Multiplex analysis of coronavirus and influenza antibodies using the ZIVA multiplex platform

Ben Miller[1], Bryan Witherbee[2], Elise Ambrose[2], Daniel Steiner[1], John Cognetti[1], Alanna Klose[1], Ethan Luta[1], Joseph Budukovski[1], Michael Bryan,[1] Amanda Darby[2] Jon Schmuke[2]

[1] University of Rochester Medical Center, Rochester, NY, [2]Adarza Biosystems, Inc. St. Louis, MO

Abstract Detection of antibodies to upper respiratory pathogens is critical to surveillance, assessment of the immune status of individuals, vaccine development, and basic biology. The urgent need for antibody detection tools has proven particularly acute in the COVID-19 era. Arrayed Imaging Reflectometry (AIR™) is a label-free protein microarray technology that relies on the target binding-induced perturbation of an antireflective coating on the surface of a silicon chip, to create a multiplex antibody detection array. ZIVA is a new multiplex protein detection platform that leverages AIR™ technology. We have used the ZIVA platform to develop an acute respiratory virus panel capable of detecting the presence of antibodies specific for coronavirus and influenza including, but not limited to, detection of antibodies to SARS-CoV-2, SARS-CoV-1, MERS, three circulating coronavirus strains (HKU1, 229E, OC43) and multiple strains of influenza. We find that the array is readily able to distinguish uninfected from convalescent COVID-19 subjects, and provides quantitative information about total IgG, as well as IgG- and IgM-specific responses.

Introduction Described here is the use of a novel, silicon chip-based label-free biosensor platform we call AIR™. Conceptually, the technique employs elements of silicon wafer science, bio-assays similar in concept to ELISA (enzyme-linked immuno-sorbant assays) and laser optics to measure Ångstrom level changes in thickness which correlate with the binding of an analyte (see Figure 1). The platform affords the ability to detect not only the *low*-level analytes, but also the *high* levels in the same assay. The method uses a novel instrument we call ZIVA™ which automatically washes, dries, images and calculates values of a specific assay panel.

In these studies, we show the ability of AIR™ technology to streamline research on infectious disease and test for antibodies against diseases like influenza, MERS, SARS, and COVID-19 in one well, minimizing the amount of sample needed and decreasing the amount of time needed to get results.

How AIR™ Works

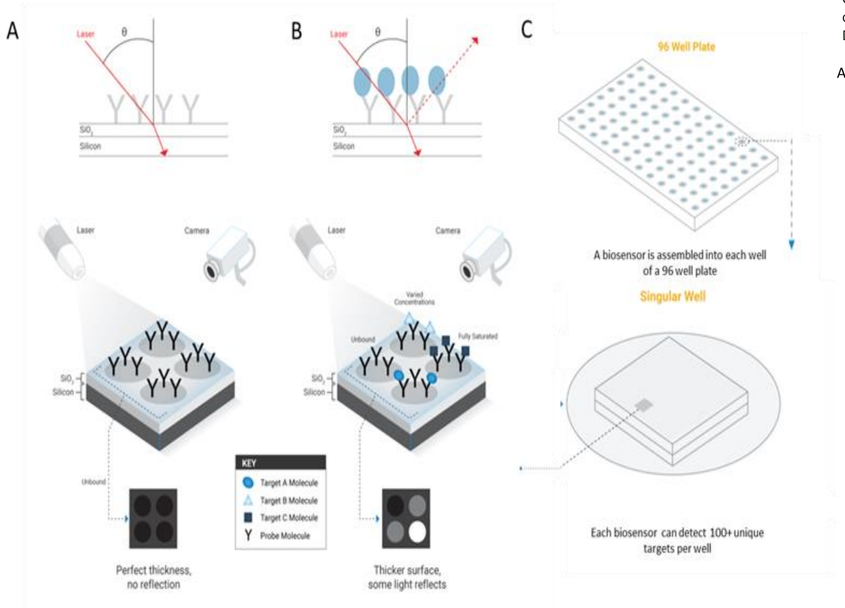

Figure 1: Basic principle of AIR™ technology to achieve high density multiplex arrays.
A) Using a silicon dioxide substrate with thickness specific to an array of antibody probes and an anti-reflective coating, near-zero reflectance is achieved;
B) Upon antigen binding to unique antibodies arrayed on individual spots, concentration dependent signal is detected using a CCD camera. When the biosensor is illuminated with s-polarized light at a fixed wavelength and angle, pico-scale thickness changes can be measured. Assays utilizing AIR™ allow for analysis of both high level and low level analytes within the same array;
C) Individual biosensors personalized for each array, allow for high sensitivity and high plex (100+ unique targets), simplifying research efforts.

