Updated guidance on molecular tests for the diagnosis of TB and drug-resistant TB

WHO /ATS TB Treatment Guideline
21st Annual Conference, The Union – North America Region,
Vancouver, Canada, 22nd February 2017
Outline

1. WHO End TB Strategy
2. Available technologies to diagnose DR-TB
3. New diagnostic policies
4. Achieving End TB Strategy targets
Diagnosis and treatment of TB and MDR-TB in the END TB Strategy?

Early diagnosis of tuberculosis including universal access to drug susceptibility testing, and systematic screening of contacts and high-risk groups

Treatment of all people with tuberculosis including drug-resistant tuberculosis, and patient support
WHO’s recommended techniques for diagnosing TB

- **Microscopy**
  - Conventional light microscopy
  - Light-emitting diode fluorescent microscopy

- **Culture**
  - Culture on solid media
  - Commercial liquid culture systems and rapid speciation

- **Drug-susceptibility testing**
  - DST first-line anti-TB agents
  - DST for second-line anti-TB agents
  - Non-commercial methods

- **Molecular testing**
  - LPA (first and second-line)
  - TB-LAMP
  - Xpert MTB/RIF assay (Ultra)

- **LF-LAM Urine test for PLHIV**
Interest in TB is at an all time high and the pipeline of technologies is robust

- Majority of technologies developed for the intermediate and central level laboratories
- More technologies suitable for the peripheral level as are replacement for microscopy are needed
- Greater investment in conducting the field evaluation and demonstration studies in high burden setting is needed
Phenotypic methods for the diagnosis of DR-TB

Phenotypic, culture methods are based on assessment of the ability of *M. tuberculosis* to grow in culture media (solid or liquid) containing a critical concentration of specific anti-TB agents (which indicates resistance) or, conversely, its inability to grow in the same media (which indicates susceptibility).

The indirect proportion method is the most common method. Resistance is defined when at least 1% of growth is observed at the critical concentration of drug in the culture medium.

Commercial liquid culture systems for DST reduce the time to result to as little as 10 days, compared with the 28–42 days needed for DST using solid media.
Phenotypic methods for the diagnosis of DR-TB - Uncertainty

Phenotypic DST for first-line agents (isoniazid and rifampicin), and selected second-line anti-TB drugs (kanamycin, amikacin, ofloxacin, levofloxacin) is generally reliable and reproducible.

New and repurposed drugs for the treatment of MDR-TB such as bedaquiline, delamanid, linezolid clofazamine - DST methods need validation.

Other anti-TB agents such as the later generation fluoroquinolones (moxifloxacin and gatifloxacin), capreomycin, thioamides, cycloserine and pyrazinamide are becoming increasingly important in the treatment of DR-TB and there is a need for their critical concentrations to be re-evaluated.
Molecular methods for the diagnosis of DR-TB

Molecular (genotypic) methods detect specific DNA mutations in the genome of the *M. tuberculosis*, which are associated with resistance to specific anti-TB drugs.

Molecular methods have considerable advantages for programmatic management of drug-resistant TB, in particular with regard to their speed, the standardization of testing, their potentially high throughput and the reduced requirements for laboratory biosafety.

Molecular tests for detecting drug resistance to rifampicin alone or in combination with isoniazid have been recommended for use by WHO since 2008.
Molecular methods for the diagnosis of DR-TB - limitations

There is imperfect correlation between phenotypic and genotypic methods.

Molecular methods had high specificity but lower sensitivity which varies for different drugs:
- Rifampicin – *rpoB* 95% sensitivity, 99% specificity
- Isoniazid – *inhA* and *katG* ~90% sensitivity, 99% specificity
- Fluoroquinolones – *gyr A* and *gyrB* ~86% sensitivity, 99% specificity
- Secondline injectable agents – *rrs* and *eis* ~86% sensitivity, 99% specificity

The predictive values of imperfect tests depend on the pre-test probability of resistance.
Xpert MTB/RIF

2010 Policy Recommendation
Xpert MTB/RIF is recommended rather than conventional microscopy, culture and DST as the initial diagnostic test in adults presumed to have MDR-TB or HIV-associated TB.

