Simultaneous Molecular Detection of *Mycobacterium Tuberculosis* and Multidrug Resistance Using CRISPR-Cas12b-Based Nucleic Acid Assay

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Abstract

Objectives To address the unmet need for rapid, accurate diagnosis of *Mycobacterium tuberculosis* (MTB) and multidrug-resistant tuberculosis (MDR-TB), we developed and validated a CRISPR-Cas12b-based diagnostic assay. **Methods** A CRISPR-Cas12b detection system was established to target the MTB-specific IS6110 sequence, and the most prevalent rifampicin-resistant mutation *rpoB* 1349C>T and isoniazid-resistant mutation *katG* 944G>C, enabling signal readout via both fluorescent platform and lateral flow chromatography (LFC) for dual-mode detection. A total of 48 clinical samples were used to evaluate the diagnostic performance of the established multiplex-RPA-CRISPR-AaCas12b assay for TB/MDR-TB, with results compared to WHO-recommended GeneXpert MTB/RIF, phenotypic drug susceptibility testing (pDST), and sequencing as reference standards. **Results** The CRISPR-AaCas12b-based TB assay demonstrated a sensitivity of 97.14% and a specificity of 100.0%, with a total turnaround time of 30 minutes, 20 minutes for RPA amplification and 10 minutes for CRISPR cleavage. The CRISPR-AaCas12b-based MDR-TB assay exhibited a sensitivity of 94.12% and a specificity of 100.0% for detection of the *rpoB* 1349C>T mutation, and a sensitivity of 94.74% and a specificity of 87.5% for the *katG* 944G>C mutation. Crucially, the LFC-integrated assay maintained equivalent diagnostic performance for TB and associated mutations, with a readout time of only 15 minutes. **Conclusion** The established CRISPR-AaCas12b assay enables simple, accurate, and sensitive detection of TB and MDR-TB. With its streamlined workflow and low resource requirements, this method holds great promise for rapid point-of-care diagnosis in resource-limited settings, facilitating the control of TB and drug-resistant TB.

Keywords: CRISPR-Cas; tuberculosis (TB); multidrug-resistant tuberculosis (MDR-TB); lateral flow chromatography (LFC)

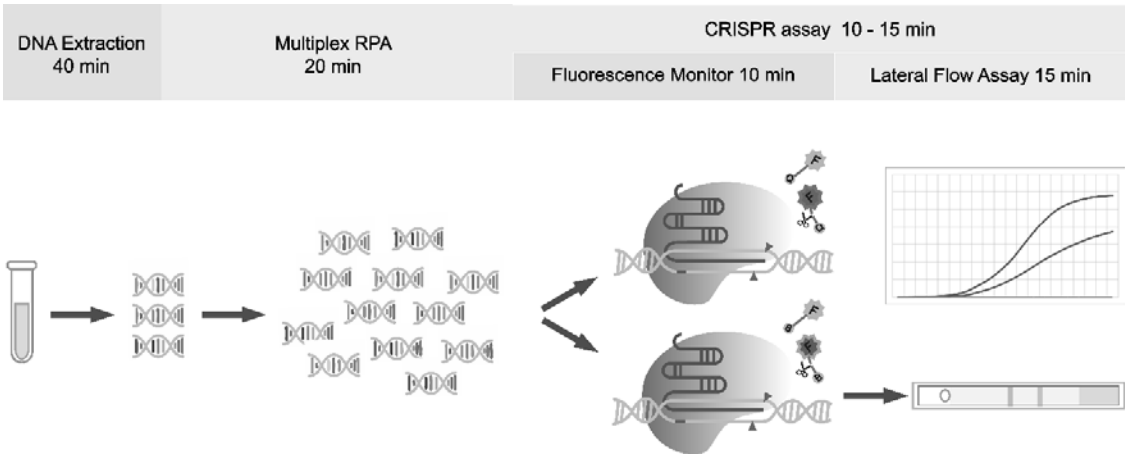

Fig 1 Schematic workflow of the multiplex-RPA-CRISPR-Cas12b assay for simultaneous detection of MTB and drug resistance.

