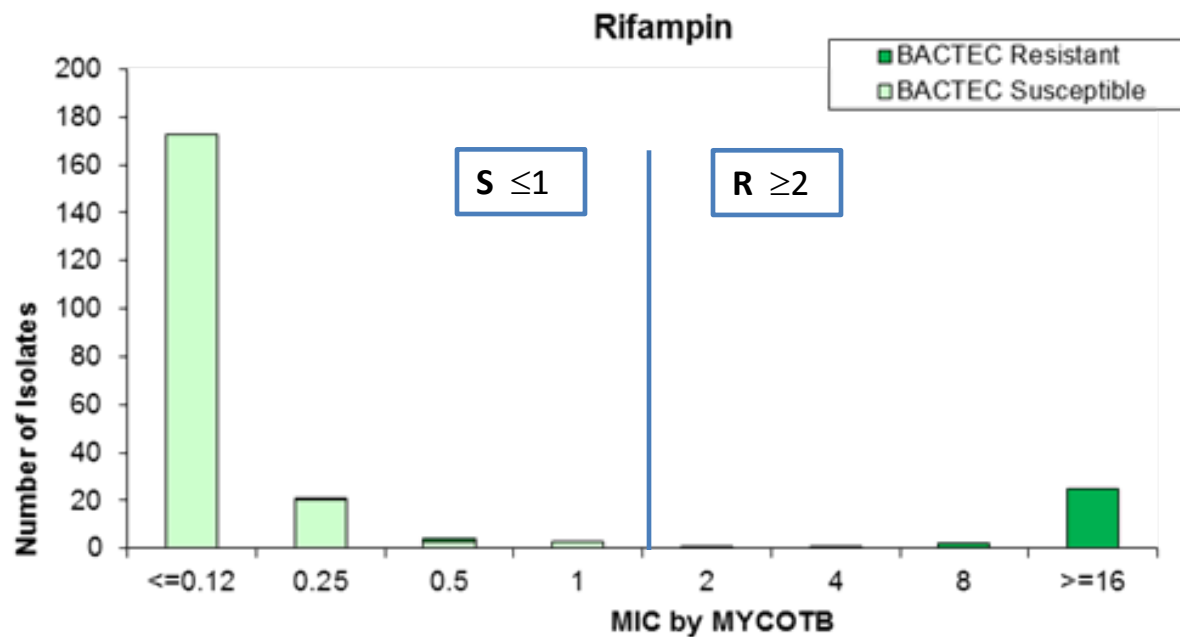
- *Analysis of Rifampin and Isoniazid: 95.7% agreement
- 10/230 discrepant isolates*
- *Analysis of four drugs (Rifampin, Isoniazid, Ethambutol, Streptomycin): 91.7% agreement
- 19/230 discrepant isolates*
- *Analysis of Ethionamide, Kanamycin, Rifabutin, Ofloxacin: 60.5% agreement
- 15/38 discrepant isolates*
- *Amikacin, Moxifloxacin, PAS and Cycloserine are on the MYCOTB plate but not performed on BACTE 460TB and therefore could not be compared*

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Validation Results - Rifampin

