M-CATCH: A Multiplex Microparticle CRISPR Assay for Mycobacterium Tuberculosis Drug Resistance Characterization

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Diagnosing drug-resistant strains Mycobacterium tuberculosis (Mtb) is critical for informing treatment plans and limiting infections. However, existing diagnostics are slow and cover a narrow range of mutations. Most critically, they have a fundamental flaw: many depend on sputum samples. The vast majority of young children and immunocompromised patients often cannot produce them reliably. I developed M-CATCH to address these challenges. M-CATCH is a multiplex CRISPR-Cas12a diagnostic assay that combines PCR pre-amplification with microdroplet microfluidics to rapidly detect TB drug resistant mutations. By targeting circulating cell free DNA (cfDNA) in serum, it enables reliable testing in all populations by replacing sputum collection with the universally accessible blood draw. Finally, the pair-merge microfluidic workflow generates uniform droplets, and merges amplified products with mutation-specific Cas12a mixtures in an integrated workflow. For validation, Mtb genomic DNA was spiked into artificial human serum samples. M-CATCH accurately differentiated rpoB H445Y (rifampicin resistance) and rplC C154R (linezolid resistance) mutations at concentrations as low as 10^2 copies/mL, surpassing the sensitivity of many current molecular assays. Further, the rpoB and rplC panels achieved an overall clinical sensitivity of 97.5% and a specificity of 100%. No cross reactivity was observed with strains of non-tuberculosis mycobacterium. Automated image analysis provided objective, high-throughput interpretation of droplet-level fluorescence. By addressing the fundamental issue with sputum and sustaining high accuracy at low DNA concentrations, M-CATCH offers a scalable, clinically relevant platform for rapid drug-resistant TB diagnosis.

Awards Won:

Third Award of $1,200

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