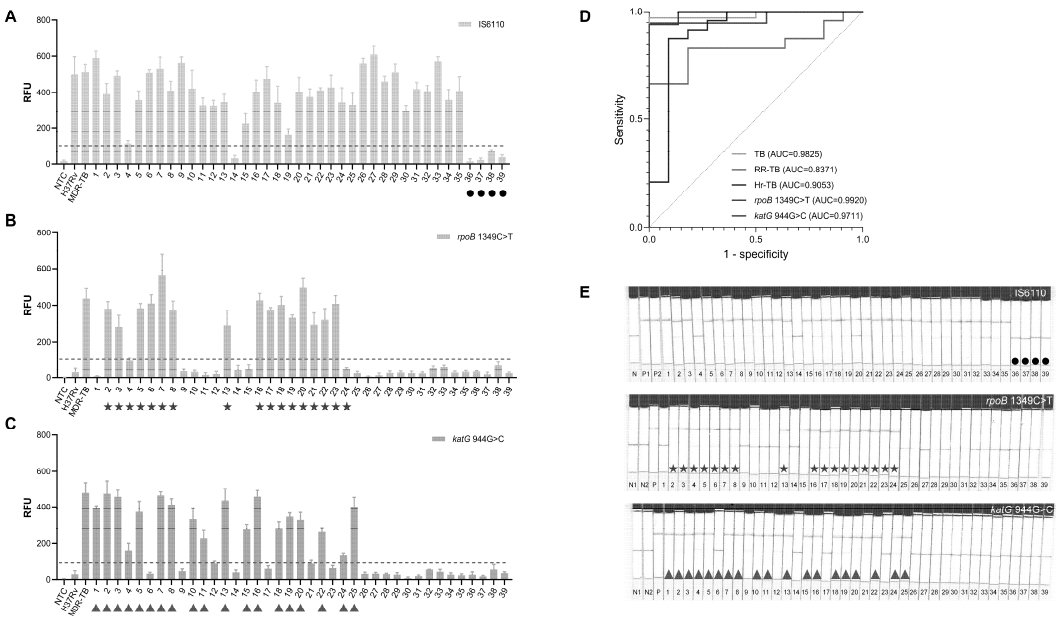

Fig 2. Detection of clinical specimens by Multiplex-RPA CRISPR-AaCas12b TB/MDR-TB assay. A) IS6110-targeted detection of MTB in clinical specimens. **B)** *rpoB* 1349C>T-targeted detection of rifampicin-resistant TB (RR-TB) in clinical specimens. **C)** *katG* 944G>C-targeted detection of isoniazid-resistant TB (Hr-TB) in clinical specimens. **D)** Receiver operating characteristic (ROC) curve analysis of Multiplex-RPA CRISPR-AaCas12b TB/MDR-TB assay in detection of MTB, RR-TB, Hr-TB, *rpoB* 1349C>T and *katG* 944G>C. **E)** Lateral flow chromatography (LFC) results of clinical specimens via Multiplex-RPA CRISPR-AaCas12b TB/MDR-TB assay: the lower red line indicates the uncleaved ssDNA probe region, while the upper red line represents the binding site for cleaved ssDNA probes. Black plots: clinical samples identified as nontuberculous mycobacteria (NTM) by culture and identification; Gray stars: samples with the *rpoB* 1349C>T mutation confirmed by sequencing; Gray triangles: samples with the *katG*

944G>C mutation confirmed by sequencing.

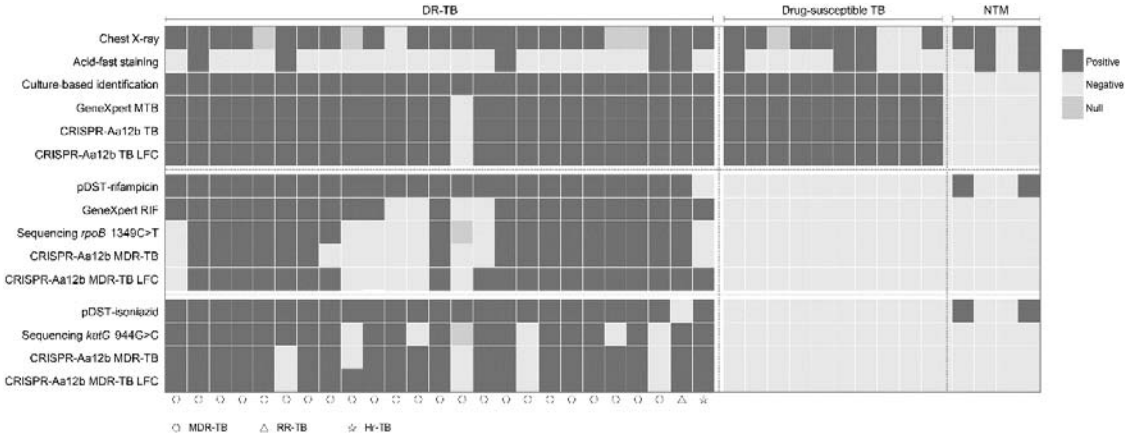

Fig 3. Diagnostic performance of the CRISPR-Aa12b assay in clinical specimens. DR-TB = drug-resistant tuberculosis; NTM = Non-tuberculous Mycobacteria; Strains (left to right): *Mycobacterium abscessus complex* (MABC), *M. kansasii*, *M. gordonae*, *M. malmoense*.