Xpert MTB/XDR: A ten-color reflex assay suitable for point of care settings to detect isoniazid-, fluoroquinolone-, and second-line injectable drug-resistance directly from *Mycobacterium tuberculosis* positive sputum.

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Abstract

Background: We describe the design, development, analytical performance and a limited clinical evaluation of the 10-color Xpert MTB/XDR assay (CE-IVD only, not for sale in the US). This assay is intended as a reflex test to detect resistance to isoniazid (INH), fluoroquinolones (FLQ), ethionamide (ETH) and second-line injectable drugs (SLID) on unprocessed sputum samples and concentrated sputum sediments which are positive for *Mycobacterium tuberculosis*. The Xpert MTB/XDR assay simultaneously amplifies eight genes and promoter regions in *M. tuberculosis* and analyzes melting temperatures (Tms) using sloppy molecular beacon (SMB) probes to identify mutations associated with INH, FLQ, ETH and SLID resistance. Results: The assay can differentiate low versus high-level resistance to INH and FLQ as well as cross-resistance versus individual resistance to SLIDs by identifying mutation-specific Tms or Tm patterns generated by the SMB probes. The assay has a limit of detection comparable to the Xpert MTB/RIF assay and succesfully detected 16 clinically significant mutations in a challenge set of clinical isolate DNA.

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In a clinical study performed at two sites with 100 sputum and 214 clinical isolates, the assay showed a sensitivity of 94-100% and a specificity of 100% for all drugs except for ETH when compared to sequencing. The sensitivity and specificity when compared to phenotypic drug-susceptibility testing were in the same range. Conclusion: Used in combination with a primary tuberculosis diagnostic test, this assay should expand the capacity for detection of drug-resistant tuberculosis near the point of care.