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Targeted next-generation sequencing approach for simultaneous *Mycobacterium* species identification and detection of resistance-associated mutations in *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (MTB) and nontuberculosis mycobacteria (NTM) infections exhibit similar symptoms, challenging pulmonary disease control. We developed the iDetect® NGS MycoChase-Hyb assay, a hybridization capture panel for simultaneous *Mycobacterium* species identification and MTB resistance detection. The assay targets *hsp65*/*rpoB* for identification and WHO-curated mutations for 15 anti-MTB drugs. Performance was assessed using 56 reference strains, 47 non-mycobacterial organisms, 115 clinical isolates, and validation methods. MTB detection limit was 313.8 copies/μL, NTMs 720.6-1066.3 copies/μL. Species identification accuracy was 96.4% with no cross-reactivity. At 1.5× LOD, coefficients of variation were 13.3-19.6% for repeatability and 14.1-19.0% for reproducibility across operators and reagent lots. Resistance detection accuracy was 100% for major drugs (isoniazid, rifampicin, fluoroquinolones). Low variant

allele frequency (VAF) mutations were detected, including *rpoB* p.His445Asp (60.2% VAF) and *rpoB* p.Ser450Leu (38.0% VAF) variants indicating heteroresistance. A *ddn* p.Met21fs mutation conferring delamanid resistance demonstrated detection of resistance to new therapeutics. The assay shows robust performance for species identification and resistance profiling, supporting routine diagnostic use.