Advances in the Diagnosis and Treatment of Tuberculosis
San Antonio, Texas

Impact of Automated Molecular Diagnostics on the Initiation of TB Treatment
David E. Griffith, MD
February 22, 2012

David Griffith, MD has the following disclosures to make:

• No conflict of interests
• No relevant financial relationships with any commercial companies pertaining to this educational activity
Global Tuberculosis Control
WHO Report 2011

• In 2010 8.8 million (8.5-9.2 million) incident cases of TB
  – 1.1 million (0.9-1.2 million) deaths HIV neg
  – 0.35 million (0.32-0.39 million) deaths HIV associated

• 2010 India and China accounted for 40% of the world’s TB cases, 50% MDR cases

• Africa an additional 24%
  • 2009: top 10 TB incidence countries in Africa
  • Some areas > 1000 cases/100,000 pop

Global Tuberculosis Control
WHO Report 2011

• 3.6% TB cases globally estimated to be MDR

• Less than 5% of new and previously treated TB patients were tested for MDR-TB

• Among 36 high burden and high MDR incidence countries, 20 had less than 1 lab capable of performing culture and DST/5 million pop
Trends in Tuberculosis—United States, 2010 (MMWR 3/24/11)

- 3.6 cases/100,000; U.S. born 1.6 cases/100,000
- Foreign born 60% TB cases, 18 cases/100,000
- 4 countries 50% of foreign born TB morbidity: Mexico, Philippines, India, Vietnam
- Foreign born account for 90% of US MDR cases

What’s all the fuss?

- 1/16/12: MSNBC headline
  - “Totally drug resistant TB strain reported”
- 1/12/12: ATS Morning Minute Headline
  - “TB strains resistant to all first and second-line drugs reported in India”
Estimated Migrants “Entering” U.S.

- Visitors without visas
  ~ 30,000,000

- Non-immigrant visas
  27,907,139

- Immigrants and refugees
  411,266

- Undocumented migrants
  ~ 275,000 ????

Status adjusters in U.S.:

N= ~ 59,000,000

## Molecular Diagnosis of Drug Resistance

**MDDR: DNA Sequencing**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF</td>
<td>rpoB</td>
<td>&gt;95</td>
</tr>
<tr>
<td>INH</td>
<td>inhA, katG, ndh, ahpC</td>
<td>80</td>
</tr>
<tr>
<td>PZA</td>
<td>pncA</td>
<td>72-97</td>
</tr>
<tr>
<td>EMB</td>
<td>embB, embC, embR</td>
<td>47-65</td>
</tr>
<tr>
<td>FQ</td>
<td>gyrA, gyrB</td>
<td>40-80</td>
</tr>
<tr>
<td>KAN</td>
<td>rrs</td>
<td>75</td>
</tr>
<tr>
<td>AMK</td>
<td>rrs</td>
<td>&gt;75</td>
</tr>
<tr>
<td>CAP</td>
<td>rrs, tylA</td>
<td>ND</td>
</tr>
</tbody>
</table>
# Early Detection of Drug Resistant TB: MDDR