2013 Policy Update
Xpert MTB/RIF is recommended rather than conventional microscopy and culture as the initial diagnostic test in all adults and children with signs and symptoms of TB.

Xpert MTB/RIF remains the only WHO-recommended diagnostic test that can simultaneously detect TB and rifampicin resistance that is suitable for use at lower levels of the health system.
Policy update on LPAs

New version 2 of the Hain MTBDRplus assay available

New manufacturer of LPA – Nipro Corporation, Tokyo

Both assays show equivalence to Hain version 1.

Guideline Development Group convened by WHO in March 2016

New guidance recommends the use of LPA as the initial test for the detection of resistance to rifampicin and isoniazid in sputum smear – positive specimens and cultures of MTBC

Examples of different line probe assays strip readouts:
- a) Hain GenoType MTBDRplus V1 and V2 strip readout
- b) Nipro NTM+MDR Detection Kit 2 strip

>500 LPA laboratories had been established in low and middle-income countries
WHO recommends the use of the SL-LPA for patients with confirmed rifampicin-resistant TB or MDR-TB as the initial test to detect resistance to fluoroquinolones and the second-line injectable drugs, instead of phenotypic culture-based drug-susceptibility testing (DST).

500 LPA laboratories had been established in low and middle-income countries.
Determining the outcomes of using the test to detect resistance conferring mutations for fluoroquinolones in different prevalences

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<th>FQ resistance prevalence</th>
<th>PPV</th>
<th>NPV</th>
<th>True positive</th>
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Sensitivity 86% Specificity 99%
Linking diagnosis and treatment of TB and MDR-TB

Patient diagnosed with rifampicin-resistant TB (using Xpert MTB/RIF or LPA) (sputum smear status unknown)

SL-LPA direct

80% interpretable results

- FQ-S / SLID-S
- FQ-R / SLID-S
- FQ-S / SLID-R
- FQ-R / SLID-R

Initiate the shorter MDR-TB regimen

Initiate an optimised longer MDR-TB regimen

20% uninterpretable results

- No result

Perform Culture

SL-LPA Indirect testing
Additional USAID priority countries:

- Armenia
- Benin
- Burkina Faso
- Cote d’Ivoire
- Georgia
- Guinea
- Haiti
- Bangladesh
- DPR Korea
- Pakistan
- Philippines
- Russia
- Viet Nam
- Azerbaijan
- Belarus
- Kazakhstan
- Kyrgyzstan
- Peru
- Rep. Moldova
- Somalia
- Tajikistan
- Ukraine
- Uzbekistan
- Botswana
- Cameroon
- Chad
- Ghana
- Guinea-Bissau
- Malawi
- Swaziland
- Uganda
- Brazil
- Congo
- Lesotho
- Liberia
- Namibia
- UR Tanzania
- Zambia
- Angola
- China
- DR Congo
- Ethiopia
- India
- Indonesia
- Kenya
- Mozambique
- Myanmar
- Nigeria
- PNG
- South Africa
- Thailand
- Zimbabwe

Countries in red lack LPA capacity (2014 data)
GeneXpert Omni and Xpert Ultra

- Small and Portable
- Durable
- Low Power Consumption
- Automatic Connectivity
- Solid State
- Integrated Battery

A multi-centre non-inferiority diagnostic accuracy study of the new Ultra assay compared with the Xpert MTB/RIF assay was evaluated by a Technical Expert Group in January 2017

Evaluation the Omni instrument expected towards the end of 2017
Current knowledge gaps

1. Correlation of phenotypic DST critical concentrations with molecular methods;
2. Incomplete cross resistance within the classes of key drugs such as the FQs;
3. Which mutations are associated with elevated MICs for certain drugs;
4. The important of knowledge of drug resistance prevalences;
5. Molecular basis of resistance for new and repurposed drugs remains uncertain
Achieving early diagnosis and universal access to DST

- Requires rapid molecular diagnosis at the first entry point to the health system.
- All bacteriologically confirmed case require a rapid DST (at least rifampicin).
- All rifampicin-resistant TB or MDR-TB require rapid second-line DST.
- Conventional microscopy and culture required for monitoring TB patients response to therapy.
- This requires a functional laboratory network with strong sample referral mechanism.
THANK YOU

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