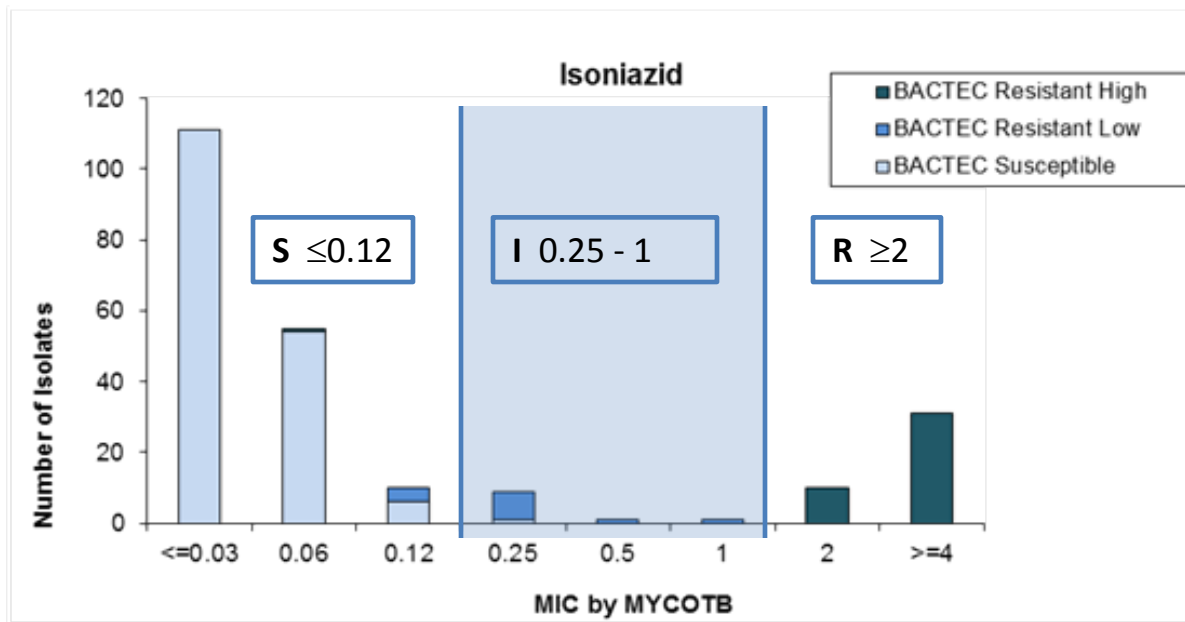


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Validation Results - Isoniazid

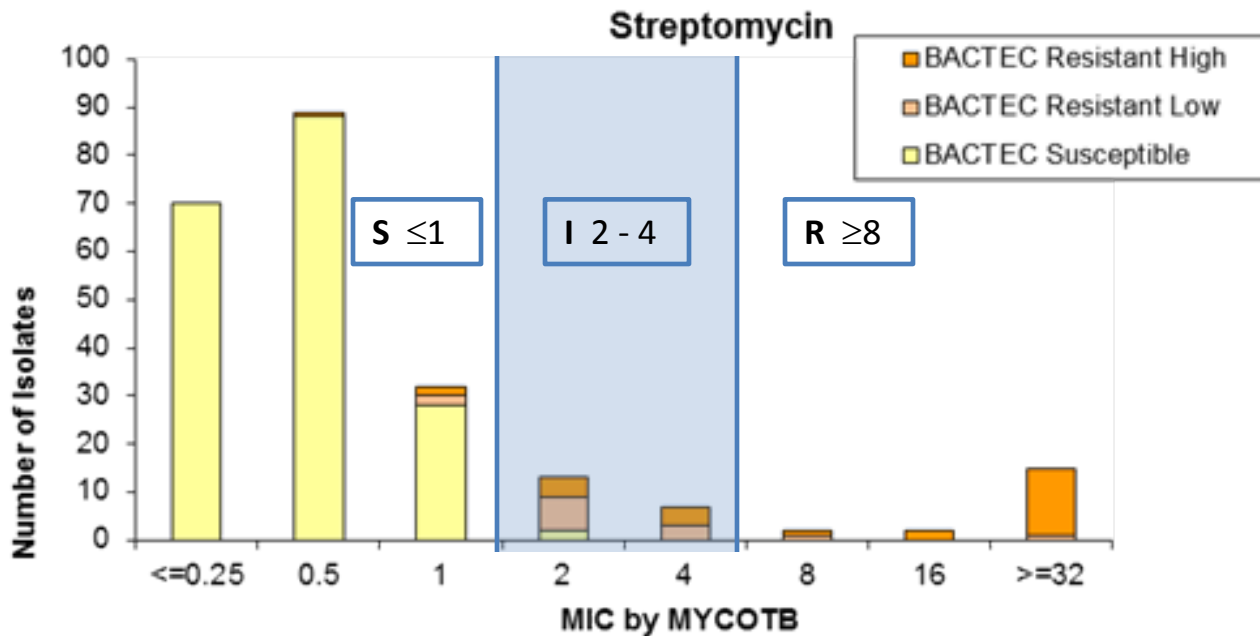


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Validation Results - Streptomycin