**Patient:**          **Submitter Specimen Identifiers:** AMCC1107153

## Results for Molecular Detection of Drug Resistance; Conventional Drug Susceptibility Test in progress.

<table>
<thead>
<tr>
<th>Locus (region) examined*</th>
<th>Result</th>
<th>Interpretation (based on in-house evaluation of 294 clinical isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pncA (promoter)</td>
<td>No mutation</td>
<td>Probable rifampin susceptible. (90% of INH-R isolates in our in-house evaluation of 294 clinical isolates have a mutation at this locus.)</td>
</tr>
<tr>
<td>katG (w213-214 deletion)</td>
<td>No mutation</td>
<td>Cannot rule out INH resistance. (95% of INH-R isolates in our in-house evaluation of 294 clinical isolates have a mutation at one or both of these loci.)</td>
</tr>
<tr>
<td>rpsL (M500V, Gly408)</td>
<td>No mutation</td>
<td>Cannot rule out ethambutol resistance. (79% of EMB-R isolates in our in-house evaluation of 294 clinical isolates have a mutation at this locus.)</td>
</tr>
<tr>
<td>pncA (promoter, coding region)</td>
<td>No mutation</td>
<td>Cannot rule out PAS resistance.</td>
</tr>
<tr>
<td>gyrA (QDRR)</td>
<td>No mutation</td>
<td>Cannot rule out fluoroquinolone resistance. (88% of FQ-R isolates in our in-house evaluation of 294 clinical isolates have a mutation at this locus.)</td>
</tr>
<tr>
<td>rrs (1600 region)</td>
<td>No mutation</td>
<td>Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 294 clinical isolates: 88% of AMK-R isolates have a mutation in the rrs locus; 88% of KAN-R isolates have a mutation in the rrs locus; 49% of CAP-R isolates have a mutation in the rrs locus.)</td>
</tr>
<tr>
<td>rrl (promoter)</td>
<td>No mutation</td>
<td>Cannot rule out resistance to second-line drugs (ethionamide, tilosmycin, amikacin). (In our in-house evaluation of 294 clinical isolates: 51% of EMB-R isolates have a mutation in the rrl locus; 51% of EMB-R isolates have a mutation in the rrl locus; 51% of EMB-R isolates have a mutation in the rrl locus.)</td>
</tr>
</tbody>
</table>

* A negative result (e.g., no mutation) does not rule out contributory mutations present elsewhere in the genome.

---

### Early Detection of Drug Resistant TB: High Risk Patient

- **2/16/11:** 42 yo man with 6 mos cough, sputum, weight loss, sweats
- **Sputum AFB smear (+), HPLC (+) for MTB**
- **1993:** cavitary abnormalities on CXR, no MTB information, I/R unsupervised 6 mos
- **2006:** sputum AFB culture (+) INH resistant MTB
- **2006:** 7 mos daily unknown TB medications, unsupervised
Without rapid identification of drug resistance what would you do?

- Start patient on IREZ daily by DOT
- Start patient on IREZ daily by DOT (+) FQ and injectable
- Start patient on IREZ daily by DOT (+) FQ, injectable and second new oral drug
- Hold antituberculosis therapy pending in vitro susceptibility results

Early Detection of Drug Resistant TB: High Risk Patient

<table>
<thead>
<tr>
<th>Locus (region) examined</th>
<th>Result</th>
<th>Interpretation (based on in-house evaluation of 284 clinical isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>spol (MtbH37Rv)</td>
<td>No mutation</td>
<td>Non-drug-resistant. 96% of MtbH37Rv strains in our in-house evaluation of 284 clinical isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>FDR genome</td>
<td>No mutation</td>
<td>Non-drug-resistant. 100% of FDR strains in our in-house evaluation of 284 clinical isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>katG (codon 152)</td>
<td>Mutation: AAC-&gt;GAC; Ser44Thr</td>
<td>Can cause lack of function of the enzyme. 100% of katG strains in our in-house evaluation of 284 clinical isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>atm (MtbH37Rv)</td>
<td>No mutation</td>
<td>Can cause lack of function of the enzyme. 99% of atm strains in our in-house evaluation of 284 clinical isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>pck (promoter, coding region)</td>
<td>No mutation</td>
<td>Can cause lack of function of the enzyme. 99% of pck strains in our in-house evaluation of 284 clinical isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>gpt (ORF)</td>
<td>No mutation</td>
<td>Can cause lack of function of the enzyme. 99% of gpt strains in our in-house evaluation of 284 clinical isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>esp (H37Rv)</td>
<td>No mutation</td>
<td>Can cause lack of function of the enzyme. 99% of esp strains in our in-house evaluation of 284 clinical isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>sbc (promoter)</td>
<td>No mutation</td>
<td>Can cause lack of function of the enzyme. 99% of sbc strains in our in-house evaluation of 284 clinical isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>gat (MtbH37Rv)</td>
<td>No mutation</td>
<td>Can cause lack of function of the enzyme. 99% of gat strains in our in-house evaluation of 284 clinical isolates have a mutation at this locus.</td>
</tr>
</tbody>
</table>
| *A negative result (i.e., no mutation) does not rule out drug resistance; present elsewhere in the genome.*
Early Detection of Drug Resistant TB: High Risk Patient

