

REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

Immobilized Crispr Chemistry For Massively Multiplexed Target Detection

Julie L Lucas MRIGlobal **Elaine Bradford** MRIGlobal **Ryan Howard** MRIGlobal **Alyssa Jespersen** MRIGlobal **Kaylee Mathiason** MRIGlobal **Isaac Moran** The Charles Stark Draper Laboratory **Logan Rubio** The Charles Stark Draper Laboratory
Chelsey Smith MRIGlobal **Michael Turo** The Charles Stark Draper Laboratory **Richard A. Winegar** MRIGlobal

Current biosurveillance and diagnostic systems aim to detect a broad range of biothreats and pathogens of interest in a timely and user-friendly manner. Traditional PCR-based detection devices can be used to detect relatively small panels of assays. Typically, it takes weeks to months to develop and transition new assays onto closed platform devices. However, as Ebolavirus, SARS-CoV-2 and Monkeypox virus outbreaks have shown, novel, emerging, and re-emerging pathogens present an ever evolving challenge. We are developing a Massively Multiplexed Detection (MMD) device to detect >500 pathogenic targets in a microfluidic platform. By using CRISPR technologies, we aim to be able to quickly reconfigure the open platform to accommodate new pathogens and/or new sequence variations within 24 hours of discovery.

The current panel for the MMD device includes upper and lower respiratory bacteria and viruses, bacterial anti-microbial resistance genes, fungal pathogens, select agents and their near neighbors, vector-borne viruses, food-borne and water-borne pathogens, zoonotic pathogens, biomarkers for disease severity, and endogenous controls. In addition, several variant assays are included, since alterations in CRISPR guide RNA can distinguish single-nucleotide polymorphisms (SNPs) with specificity. The MMD platform requires advances in automated sample preparation, microfluidics, optics, and immobilized CRISPR-based detection chemistry. In this work we will describe strategies and progress on developing optimized CRISPR-based detection chemistry.

The authors acknowledge support from the DARPA Biological Technologies Office as part of the Detect It with Gene Editing Technologies (DIGET) program funded under the Naval Information Warfare Center contract N66001-21-1-4048 which is awarded to MRIGlobal (prime). The authors thank Craig Willis, Pamela Winegar, Landon Adebisi, and Sarah Pope for programmatic support, The Charles Stark Draper Laboratory for their work on MMD device development and Mammoth Biosciences for reagent and assay design support.