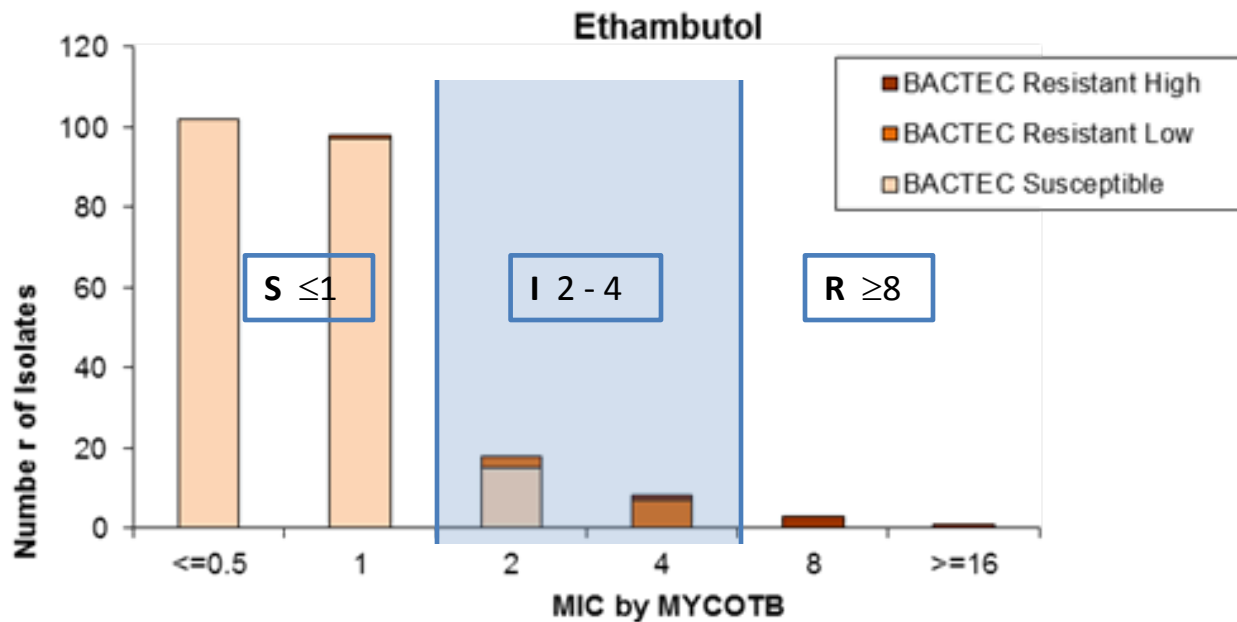


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Validation Results - Ethambutol



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Validation – Challenges

- *On a time crunch to implement a new test*
- *Have to compare two different methods that provide a different result factor (MIC vs. CC)*
- *BACTEC perform first line drugs and then second line drugs if first-line are resistant so limited data for second line drugs*
- *Not all drugs tested by both methods*

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Validation – Solutions

- *Started evaluating TREK in 2011 to give plenty of time before discontinuation of BACTEC*
- *Determined tentative MIC breakpoint:*
 - *Susceptible - equivalent to or lower than critical concentration by BACTEC*
 - *Resistant - higher than the critical concentration by BACTEC*
- *For drugs with limited data or not evaluated*
 - *Disclaimer*
 - *Correlate with clinical picture/Educate physicians*
 - *Alternative testing – Molecular, CDC?*

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Implementation – October 2012

- *Implemented TREK method in November to allow for a 2-month overlap with BACTEC*
- *Ensured up-to-date training of staff in the laboratory*
- *Provided information and education to TB Control Program, Providers in the State of Florida*
 - *Discussed with our TB Control Program and TB Physician's Network*
 - *Fact sheet/Notification of change faxed out with every report*

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Implementation

- *Adapted our molecular AST algorithm*
 - *Pyrazinamide testing – pncA DNA Sequencing*
 - *Hain GenoType[®] MTBDRplus Assay – reflex test*
 - *First-time TB-suspect, NAAT positive specimen (irrespective of AFB smear result)*
 - *First-time TB-suspect, culture for identification, M. tuberculosis complex ID*
- *Developed reporting format for TREK results with our TB Physician's Network (and other appropriate stakeholders)*

→ Full implementation January 2013

Antimicrobial Susceptibility Testing

TREK Sensititre[®] MIC Microtiter Plate Method



Pros

- *Simple, cost-effective test method*
- *Minimal/inexpensive test equipment*
- *All drugs reported at same time*
- *Result is an MIC, which can be useful for clinicians*

Cons

- *Slow turnaround time (requires solid culture)*
- *Cannot test PZA*
- *Interpretation of results a challenge*
- *Not an FDA-approved test*

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Future

- *Develop method for broth inoculation of MYCOTB plates to reduce TAT*
- *Develop customized MYCOTB plate*
 - *Kanamycin off, Capreomycin on?*
 - *Bedaquiline?*
- *Continued evaluation of tentative breakpoint determination and of reporting*
 - Example 1. Interpretation for Amikacin, Moxifloxacin, PAS and Cycloserine*
 - Example 2. Intermediate determination for some drugs e.g. Isoniazid, Ethambutol, Streptomycin*

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Thank You!

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