Covid-19 Grant Outcomes and Accomplishments Final Technical Report

Project
23-01

PI
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Personnel
The following eight UNC-Chapel Hill personnel were paid from this award:

Name	Title
Yury Desyaterik	Research Associate
Bidhan Dhar	PostDoc
Sandra Elmore	Research Scientist
William Henley	Research Associate
Vipresh Jain	Software Engineer
John Perry	Research Scientist
Angela Proctor	Research Associate
David Thrower	Research Scientist

Technical Progress Summary

The goal of this project executed by the Ramsey group at UNC-Chapel Hill was development of high throughput, highly multiplexed nucleic acid and protein assays for SARS-CoV-2 and other respiratory diseases. The focus of the work was to address targets relevant to the diagnosis of COVID-19 via development of both Nucleic Acid-Spatially Isolated Reactions in

a Complex Array (NA-SIRCA), as well as serology assays relevant to viral detection utilizing the unique digital array technology of the Ramsey lab.

The NA-SIRCA, also described as digital array polymerase chain reaction (daPCR), functions as shown in Figure 1.

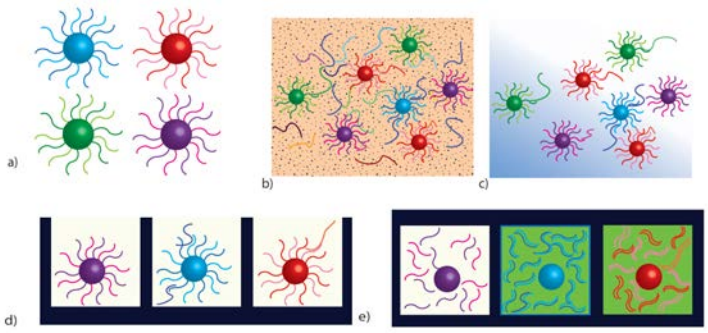

Figure 1: Schematic of the NA-SIRCA strategy. a) Sets of streptavidin-functionalized beads are labeled with unique primer pairs and fluorescent dye combinations. b) A mixture of bead sets is incubated with sample to capture their conjugate NA target before c) being washed to remove the sample matrix. d) The beads and captured targets are magnetically loaded into isolated microwells with master mix before e) sealing the wells from one another. Thermocycling releases the primers from the beads, and amplicons are detected by intercalating dye fluorescence.

Working with our clinical collaborators, the Ramsey lab evaluated published assays for SARS-CoV-2 and designed the assays to integrate appropriate primer pairs for SARS-CoV-2 into a multiplex digital array PCR assay using published sequences. In addition, we re-evaluated a previously developed 12-multiplex respiratory assays for endemic viruses for potential incorporation into a comprehensive multiplex assay. In total, this project investigated 34 primer sequences for SARS-CoV-1, SARS-CoV-2, and MERS to evaluate the potential for these various sequences to be incorporated into a multiplex assay capable of detection of SARS-CoV-2 and differentiation from similar viral pathogens.

Although COVID-19 related hiring restrictions and shortages of assay-related lab supplies and reagents hindered progress, adjustments in personnel allocation and process redesign still enabled significant progress on this effort. Results included functionalization of encoded bead sets and testing with all primer pairs for the NA-SIRCA assay. To date, testing has demonstrated low to moderate background signal for most primer pairs. We did not attempt to modify underperforming sequences, as the purpose of testing many different pairs was to identify the best performing pairs for assay testing. We also experienced delays in receiving COVID-19 positive human samples for molecular assay evaluation. However, this funding has been enabling for critical assay development work, and the preliminary work executed has been leveraged toward two additional COVID-19 related projects funding by federal agencies, as detailed below.

The protein SIRCA functions in a manner similar to the nucleic acid assays, but uses nucleic acid tagged antibodies for detection. For serology assays, protein antigens are attached to our encoded bead sets. Again, working closely with our clinical collaborator, we identified

candidate assays for SARS-CoV-2, SARS-CoV-1, and endemic coronaviruses. Assays were developed using the protein SRICA approach and tested in the laboratory. In addition, serology assays developed under this funding were tested in serum using de-identified serum specimens (not human subjects research), yielding promising results, as shown in Figure 2.

