THE USE OF MOLECULAR LINE PROBE ASSAY FOR THE DETECTION OF RESISTANCE TO SECOND-LINE ANTI-TUBERCULOSIS DRUGS

EXPERT GROUP MEETING REPORT
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Executive summary

Background

Genotypic (molecular) methods have considerable advantages for scaling up programmatic management and surveillance of drug-resistant TB, offering speed of diagnosis, standardised testing, potential for high through-put, and fewer requirements for laboratory biosafety. Molecular line probe assay (LPA) technology for rapid detection of multi-drug resistant tuberculosis (MDR-TB) was endorsed by the World Health Organization (WHO) in 2008. In 2009, Hain Lifescience introduced a new LPA, the Genotype MTBDR*sl*® test, for the rapid determination of genetic mutations associated with resistance to fluoroquinolone, aminoglycosides (kanamycin, amikacin), cyclic peptides (capreomycin), ethambutol, and streptomycin. The assay format is similar to the Genotype MTBDR*plus* assay for the detection of mutations conferring rifampicin and isoniazid resistance, endorsed by WHO in 2008, and allows for testing and reporting results within 24 hours.

In September 2010, FIND presented the results of its field evaluation studies to an Expert Group convened by WHO, that additionally considered data from other published and unpublished studies. The FIND studies were conducted at the US Centers for Disease Control and Prevention (CDC), the Korea International Tuberculosis Research Center (ITRC), and the University of Cape Town (UCT). The Expert Group concluded that although the available data suggested possible use of the assay for testing culture isolates, too few data on direct testing on sputum specimens were available to develop policy guidance on its use. As well as a paucity of data on direct testing, the Expert Group recommended that additional data from other geographic locations as well as genetic sequencing information from isolates with discordant LPA and phenotypic DST results were needed.

Subsequently, FIND implemented a study of direct testing at ITRC (150 sputum specimens), at Hinduja Hospital in Mumbai, Infis (170 sputum specimens), and provided additional support to UCT for a study that included direct testing of 270 sputum specimens. In addition, the National Health Laboratory Services in Cape Town, South Africa, provided FIND with the results of direct testing on 657 specimens.

In March 2012, WHO again convened an Expert Group that evaluated the utility of the Genotype MTBDR*sl* as a replacement test for conventional drug susceptibility testing (DST). This report summarizes the evidence evaluated by the Expert Group, from 11 published and 7 unpublished studies on the MTBDR*sl*® assay, including results from direct testing on clinical specimens and indirect testing of *M. tuberculosis* isolates. Pooled estimates for sensitivity and specificity for each class of second-line anti-TB drug were determined, for both direct and indirect testing.

Summary of results

Diagnostic accuracy for the detection of fluoroquinolone resistance: Thirteen studies evaluated indirect testing for fluoroquinolone resistance among 2,354 individuals. Eight of these studies used a cross-sectional design and five studies used a case-control design. Sensitivity varied from 57.1% to 97.4% and specificity from 77.3% to 100.0%. One small study, Lacoma *et al*. 2011 (n=29) that evaluated DST for moxifloxacin, had outlier estimates for sensitivity (57.1%) and specificity (77.3%). When this study was excluded, the range in sensitivity and specificity estimates was still wide at 70.3% to 97.4% and 88.1% to 100% respectively. 11 studies specifically evaluated ofloxacin resistance among 2,110 individuals. Sensitivity varied from 70.3% to 97.4% and specificity from 88.1% to 100.0%.

Seven studies evaluated the diagnostic accuracy for the detection of fluoroquinolone resistance with direct testing among 1,121 individuals. Sensitivity varied from 37.5%-100.0% and specificity from 93.7% to 100.0%. Six of these studies specifically evaluated ofloxacin resistance among 1,069

individuals. Sensitivity varied from 68.2% to 100.0% and specificity from 93.7% to 100.0%. One small study, Lacoma *et al*. 2011 (n=52) that evaluated DST for moxifloxacin, had a sensitivity estimate of 37.5%. When this study was excluded, the range in sensitivity estimates remained wide at 68.2% to 100.0%.

Overall, indirect testing for fluoroquinolones showed a pooled sensitivity of 88.8% (95%CI 82.7, 92.9) and pooled specificity of 97.9% (95% CI 94.8, 99.2). Direct testing for fluoroquinolones showed a pooled sensitivity of 83.5% (95%CI 69.1, 91.9) and pooled specificity of 97.4% (95% CI 95.7, 98.4).

Diagnostic accuracy for the detection of kanamycin resistance

Ten studies evaluated indirect testing for kanamycin resistance among 1,976 individuals. Six of these studies used a cross-sectional design and four studies used a case-control design. Sensitivity varied from 25.0% to 100.0% and specificity from 86.4% to 100%. Four studies evaluated the diagnostic accuracy for the detection of kanamycin resistance with direct testing among 400 individuals. Sensitivity varied from 25.0% to 100.0% and specificity from 86.4% to 100.0%.

Overall, indirect testing showed a pooled sensitivity of 67.0% (95%CI 50.4, 80.2) and pooled specificity of 99.4% (95% CI 97.0, 99.9). Direct testing showed a pooled sensitivity of 96.2% (95%CI 67.5, 99.7) and pooled specificity of 99.0% (95% CI 78.4, 100.0).

