MOLECULAR LINE-PROBE ASSAY FOR THE DETECTION OF RESISTANCE TO ISONIAZID AND RIFAMPICIN (LPA)

BACKGROUND

- Multidrug-resistant tuberculosis (MDR-TB) is a public health crisis and a major threat to global TB control.
- Globally in 2015, there were 480,000 new cases of MDR-TB and an additional 100,000 cases with rifampicin-resistant TB newly eligible for MDR-TB treatment.
- WHO recommends the use of LPA as a rapid diagnostic test for detection of rifampicin and isoniazid resistance. The WHO recommended commercially available tests include GenoType MTBDRplus VER 1 and 2 (Hain Lifescience, Germany), Nipro NTM+MDRTB detection kit 2 (Nipro, Japan).

ABOUT THE TESTS

- The Hain version 1 and 2 assays include probes to identify Mycobacterium tuberculosis complex (MTBC), and detect mutations in the rpoB gene (associated with rifampicin resistance); in the katG gene and in the inhA promoter region (associated with isoniazid resistance). The probes used are the same for both versions of the assay.
- The Nipro assay allows detection of MTBC and resistance to rifampicin and isoniazid. This assay also differentiates M. avium, M. intracellulare and M. kansasii from MTBC and from other non-tuberculous mycobacteria.

COSTS

- FIND has negotiated a preferential price of Euro 7.50 (approx. USD 10) for the MTBDRplus strips in 138 countries (http://www.finddx.org/pricing/); however, doing the test requires other laboratory consumables and supplies which may push the cost up to between USD20 and USD30.
- The cost of the equipment ranges between ~USD 8,000 and ~USD40,000 depending on its capacity and whether results are read automatically or not.

DETECTION OF RESISTANCE TO ISONIAZID

- Resistance-conferring mutations in inhA and katG genes account for approximately 90% of isoniazid resistance detected by phenotypic DST methods. Different mutations are associated with different levels of minimal inhibitory concentrations (MICs) for isoniazid in phenotypic testing.
- Mutations in the promoter region of the inhA gene are normally associated with low-level resistance to isoniazid, which is generally exceeded by serum concentration after normal dosing of drug. This mutation also confers cross-resistance to ethionamide and prothionamide.
- The presence of katG mutations alone is associated with elevated MICs, and can be overcome by a high dose of isoniazid. Although resistance associated with katG is almost always encoded by the same mutation (S315T), MICs vary considerably. In only a minority of strains with this mutation are therapeutically achievable levels exceeded.
- A combination of mutations in inhA and katG are associated with very high level resistance to isoniazid in phenotypic testing.

Presence of either inhA or katG mutations alone should not exclude patients from enrolment on for the WHO recommended shorter MDR-TB regimen


For more information please visit: www.who.int/tb © World Health Organization October 2016

Courtesy of Hain Lifescience, Nehren, Germany
WHO
RECOMMENDATIONS
ON THE USE OF THE LPA

http://www.who.int/tb/areas-of-work/laboratory/policy_statements

POLICY RECOMMENDATION
For persons with a sputum smear-positive specimen or a cultured isolate of *M. tuberculosis* complex (MTBC), commercial LPAs may be used as the initial test instead of culture-based DST to detect resistance to rifampicin and isoniazid (conditional recommendation, moderate certainty in the evidence for the test’s accuracy).

REMARKS
- These recommendations apply to the use of LPAs for testing sputum smear-positive specimens (direct testing) and cultured isolates of MTBC (indirect testing) from both pulmonary and extrapulmonary sites.
- LPAs are not recommended for the direct testing of sputum smear-negative specimens.
- These recommendations apply to the detection of MTBC and the diagnosis of MDR-TB but acknowledge that the accuracy of detecting resistance to rifampicin and isoniazid differs and that the overall accuracy of MDR-TB diagnosis is therefore reduced.
- Conventional culture-based DST remains necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance.
- Culture-based DST for isoniazid may still be used to evaluate patients when the LPA result does not detect isoniazid resistance. This is particularly important for populations with a high pre-test probability of resistance to isoniazid.
- These recommendations apply to the use of LPA in children.

ESTABLISHING LPA AT COUNTRY LEVEL
- LPA is suitable for use at national/central reference laboratories or those with proven capability to conduct molecular testing. Expansion to more decentralised laboratories could be considered depending on availability of suitable laboratory infrastructure, specially trained personnel and adequate quality assurance of testing.
- Adequate laboratory infrastructure and equipment must be available, including the necessary biosafety precautions and prevention of contamination: specimen processing for culture and manipulation of cultures require TB containment laboratories with appropriate biological safety cabinets. 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 availability of second-line TB agents is critical in the event that resistance to rifampicin or isoniazid, or both, is detected.
- For patients with confirmed rifampicin resistance TB or MDR-TB, second-line LPAs are recommended to detect additional resistance to second-line anti-TB agents.

Estimated incidence of MDR/RR-TB for countries with at least 1000 incident cases, 2015