Validation of 2nd line drug susceptibility testing

Ed Desmond
California Dept. of Public Health
XDRTB!!!

- KwaZulu-Natal outbreak, 2006
  - 53 HIV-infected patients, 52 deaths
  - Average survival from specimen collection 25 days (Lancet 2006 368:964)

- Case definition of XDRTB:
  - Resistant to INH and rifampin
  - Resistant to fluoroquinolone and at least one of the injectable drugs: amikacin, kanamycin, capreomycin

- California has had 18 XDRTB cases and 77 resistant to fluoroquinolone or injectable drugs, from 1993-2006
Current methods too slow!

- If susceptible testing for 2nd line drugs is done by agar proportion, TAT from specimen collection would be:
  - 2 weeks for primary isolation & ID
  - 2 weeks for 1st line drug susceptibility testing
  - 4-5 weeks to send to ref lab & get 2nd line results back

  Total of 8-9 weeks (may be too late for patient)
Testing susceptibility of second-line drugs in MGIT

TAT from collection to second-line drug result could be reduced from 8-9 weeks to 5-6 weeks

- 3 weeks quicker
How many cultures need 2\textsuperscript{nd} line drug susceptibility testing?

- 13,293 reported TB cases in U.S. 2007
- About 80\% culture +: 10,600

CLSI criteria for 2nd line drug susceptibility testing:
- Resistant to rifampin, or
- Resistant to any 2 first line drugs
- First line drugs = INH, Rif, Ethamb, PZA
- About 5\% of cultures meet these criteria

- About 530 per year
How many laboratories should be doing 2nd line drug susceptibility testing?

- No cut & dried answer
- Testing should be done by rapid method
- Validation and QC are labor-intensive
- What should be the minimum number of 2nd line panels per year?
  - 50?
  - 20?
Validation of a test system not cleared by U.S. FDA

CLIA regulation §493.1253, part (2):

Establishment of performance specifications. Each laboratory that introduces a test system not subject to FDA clearance or approval…must, before reporting patient test results, establish for each test system the performance specifications for the following performance characteristics, as applicable:
Validating test system not cleared by FDA, cont’d

Establish for each test system:

i. **Accuracy**

ii. **Precision**

iii. **Analytical sensitivity**

iv. **Analytical specificity to include interfering substances**

v. **Reportable range of test results**

vi. **Reference intervals (normal values)**

[§493.1253, cont’d]
Draft interpretive guidelines for CLIA inspectors

“Establishment of accuracy may be accomplished by:

- Testing reference materials or comparing results of tests performed using an established reference method, or…”

- “Comparing split sample results with results obtained from a method which is shown to provide clinically valid results”
Draft interpretive guidelines for CLIA inspectors, cont’d

“Laboratory needs to determine the test system’s precision and have mechanisms for determining analytical specificity, sensitivity, and interfering substances.”

Precision (reproducibility) must be established

- “Repeat testing of patient samples
- Testing of QC material in duplicate and over time”
Determination of calibration and control procedures:

“..the laboratory defines the frequency for calibration and control performance as well as the type, number, and concentration of calibration and control materials used to monitor, detect error, and evaluate method performance.”
“(c) At least twice annually, the laboratory must verify the accuracy of…
(2) Any test or procedure… for which compatible proficiency testing samples are not offered by a CMS-approved proficiency testing program.”
Validation of second-line drug susceptibility testing in MGIT

VA West Haven, CT & CA MDL

- Verify drug stability in MGIT in test conditions
- Collect susceptible (S) and resistant (R) strains
- Determine MIC of H37Rv (suscept. lab strain)
- Determine MICs of S and R strains
  - Near breakpoint, test dilutions closer together than serial 2-fold
- Choose test concentrations which inhibit growth of S strains, while R strains will grow
Validation of second-line drug susceptibility testing in MGIT, cont’d

- Test clinical strains by new and reference method
- Verify reproducibility in both laboratories
- Verify reproducibility with different lots of medium and drugs
- Design and implement a proficiency testing program
Stability of drugs in susceptibility testing


