Frequently asked questions about the molecular line probe assay for the detection of resistance to second-line anti-tuberculosis drugs (SL-LPA)

Version: 6 May 2016

These notes are to be read alongside the WHO policy guidance: “The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs – 2016 (WHO/HTM/TB/2016.07) published by the Global TB Programme of the World Health Organization (WHO) in 2016 (see references).

Why is it important to address drug-resistant TB?
Drug resistance is fuelled by inadequate treatment; once TB bacteria acquire drug resistance they can spread from person to person in the same way as drug-susceptible TB. While 6-month standardized regimens with first-line drugs can effectively treat the majority of TB patients, those with strains resistant to rifampicin (the most effective TB drug) and those with multidrug-resistant TB (MDR-TB) require specialized treatment involving second-line drugs. These treatment regimens are much longer and costly, and involve drugs which are more toxic and less effective than first-line drugs. Once the second-line drugs are compromised due to resistance, treatment options for patients become very limited and even more toxic and expensive.

MDR-TB is a major global public health problem and threatens progress made in TB care and prevention in recent decades. Each year MDR-TB leads to about 480,000 new cases and 190,000 deaths worldwide. By some estimates, MDR-TB and other forms of antimicrobial resistance could become a more important cause of death than cancers in the world by 2050, costing US$ 100 trillion to control if no action is taken. Extensively drug resistant TB (XDR-TB) has been reported by 105 countries. About 9.7% of patients with MDR-TB have XDR-TB globally. However, XDR-TB is more common among MDR-TB patients in some countries in Eastern Europe and Central Asia.

How is TB drug resistance detected?
TB drug resistance is detected in the laboratory using a variety of tests. Conventional so-called ‘phenotypic testing’ identifies resistant bacteria that survive despite the presence of specific TB drugs which are added to the substrate in which they grow (called culture medium). So-called ‘genotypic testing’ detect DNA mutations in the bacteria themselves that are responsible for drug resistance.

Which molecular tests are recommended by WHO for the diagnosis of MDR-TB?
The first molecular test to be recommended by WHO (in 2008) for detection of resistance was a commercial line probe assay (LPA) - GenoType® MTBDRplus, Hain Lifescience, Nehren, Germany, for detection of resistance to rifampicin. This was followed by Xpert MTB/RIF, recommended by WHO in 2010 for simultaneous detection of TB and rifampicin resistance and updated in 2013 with more extensive recommendations http://www.who.int/tb/publications/xpert-mtb-rif-assay-diagnosis-policy-update/en/.

1 Combined resistance to at least isoniazid and rifampicin
2 Resistance to any fluoroquinolone, and at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin), in addition to multidrug resistance
In May 2016, WHO issued new policy which recommends LPA for the detection of resistance to second-line TB drugs among patients diagnosed with rifampicin-resistant TB or MDR-TB. This SL-LPA detects additional resistance to fluoroquinolones (such as levofloxacin or moxifloxacin) and second-line injectable drugs (kanamycin, capreomycin, amikacin). The new WHO recommendation applies to the use of the commercial Genotype® MTBDRsl assay, Hain Lifescience, Nehren, Germany SL-LPA only. Other LPAs for the detection of resistance to second-line anti-TB agents were not evaluated.

**Why is WHO releasing its recommendations on the SL-LPA now?**

The SL-LPA has been available for several years and were evaluated in a number of studies. In September 2013, a World Health Organization (WHO) Guideline Development Group reviewed the evidence (11 published and 7 unpublished studies) and recommended that SL-LPA not be used as a replacement test for phenotypic drug susceptibility testing (DST) and noted, in addition, that the assay did not allow for detection of specific resistance to individual drugs with the second-line injectable drugs or fluoroquinolone classes (http://www.who.int/tb/publications/who_hm_tb_2013_01/en/). Subsequently, at least two systematic reviews have been published, and a new version of the test (version 2.0) has become commercially available. A Cochrane review on the diagnostic accuracy of version 1.0 of Genotype® MTBDRsl assay (Theron 2014) included 21 studies published up to 30 January 2014. Several additional studies have since been performed, including studies that have evaluated MTBDRsl version 2.0, necessitating an updated systematic review.

**Why is the use of SL-LPA important?**

Similar to any other diagnostic test, the SL-LPA is not a perfect test. Nevertheless, it is a rapid test that allows quick triage of MDR-TB patients into either the shorter MDR-TB regimen or the conventional longer regimen. Detection of any second-line resistance by the SL-LPA means that MDR-TB patients should not be enrolled on the shorter regimen as this could jeopardise their treatment outcome and fuel the development of XDR-TB. These patients therefore need to be put on conventional MDR-TB regimens according to existing WHO policy guidance. Patients detected with XDR-TB by the SL-LPA should also not be enrolled on the shorter regimen but require carefully designed individual regimens to optimise their chances of success.

**What are the prerequisites for establishing SL-LPA at country level?**

Adoption of LPA should be phased in, starting at national/central reference laboratories or those with proven capability to conduct molecular testing. Expansion could be considered, within the context of country laboratory strengthening plans, availability of suitable personnel in peripheral centres and quality of specimen transport systems.

Adequate and appropriate laboratory infrastructure and equipment should be provided, ensuring that required precautions for biosafety and prevention of contamination are met: specimen processing for culture and procedures for manipulation of cultures must be performed in biological safety cabinets (BSCs) in TB-containment laboratories. Laboratory facilities for LPA require at least three separate rooms - one each for DNA extraction, pre-amplification procedures, and amplification and post-amplification procedures. Restricted access to molecular facilities, unidirectional work flow, and stringent cleaning protocols must be established to avoid contamination.

Appropriate laboratory staff should be trained to conduct LPA procedures. Supervision of staff by a senior individual with adequate training and experience in molecular assays is strongly recommended. A programme for external quality assessment of involved laboratories should be developed as a priority. Mechanisms for rapid reporting of LPA results to clinicians must be established to provide patients with the benefit of an early diagnosis. The infrastructure available for 1st line LPA can be used also for 2nd line LPA.
By 2014 approximately 400 LPA laboratories had been established in low and middle-income countries, as reported to WHO.

**How much does testing with SL-LPA cost?**

FIND (an international non-profit organization that enables the development and delivery of much-needed diagnostic tests for poverty-related diseases) has negotiated a preferential price for a group of eligible countries at € 7.50 per one test (a strip) - GenoType® MTBDRsl 1.0 [http://www.finddx.org/pricing/](http://www.finddx.org/pricing/). The operational cost for the health system of doing the assay which includes equipment and consumables is estimated at USD 20 per test. Warranty and human resources are excluded from this estimation. The human resources cost is very country specific and may add large variability to this estimation. The additional costs which may apply include custom and other clearance fees, transportation from the port of entry to the final destination, infrastructure costs, training for laboratory staff and clinicians.

**Can SL-LPA procurement be supported by donor organizations?**

It is essential that the introduction of the SL-LPA is coordinated at the country level. Technical agencies and donors need to work within the framework of national TB and HIV/AIDS programmes to assist in the implementation of the new tool. Many international donors have been active in supporting countries during the implementation of new technologies to diagnose TB. The Global Fund for AIDS, Tuberculosis and Malaria, UNITAID, US President’s Emergency Plan for AIDS Relief and the United States Agency for International Development are some of the largest supporters of TB diagnostic technologies in affected countries.

**References**


Factsheet on molecular line probe assays for the detection of resistance to second-line antituberculosis drugs (SL-LPA)