- No medications started with notification of MTB in sputum
- MTB isolate obtained from reference lab with some effort by KJ and DD
- No rpoβ mutation!
- Patient started on daily Rmp/PZA/Emb/Moxi by DOT
Early Detection of Drug Resistant TB: High Risk Patient

• 31 yo female from Korea who presented with hoarseness, cough, fever
• Treated 12 mos for TB in Korea 7 years ago with unknown medications
• Patient’s mother (?) treated with linezolid, cycloserine, Eth, clofazimine (huh?)
• Patient found to have abnormal CXR, sputum AFB smear (+), HPLC (+) for MTB
• 8/20/10: started on IREZ

Without rapid identification of drug resistance what would you do?

• Begin standard 4-drug (IREZ) therapy
• Begin standard 4-drug (IREZ) therapy (+) FQ and injectable
• Begin standard 4-drug (IREZ) therapy (+) FQ, injectable and 1-2 other 2nd line drugs
• Hold anti-TB medications pending in vitro susceptibility results
**Laboratory Results Report**

**Date Received:** 09-16-2010

**Lab Name:** NPHL

**Patient Information**
- **Name:** [Redacted]
- **DOB:** [Redacted]
- **Address:** [Redacted]
- **City:** [Redacted]
- **State:** [Redacted]
- **ZIP:** [Redacted]

**Screening Information**
- **Accession Number:** [Redacted]
- **Type:** SPUTUM
- **Source:** [Redacted]
- **Submission Date:** 09-12-2010
- **Specimen ID:** [Redacted]
- **Sample Type:** [Redacted]

**Test Results**

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorochrome Stain</td>
<td>M. tuberculosis (positive)</td>
</tr>
<tr>
<td>Culture</td>
<td>M. tuberculosis composite by mycobec dry powder</td>
</tr>
<tr>
<td>Sensitivity Testing</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

**Notes**
- Include sputum to the CBO for secondary drug susceptibility testing.
Early Detection of Drug Resistant TB: High Risk Patient

- Patient hospitalized for protection of public health
- 9/16/10: regimen changed to moxi, cap, Emb, PZA, Eth
- Repeat in vitro susceptibilities confirmed IRE resistance and E stopped
- Patient has done well
Early Detection of Drug Resistant TB: High Risk Patient: Nightmare scenario

- 24 yr old Indian immigrant with history of prior TB therapy in India X2 with undocumented medications, susceptibilities, and duration
- Asymptomatic, evaluated for immigration screening after arrival in the U.S. 5/11 (B1, smear +, cult - MTB)
- About to start graduate school at time of diagnosis
- CXR: Non-cavitary abn, sputum AFB smear (+)
- 8-4-11: started IREZ
- 8-22-11: Suspected INH, rifampin, EMB resistance