Diagnostic accuracy for the detection of amikacin resistance

Seven studies evaluated indirect testing for amikacin resistance among 1,213 individuals. Four of these studies used a cross-sectional design and three studies used a case-control design. Sensitivity varied from 80.4% to 100.0% and specificity from 94.2% to 100%. Six cross-sectional studies evaluated the diagnostic accuracy for the detection of kanamycin resistance with direct testing among 1021 individuals. Sensitivity varied from 75.0% to 100.0% and specificity from 89.4% to 100.0%.

Overall, indirect testing showed a pooled sensitivity of 89.6% (95%CI 84.0, 93.5) and pooled specificity of 99.5% (95% CI 96.1, 100). Direct testing showed a pooled sensitivity of 93.2% (95%CI 76.8, 98.3) and pooled specificity of 99.4% (95% CI 95.7, 100.0).

Diagnostic accuracy for the detection of capreomycin resistance

Nine studies evaluated indirect testing for capreomycin resistance among 1,539 individuals. Five of these studies used a cross-sectional design and four studies used a case-control design. Sensitivity varied from 21.2% to 100.0% and specificity from 80.5% to 100%. Four studies, predominately cross-sectional in design, evaluated the diagnostic accuracy for the detection of capreomycin resistance with direct testing among 461 individuals. Sensitivity varied from 66.7%-100.0% and specificity from 86.2% to 100.0%.

Overall, indirect testing showed a pooled sensitivity of 80.3% (95%CI 64.7, 90.1) and pooled specificity of 97.1% (95% CI 92.5, 98.9). Direct testing showed a pooled sensitivity of 97.4% (95%CI 70.4, 99.8) and pooled specificity of 96.6% (95% CI 88.9, 99.0).

Diagnostic accuracy for the detection of extensively drug resistant – TB (XDR-TB)

Six predominately cross-sectional studies evaluated the utility of indirect testing for the detection of XDR-TB among 1,652 individuals. One study used a case-control design. Sensitivity varied from 22.6% to 100.0% and specificity from 93.9% to 100%. Four studies with cross-sectional design evaluated the diagnostic accuracy for the detection of XDR-TB with direct testing among 840 individuals. Sensitivity varied from 80.0%-95.2% and specificity from 91.8% to 100.0%.

Overall, indirect testing showed a pooled sensitivity of 63.3% (95%CI 36.8, 83.5) and pooled specificity of 98.5% (95% CI 96.0, 99.4). Direct testing showed a pooled sensitivity of 90.2% (95%CI 79.0, 95.8) and pooled specificity of 96.6% (95% CI 93.8, 99.9).

Expert Group findings

The Expert Group concluded that the Genotype MTBDR*sl* assay shows moderate test sensitivity for the detection of fluoroquinolone and second-line injectable resistance, with high test specificity. There was significant heterogeneity in the sensitivity for the detection of kanamycin across studies, resulting in the assay being considered to be insufficient. Despite high pooled specificity estimates for all second-line drugs evaluated, the lower pooled sensitivity estimates mean that negative results for resistance cannot be considered to reliably rule-out resistance, as rates of false-negative results were variable among the reported studies and quite high for the detection of resistance to kanamycin.

The Expert Group found that while the test has the potential to be used as a rule-in test for XDR-TB where capacity to use line probe assays is available, it cannot be used as a replacement test for conventional phenotypic drug susceptibility testing (DST). Furthermore, the Expert Group noted that there is incomplete cross-resistance between the second-line injectables, and that the assay does not allow for specific resistance to individual second-line injectables to be determined. Due to the concerns regarding incomplete cross-resistance, the Expert Group concluded that the results of the Genotype MTBDR*sl* assay could not be reliably used to adjust and optimize a Category IV treatment regimen[1].

The Expert Group noted that given high assay specificity for detecting resistance to fluoroquinolones and second-line injectables the results of the Genotype MTBDR*sl* assay could be used to guide the implementation of additional infection control precautions pending the results of phenotypic DST results.

Furthermore, the Expert Group also concluded that phenotypic DST should remain the reference standard for XDR-TB until more data are available, and that countries without LPA capacity should not invest resources in establishing Genotype MTBDR*sl* capacity in the interim.

The GRADE process was used to evaluate the quality of the evidence presented to the Expert Group to determine the suitability of Genotype MTBDR*sl*® assay as a replacement test for conventional phenotypic second-line DST. The quality of evidence was determined to be very low quality. The evidence was downgraded due to inconsistency in the results across studies, imprecision in the confidence intervals for pooled sensitivity and specificity estimates and for indirectness.

Expert Group Recommendations

The Expert Group recommended that the Genotype MTBDR*sl* assay cannot be used as a replacement test for conventional phenotypic DST

Strong recommendation - Very Low Quality of Evidence

Remarks:

1. The Genotype MTBDR*sl* may be used as a rule-in test for XDR-TB but cannot be used to define XDR-TB for surveillance purposes;
2. As cross-resistance between the second-line injectables is incomplete, the Genotype MTBDR*sl* cannot be used to identify individual drugs to be used for treatment;

[1] World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis – 2011 update. WHO/HTM/TB 2011.6. Geneva, Switzerland: WHO, 2011

3. The Genotype MTBDR*sl* may be used to guide infection control precautions while awaiting confirmatory results from conventional phenotypic testing.

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