Laboratory Techniques for Rapid Diagnosis of Drug-Susceptible and Drug-Resistant TB: Implementation Results in the Region

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Accelerating TB/HIV Collaborative Activities in EURO
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Disclosure

I have no personal financial conflicts of interest to report. However, my employer, FIND, has contractual relationships with a number of companies whose products are mentioned in this presentation. These agreements call on FIND to support development work and undertake studies of their TB diagnostics assays. In turn, these companies are to provide their tests at favorable prices to the public sector in developing countries.
WHO Policy on Implementing New Tools*

- Identifying the need for a policy change
  - WHO monitoring of technical developments
  - Requests from interested outside parties
- Reviewing the evidence
  - Use of standardized criteria for assessing available data
  - Systematic review and meta-analyses
  - GRADE approach for rating the strength of a recommendation
- Convening an expert panel to review evidence and draft recommendations
- Assessing draft policy and evidence by STAG-TB
- Formulating and disseminating policy

Implementing New TB Diagnostics

• WHO Guidance on New Tools
  – Liquid culture/DST and rapid species identification (2007)
  – Line probe assays for MDR TB detection (2008)
  – LED fluorescence microscopy (2009)
  – Non-commercial culture/DST systems (2009)
  – Frontloaded microscopy (2009)
  – Automated molecular testing for TB and MDR TB*
  – Line probe assays for XDR detection*
  – Interferon-gamma release assays*
  – Commercial serologic tests*

• Implementation and scale up
  – Expand TB
  – Treat TB (Union)
  – Stop TB Partnership INAT sub-working group

*To be considered by STAG-TB 2010
Liquid Culture/DST and Rapid Species Identification

- Compared to solid culture/DST
  - More rapid time to detection
  - More sensitive
  - Higher contamination rate
  - Rapid method for species ID needed
- WHO endorsement in 2007
- FIND-BD agreement on preferential pricing for HBCs
- Guidance for SL DST developed
### MGIT 960 Second-line DST Established Critical Concentrations

<table>
<thead>
<tr>
<th>Drug</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
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<tbody>
<tr>
<td>Amikacin</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Kanamycin</td>
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<td>2.5</td>
<td>ND</td>
</tr>
<tr>
<td>Capreomycin</td>
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<td>1.25</td>
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<td>Ethionamide</td>
<td>5.0</td>
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<td>ND</td>
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<tr>
<td>Proteinamide</td>
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<td>ND</td>
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<td>1.0</td>
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<tr>
<td>Ribabutin</td>
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<tr>
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</tr>
<tr>
<td>Linezolid</td>
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<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Line-Probe Assays for MDR TB

- PCR test that detects *M. tuberculosis* and genetic mutations conferring rifampin and isoniazid resistance directly from AFB smear-positive sputum specimens
- Three published studies of earlier generation of the assay indicated good performance on AFB+ sputum
- Second generation assay includes additional probe for INH-R and more sensitive amplification control
- Subsequent evaluation studies suggest high sensitivity (>90%) and specificity (>95%) for MDR TB
- FIND demonstration study indicate that the assay can be implemented in HBCs
WHO Policy Statement on LPAs

- Integrated national plan for LPAs together with MDR-TB management and lab capacity strengthening
- Use of LPAs on smear-positive sputum and cultures (insufficient evidence on smear negatives)
- LPAs do not replace culture + DST
- Commercial assays recommended
- Lab infrastructure, procedures and biosafety
- Human resources
- Training, technical support and supplies
- EQA
Sputum AFB microscopy

AFB +
Rapid RIF test
  RIF susceptible
  Treat drug susceptible TB
  Refer for second-line DST
  RIF resistant

AFB -
MGIT+Capilia
  Negative
  Positive
    Rapid RIF test
      RIF susceptible
      Treat drug susceptible TB
      Refer for second-line DST
      RIF resistant

TB suspect
  Treat drug susceptible TB
  Refer for second-line DST
GenoType® MTBDRs/ Assay
Validation Study of MTBDRs/ Test*