Results for Molecular Detection of Drug Resistance; Conventional Drug Susceptibility Test in progress.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutations</th>
<th>Susceptibilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>ropB</td>
<td>TGT17: Ser337del</td>
<td>Rifampin resistant (100% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are RFP-R)</td>
</tr>
<tr>
<td>641G (promoter)</td>
<td>No mutation</td>
<td>Isolates resistant (100% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are RFP-R)</td>
</tr>
<tr>
<td>attb (McGBL, 168/180)</td>
<td>Mutation: ATT20-GTT20, indel20/40</td>
<td>Possibly attenuated resistant (25% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are EB/INH)</td>
</tr>
<tr>
<td>pvpA (promoter, coding region)</td>
<td>Mutation: GAC-GCC, HaeIIIPro Silent Mutation; TCC-TCC, Ser111Ser</td>
<td>Genotypic rule out PZA resistance. The significance of the HaeIIIPro mutation in predicting resistance to PZA is unknown. The Ser111Ser mutation is a synonymous (silent) single-nucleotide polymorphism (SNP) and does not result in an amino acid change and is not considered clinically significant.</td>
</tr>
<tr>
<td>pynA (R3P)</td>
<td>Mutation: Q89-G77, real-time</td>
<td>Probably resistant (50% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are EB/INH)</td>
</tr>
<tr>
<td>rrs (1400 region)</td>
<td>Mutation: A1475G</td>
<td>Amikacin resistant and Kanamycin resistant (100% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are AMK-R and KAN-R)</td>
</tr>
<tr>
<td>rrl (promoter)</td>
<td>No mutation</td>
<td>Positively Ciprofloxacin resistant. (In our studies, 49% of isolates with this mutation are CIP-R, 13% are CIP-S, 38% are CIP-I)</td>
</tr>
<tr>
<td>rfa (promoter)</td>
<td>No mutation</td>
<td>Possible Clarithromycin resistant. (In studies, 13% of isolates with this mutation are CLA-R, 38% are CLA-S, 49% are CLA-I)</td>
</tr>
</tbody>
</table>
Early Detection of Drug Resistant TB:
High Risk Patient: Nightmare scenario

• Patient hospitalized in isolation
• MTB Resistant to: I,R,E,Z, Rbt, Oflox, Kan
• In vitro susceptibilities: Cap (1% R), Moxi
  MIC 2 μg/ml (R to levo)
• Started on Cap, Moxi, Eth, PAS, cycloserine
  and linezolid
• Generally feeling well, neuropathy with
  linezolid
• Sputum converted to AFB culture - at ~ 2
  months

Early Detection of Drug Resistant TB:
High Risk Patient

• 37 yo HIV seropositive, hepatitis C
  seropositive man from Burma (refugee)
• Treated in 2009 for TB in Burma
• 3 reported treatment regimens, unknown
  durations and conditions (i.e., ± DOT)
  – Stm, Emb, Oflox
  – INH, Rmp, PAZ, Emb, Oflox, Emb
  – INH, Rmp, Oflox
• Respiratory arrest in ED
Without rapid identification of drug resistance what would you do?

• Begin standard 4-drug (IREZ) therapy
• Begin standard 4-drug (IREZ) therapy (+) FQ and injectable
• Begin standard 4-drug (IREZ) therapy (+) FQ, injectable and 1-2 other 2nd line drugs
• Hold anti-TB medications pending in vitro susceptibility results
Early Detection of Drug Resistant TB: High Risk Patient

- Patient started on IREZ at time of initial diagnosis
- TB medication held at admission to ICU with submission of MTB isolate to CDC
- Started on IRE (+) FQ after CDC report
- Returned home to complete outpatient TB therapy
Early Detection of Drug Resistant TB:
High Risk Patient

- 8/7/11: 31 year old female from India with headache, fever, recent mental status changes and breast abscess
- Previous therapy for TB breast abscess
- 8/8/11: started IREZ (+) Moxi, Am
- 9/19/11: breast abscess grew MTB with preliminary resistance to IRE
- 9/20/11 Eth, Lin added to the regimen
Without rapid identification of drug resistance what would you do?