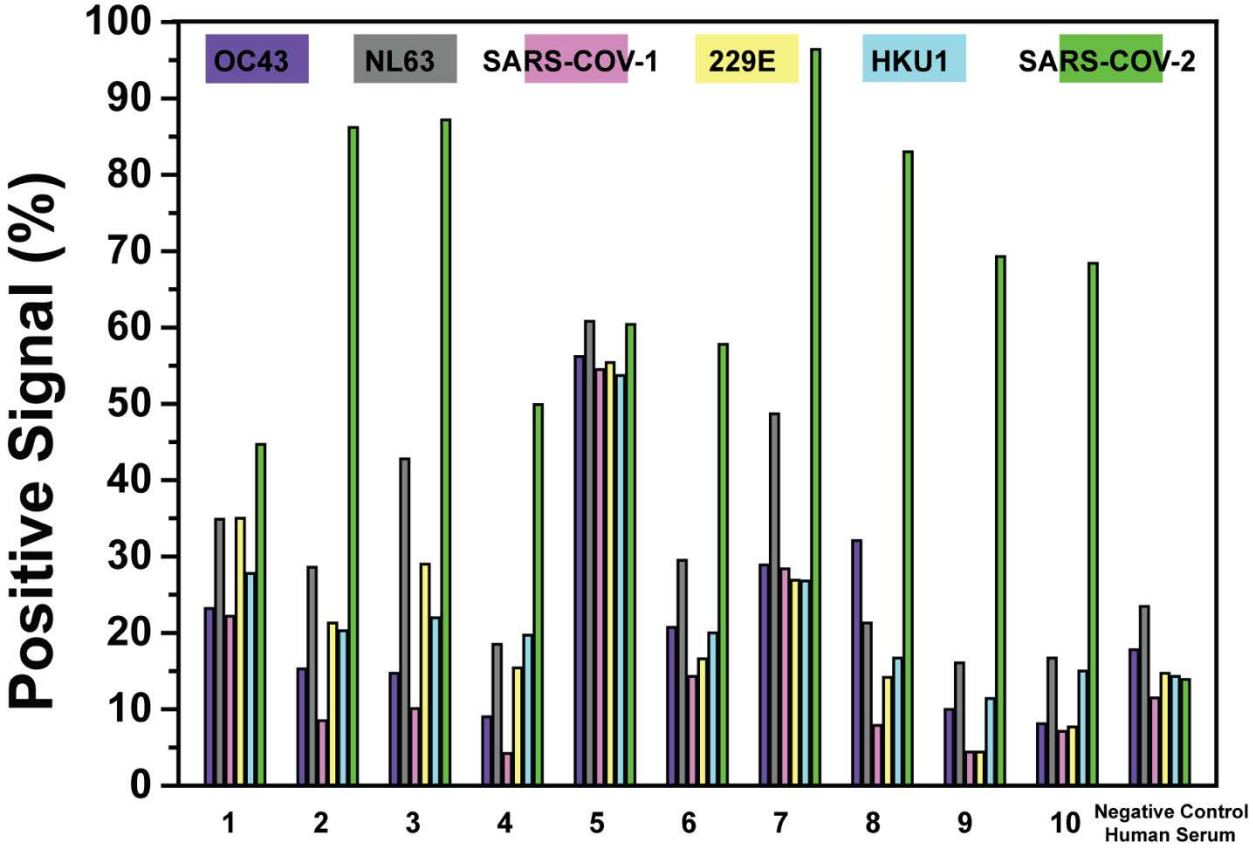

Figure 2. Serology assays from 10 de-identified SAR-CoV-2 positive specimens and a negative control.

Finally, during the course of this project, substantial progress was made toward developing the instrumentation required for facile, automated execution of these nucleic acid and protein assays, with the goal of developing a commercial, FDA-approved instrument. We note that this work leveraged additional funding for development of this digital assay instrumentation for non-COVID applications. Key progress included demonstration of an automated liquid handler (ALH); development and optimization of key components for thermocycling and optical readout; and development of control and user interface software.

Additional Funding

This NCPC award has aided us in securing additional funding (≈$1.9M) from the US Department of Defense as a supplement to an Other Transactional Agreement (OTA) to expand this NCPC

sponsored effort to develop new and improved COVID-19 diagnostics and research tools. This additional funding will enable more expansive development of the NCPC assays and improve our ability to implement the assays as a commercial solution.

In addition, this NCPC award was leveraged toward a collaborative effort with Prof. Rachel Noble, resulting in a NIH award (>$1.6M) for SARS-CoV-2 detection in wastewater using the Ramsey lab digital array PCR approach.