- 106 clinical isolates and 64 AFB+/- sputum specimens tests by LPA, DNA sequencing, and DST on solid and liquid media
- Isolates
  - FQ resistance detected by LPA in 29/32 (91%) FQ-R strains
  - AK/CM resistance detected by LPA 39/46 (85%) for AK/CR-R strains
  - Specificity = 100%
  - No discrepancy with sequencing
- Sputum specimens (42 AFB+, 12 scanty, 10 AFB-)
  - FQ resistance detected by LPA in 8/9 (89%) FQ-R strains
  - AK/CM resistance detected by LPA 6/8 (75%) for AK/CR-R strains
  - Specificity = 100%
- Performance for EMB-R is less good

Hillemann et al.  JCM (epub), 22 April 2009
Non-Commercial Culture/DST Systems

- Rapid DST systems requiring less sophisticated lab infrastructure and technical expertise
- WHO endorsement of microscopic observation of drug susceptibility (MODS) and nitrate reductase assays (NRA) for direct testing of sputum specimens and MODS, NRA and colorimetric redox indicators (CRI) for indirect testing on clinical isolates
- Seen as interim solution in resource-constrained settings while capacity for genotypic and/or automated liquid culture and DST are being developed
Automated Molecular Testing for TB and RIF-R: GeneXpert® MTB/RIF Test

Workflow
• sputum
• simple 1-step external sample prep. procedure
• time-to-result < 2 h
• throughput: ≥ 16 tests / day / module
• no need for biosafety cabinet
• integrated controls
• true random access

Performance
• specific for MTB
• sensitivity better than smear, similar to culture
• detection of rif-resistance via rpoB gene

Product and system design
• test cartridges for GeneXpert System
• several GeneXpert modules can be combined in 1 workstation
• swap replacement of detection unit
• ~1 day technician training for non-mycobacteriologists
**Xpert® MTB/RIF - high tech for low tech settings**

Concentrates bacilli & removes inhibitors

1. Sputum liquefaction & inactivation with 2:1 SR
2. Transfer of 2 ml after 15 min
3. Sample is automatically filtered & washed
4. Ultrasonic lysis of filter-captured organisms to release DNA
5. DNA molecules are mixed with dry PCR reagents
6. Nested real-time amplification & detection in integrated reaction tube

**Time-to-result: 1 h 45 min**

*Find,* for Innovative new diagnostics
EXPAND TB - A UNIQUE PARTNERSHIP MODEL

- Policies, norms international standards
- Participate in lab assessments
- Provide long-term, on-site monitoring
- Develop indicators and tools for M&E

- Negotiate with partners to ensure lowest prices
- Ensure customer support in place
- Share know-how from product development process
- Provide long-term, on-site mentoring for technology transfer

- Coordinate and manage procurement and delivery
- With FIND, engage industry to ensure affordability and sustained price decreases
- Collaborate with WHO pre-qualification to include diagnostics

Funding for essential instruments, reagents, supplies

- Logistics and supplies
- Human Resources (Guidelines Technology transfer)
- Infrastructure
- Quality Assurance
- Linked referral systems and reporting

Global Laboratory Initiative

FIND

Global Drug Facility
TARGETS

- Reduce the Dx Gap
- Service up to 1/3 world population
- 30% MDR-TB estimated prevalence
- 27 countries
- Funding M$87 to WHO-GLI, WHO-GDF, FIND
- Assess and strengthen 101 labs
3 MAIN OBJECTIVES

- Improve control of MDR-TB
- Improve market dynamics
- Integrate tools in TB control programmes
3 PROJECT PHASES

Phase 1: Laboratory Preparedness
- Political commitment -- signing MOU - Prerequisite
- Lab assessments
- Infrastructure/biosafety
- Quality Assurance
- SOPs

Phase 2: Introduction of new diagnostics
- Procurement of commodities
- Integration of new diagnostics into screening and treatment guidelines (Training, Validation, Knowledge transfer)

Phase 3: Impact Assessment
- Continued support and oversight of technology transfer
- Impact measured and reported
- Ensuring GLP, IQC and EQA measures
SELECTION OF COUNTRIES

- High-burden MDR-TB Countries
- UNITAID eligible countries
- GLC approved project
- Partner support infrastructure & tech transfer
WORLDWIDE COMMITMENT

2009: 6 countries
2010: 18 countries including India (43 labs)
2011: 3 countries
EXPAND TB in EURO

- 4 CAR: Uzbekistan, Kyrgyzstan, Kazakhstan, Tajikistan and additionally Azerbaijan, Georgia, Belarus and Moldova
- All countries have implemented automated liquid culture and DST
- LPA technology in various phases of implementation