- Continue IREZ (+) FQ, Am, Eth, Lin
- Stop REZ, continue PZA, FQ, Am, Eth, Lin
- Continue PZA, FQ, Am, Eth, Lin (+) cycloserine, PAS
- Hold TB therapy until second line in vitro susceptibilites available

Early Detection of Drug Resistant TB:
High Risk Patient: Missed Opportunity
Early Detection of Drug Resistant TB: High Risk Patient: Missed Opportunity

• 10/14/11: CDC study requested 10/14/11
• 10/21/11: In vitro susceptibilities:
  – Resistance to IRE, Sm, Eth, oflox, rbt
  – Susceptible to Am, Km, Cap, PAS, cycloserine
• NJH reported levo/moxi MIC 2μg/ml
• Current medications: Lin, levo, cycloserine, Am, PZA
• Neurologic status improved and stable

Early Detection of Drug Resistant TB: Public Health Emergency

• 7/26/11: 39 yo high school teacher with 10 mos cough, recent fever, sweats, dyspnea; lived in South Africa for 10 years
  – Cavitary changes on chest CT, sputum and BAL AFB smear (+), NAAT (+) MTB
• 8/3/11 presented with pneumothorax and cavitary densities on CXR
  – Started on IREZ
Early Detection of Drug Resistant TB: Public Health Emergency

• Contact investigation started in school involving hundreds of students, teachers and administrators
• Contact investigation yields:
  – ~ 13 active TB cases
  – ~ 250 LTBI cases
• What do you tell the mob of parents with torches and pitchforks at the school?

Without rapid identification of drug resistance what would you do?

• “Everyone stay calm, take some deep breaths and go home”
  – Headline: Public Health Official Murdered by Angry Mob
• Wait on MTB susceptibilities to start LTBI therapy
• Begin LTBI therapy ASAP with INH
• Begin LTBI therapy ASAP with Rmp
• Start IREZ for TB cases or
• Wait on MTB susceptibilities to start TB therapy?
Early Detection of Drug Resistant TB: High Risk Patient

Results for Molecular Detection of Drug Resistance: Conventional Drug Susceptibility Test in progress.

<table>
<thead>
<tr>
<th>Marker (region) examined</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB (promoter)</td>
<td>No mutation</td>
<td>Can not rule out 9th resistance. 98% of MHTB isolates have an mutation at this locus.</td>
</tr>
<tr>
<td>katG (pyrD codon)</td>
<td>No mutation</td>
<td>100% of MHTB isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>rrs (ribosomal)</td>
<td>No mutation</td>
<td>All susceptible to all 1st line drugs</td>
</tr>
<tr>
<td>rpoB (promoter, coding region)</td>
<td>Mutation (STC), variable</td>
<td>Can not rule out 9th resistance. The significance of the mutation detected regarding sensitivity to 9th is unknown.</td>
</tr>
<tr>
<td>gyrA (GRF)</td>
<td>No mutation</td>
<td>Can not rule out 9th resistance. 98% of MHTB isolates have an mutation at this locus.</td>
</tr>
<tr>
<td>ms (45 spleen)</td>
<td>No mutation</td>
<td>100% of MHTB isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>uup (genotype)</td>
<td>No mutation</td>
<td>100% of MHTB isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>spa (antigen D)</td>
<td>No mutation</td>
<td>100% of MHTB isolates have a mutation at this locus.</td>
</tr>
</tbody>
</table>

*Negative results (e.g., no mutations) do not rule out contributory mutations present elsewhere in the genome.

Early Detection of Drug Resistant TB: Public Health Emergency

- No MTB mutations associated with drug resistance
- Culture grows M TB sensitive to all 1st line drugs
- TB cases started on IREZ
- LTBI cases started (mostly) on INH
- The ripples are still being felt
Early Detection of Drug Resistant TB: Suspected Treatment Failure and Acquired Drug Resistance

- 36 yo female with 3 months cough, sputum, fatigue, weight loss
- TST and QFT (+)
- Chest radiograph: biapical cavitary densities
- 3 year old and 3 month old contacts in the home

Early Detection of Drug Resistant TB: Suspected Treatment Failure and Acquired Drug Resistance

- 1/11/11 Daily IREZ daily by DOT
- 2/16/11 TIW IREZ
- 2/24/11 MTB isolate INH resistant
- INH stopped, REZ continued daily by DOT
- 2/28/11 MTB isolate reported as resistant to EMB
- Conclusion: 1 month Rmp/PZA
Without rapid identification of drug resistance what would you do?