- Determined stability of drugs in storage, solutions at 4 C or -20 C
  - INH, EMB, ETA stable for 1 year at 3-7 C
  - KM, Capreol stable at -20 C for 1 yr

- Measured stability of drug in test medium, stored at 4 C or 37 C
  - Dilute drug in medium to MIC of stock strain +/- 1 dilution
  - Compare growth of stock strain in fresh vs. stored medium
Some drugs lose potency/activity during incubation at 37 C

- EMB loses half in 2 to 4 days
- INH, rif, ethionamide lose half in 2 weeks

Loss of activity over several days or weeks does not prevent meaningful drug susceptibility testing

Aminoglycoside antibiotics very stable in Griffith study

Fig 1. Levofloxacin
Fig. 2  Amikacin
Fig. 4 Ethionamide

![Graph showing the distribution of strains based on MIC (μg/mL).](chart)

- **MIC (μg/mL)**: Use agar proportion as reference.
- **R** and **S** indicate resistant and susceptible strains, respectively.
FIGURE 1. The minimal inhibitory concentrations (MICs) of ethionamide of probably sensitive (○) strains, obtained pre-treatment from patients, and strains that are probably resistant (□), obtained after treatment of patients with ethionamide. Data taken from [6].
Median number of drug-resistant mutants in “wild type” strains of M. tb, per $10^6$ bacilli

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (ug/mL)</th>
<th>Mutants per $10^6$ bacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>0.1</td>
<td>41</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2.0</td>
<td>5,000</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>10.0</td>
<td>30,000</td>
</tr>
</tbody>
</table>

Source: WHO guidelines for drug suscept. testing for 2nd line drugs, 2001, p 8
# MGI T test concentrations

<table>
<thead>
<tr>
<th>Drug</th>
<th>Levofloxacín</th>
<th>Amikacin</th>
<th>Capreomycin</th>
<th>Ethionamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (ug/mL)</td>
<td>1.5</td>
<td>1.5</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

**WHO 2007 guidelines for MGIT 960**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Levo</th>
<th>Amikacin</th>
<th>Capreomycin</th>
<th>Ethionamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc ug/mL</td>
<td>No recomm.</td>
<td>1.0</td>
<td>2.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Inoculating a MGIT drug sus test from a primary MGIT tube

BD package insert calls for mixing the MGIT tube and using a sample of this or a 1:5 dilution of it (day 3 to 5 after positivity) as inoculum

- Problem: variable amounts of *M. tb* cells likely have been removed for smear and AccuProbe
- Problem: anecdotes of inconsistent results
- Problem: studies in which no *M. tb* cells were removed from inoculum tube don’t really evaluate accuracy of this method as performed using primary cultures/clinical isolates
Calif. protocol for inoculum prep from 1° MGIT

Pipet 2 mL from bottom of primary MGIT tube into a 1 dram vial & let settle for 20 min.

Pipet supernate to another 1 dram vial & let settle for 15 min.

Pipet supernate to another 1 dram vial and dilute to McFarland 0.5 using normal saline; dilute 1:5

Protocol compensates for variable amounts removed from primary tube, and works with single cells or small clumps.

Full disclosure: BD provided free MGIT medium for evaluation of this protocol.
Proficiency testing

- Save strains shown to be susceptible and resistant by reference method (agar proportion)
- Twice per year supervisor challenges drug susceptibility testing personnel with a set of (5?) cultures, varying proportions with each sample set
- When cultures are sent to CDC laboratory for confirmation or additional drugs, compare CDC’s agar proportion results with your own
Molecular beacons quality assurance

- Detection of INH and rifampin resistance in acid-fast smear positive sputum sediments or pure cultures
- Perform followup culture and drug susceptibility testing on all specimens
- Compare culture and molecular beacons results
  - When discrepancies occur, sequence appropriate genes to determine if molecular method was in error
Thank you