- Continue Rmp, PZA
- Continue Rmp, PZA (+) FQ and injectable
- Continue Rmp, PZA (+) FQ, injectable and another oral drug
- Hold all TB drugs pending repeat susceptibility results on new MTB isolate
- ? Therapy for 3 mos old and 3 yo children

Early Detection of Drug Resistant TB: Suspected Treatment Failure and Acquired Drug Resistance

<table>
<thead>
<tr>
<th>Locus (region)</th>
<th>Result</th>
<th>Interpretation (based on in-house evaluation of 154 clinical isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB (R018)</td>
<td>No mutation</td>
<td>Probably rifampicin susceptible. 96% of R018 isolates in our in-house evaluation of 254 clinical isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>rmpA (promoter region)</td>
<td>No mutation</td>
<td>Cannot rule out rmpA resistance. 96% of FQ-R isolates in our in-house evaluation of 254 clinical isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>rrs (1600 region)</td>
<td>No mutation</td>
<td>Cannot rule out drug resistance to aminoglycosides (kanamycin, streptomycin, amikacin), 96% of FQ-R isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>rps (promoter region)</td>
<td>No mutation</td>
<td>Cannot rule out resistance to aminoglycosides (kanamycin, streptomycin, amikacin), 96% of FQ-R isolates have a mutation at this locus.</td>
</tr>
<tr>
<td>rhlB (odD)</td>
<td>Mutation: GAC→GCG, Asp324Ala</td>
<td>Probable aminoglycoside resistant. 96% of strains in our in-house evaluation of 254 clinical isolates with this mutation are FQ-R.</td>
</tr>
</tbody>
</table>

*Note: A negative result for rmpA does not rule out resistance to aminoglycosides in all cases.
Early Detection of Drug Resistant TB: Suspected Treatment Failure and Acquired Drug Resistance

• Patient put on daily Rmp/PZA/Moxi/Stm
• No rpoβ mutation!
• No apparent emergence of rifamycin resistance during one month Rmp/PZA
• Patient placed on daily Rmp/PZA/Moxi
• Children treated with Rmp window prophylaxis
Early Detection of Drug Resistant TB:
Suspected Treatment Failure and Acquired Drug Resistance

• 4/6/11: 4 months cough, sputum, sweats, weight loss treated for CAP (FQ), extensive cavitary changes radiographically
• 4/14/11 sputum AFB culture (+) MTB, susceptible, IREZ daily by DOT
• Slow clinical and radiographic response, persistent AFB smear and culture (+) > 2 mos after starting therapy
• Well educated professional, high profile CI

Without rapid identification of drug resistance what would you do ?

• Continue IREZ daily by DOT pending repeat susceptibilities
• DC Emb/PZA continue daily IR
• DC Emb/PZA start TIW IR
• Add FQ (+) injectable to regimen of IREZ
Early Detection of Drug Resistant TB: Suspected Treatment Failure and Acquired Drug Resistance

- No mutations associated with antibiotic resistance
- Continued on IREZ daily
- Sputum converted to AFB smear and culture (-) at 3 months
- Result helpful to physicians in subsequent management of the patient
For whom is rapid identification of drug resistance indicated?

• A patient previously treated with any known drug resistance
• A patient previously treated with unsupervised therapy
• A foreign born individual
  – with a history of previous therapy
  – from an area with high prevalence of drug resistant TB
• A patient failing therapy
• A complex social/public health situation

CDC criteria for acceptance of specimens for rapid molecular testing for drug resistance

• MTB isolate available, AFB smear (+) or (-) but NAAT (+) for MTB.
• High risk patients (early rather than later)
• Cases of public health importance
• Known Rmp resistance
• Mixed or nonviable cultures
• Other reasons
Limitations of Molecular DST: DNA Sequencing

- Validated for MTB confirmed specimens
- Predictive value of mutations variable
- If no mutation detected, cannot rule out resistance
- Not all mutations confer resistance
- Molecular DST can be used to guide treatment until conventional DST results available.
- MUST CONFIRM RESULTS WITH CONVENTIONAL DST

Rapid Molecular Detection of TB and Rifampin Resistance
Boehme et al NEJM, 2010; 363: 1005

- Xpert MTB/RIF: an automated molecular test for MTB and resistance to rifampin
- Uses real-time PCR assay to amplify an MTB-specific sequence of the \( rpo\beta \) gene, which is probed with molecular beacons for mutations within the rifampin-resistance determining region
- 5 trial sites: Peru, Azerbaijan, (2)South Africa, India
Rapid Molecular Detection of TB and Rifampin Resistance
Boehme et al NEJM, 2010; 363: 1005

- Among culture (+) patients 1 MTB/Rif identified:
  - 551/561 (98%) smear (+) and
  - 124/171 (73%) smear (-) TB (90% with 3)
- MTB/Rif correctly identified:
  - 200/205 (98%) Rif resistant patients and
  - 504/514 (98%) with Rif-sensitive MTB.
- The MTB/Rif test provided sensitive detection of TB and rifampin resistance directly from untreated sputum in < 2 hours with minimal hands on time
Rapid Molecular Detection of TB and Rifampin Resistance
Boehme et al NEJM, 2010; 363: 1005

- The costs of MTB/Rif (instruments and tests) considerably higher than those for microscopy
- MTB/Rif testing could be less costly than implementation of culture and drug susceptibility testing
- Simple to perform with minimal training and not prone to cross-contamination
- Specimen processing is simplified to a single nonprecise step that both liquefies and inactivates sputum: eliminates the necessity for a “biosafety cabinet”

TB Diagnosis-Time for a Game Change
Small and Pai NEJM, 2010; 363: 1070

- MTB/Rif assay:
  - Relatively high cost
  - Tests only for rifampin resistance
  - Detects a relatively small number of mutations
  - Inability to indicate which patients are smear (+) for infection control intervention and treatment monitoring
Tuberculosis Consultation:
Rapid identification of drug resistant TB

Heartland National TB Center (HNTC)
1-800-TEX-LUNG (1-800-839-5864)
Southeastern National TB Center (SNTC)
1-800-428-4696
Curry International TB Center
1-877-390-6682
New Jersey Medical School Global TB Institute
1-800-428-3627
Tuberculosis Consultation: Rapid identification of drug resistant TB

• Texas Dept of State Health Services (DSHS) Mycobacteriology Lab, Austin, TX
  – denise.dunbar@dshs.state.tx.us (512-776-7342)
  – ken.jost@dshs.state.tx.us (512-776-7580)

• Centers for Disease Control and Prevention (CDC), Atlanta, GA
  – Beverly Metchock
    • bem1@cdc.gov
    • 404-639-1285

• California Dept. of Public Health, Microbial Diseases Lab
  – Ed.Desmond@cdph.ca.gov
  – 510-412-3781

• Florida Dept. of Health Bureau of Laboratories
  – max_salfinger@doh.state.fl.us
  – 850-245-4517
TB: a portal through which to view the future
Sbarbaro J, ARRD 1982; 125: 127

• Described the discovery of streptomycin and INH and noted:
  – “it took until 1961, nine years after the presentation of evidence that we had achieved a significant scientific breakthrough, for the Committee on Therapy of the American Trudeau Society to issue a statement that essentially eliminated bed rest as a therapeutic principle. Now, please understand that I am not ridiculing or criticizing the professionals of the past...clearly, we professionals change slowly.”