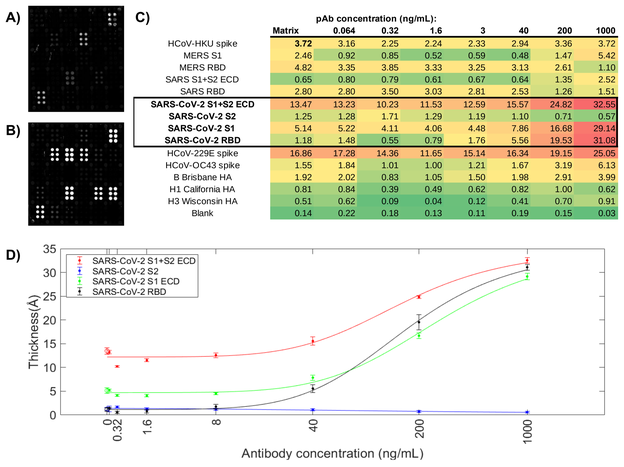

Figure 2: Response of a commercial anti-SARS-CoV-2 rabbit polyclonal antibody (pAb) on the array.
A) Array exposed to 20% FBS + 10% PNHS;
B) Array exposed to 1 µg/mL anti-SARS-CoV-2 pAb in 20% FBS + 10% PNHS. Strong responses to SARS-CoV-2 S1+S2 ECD, S1, and RBD are observed, as well as smaller cross-reactive responses to HCoV-229E, HCoV-OC43, and MERS spike proteins;
C) Quantitative data for the titration. Concentrations of pAb are provided at the top of each column in ng/mL; response values at each concentration for each antigen are provided in Ångstroms of build.
D) Titration curves for the four SARS-CoV-2 antigens with standard deviation of replicate probe spots at each concentration.

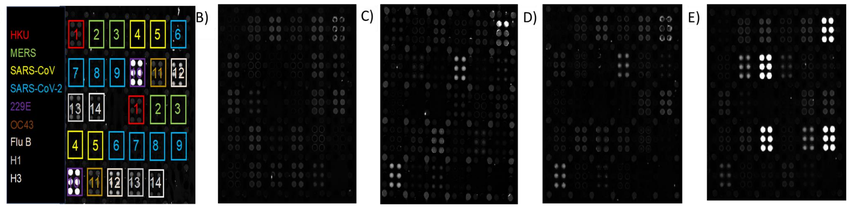

Figure 3: Control and Patient Representative AIR™ Images.
Layout Key for Biosensor (A). Six Replicate spots were printed for each antigen at various locations on the chip. Each group is surrounded by negative control reference spots (anti-FITC) with Blank (background) areas included as additional negative controls. The following were arrayed: Human Coronavirus Spike Glycoprotein (HKU); MERS-CoV spike glycoprotein, S1 domain; MERS-CoV spike glycoprotein, RBD; SARS-CoV spike glycoprotein S1 domain; SARS-CoV spike glycoprotein, RBD; SARS-CoV spike glycoprotein, S1+S2 ECD; SARS-CoV-2 spike glycoprotein, S2 ECD; SARS-CoV-2 spike glycoprotein, S1 domain; SARS-CoV-2 spike glycoprotein, RBD; human coronavirus spike glycoproteins S1+S2 ECD (HCoV-229E and HCoV-OC43 isolates); influenza B/Brisbane/2008 Hemagglutinin; Influenza A/CA/2009 (H1N1) hemagglutinin; influenza A/WS/2005 (H3N2) influenza. Representative AIR™ array images (100 ms exposures) of (B) 5% FBS; (C) 10% PNHS; (D) a negative single-donor sample, and (E) one convalescent serum sample. Strong responses to SARS-CoV-2 antigens are readily observed in (E), but not in (B), (C), or (D), while responses to circulating coronaviruses HKU, OC43, and 229E are observed in (C), (D), and (E). In each case, samples were diluted 1:20 in Adarza® diluent, and incubated with the arrays overnight at 4 ºC. All arrays in this figure were imaged at an exposure of 100 ms.

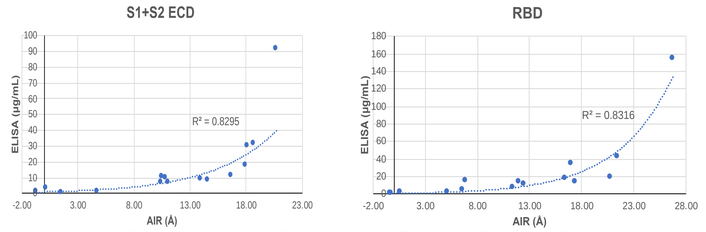

Figure 4: Correlation of AIR and ELISA data for SARS-CoV-2 S1+S2 ECD (left) and RBD (right). Exponential trend lines and associated R^2 values are indicated. Graphs illustrate data collected by ZIVA through AIR technology correlate with predicted ELISA data.

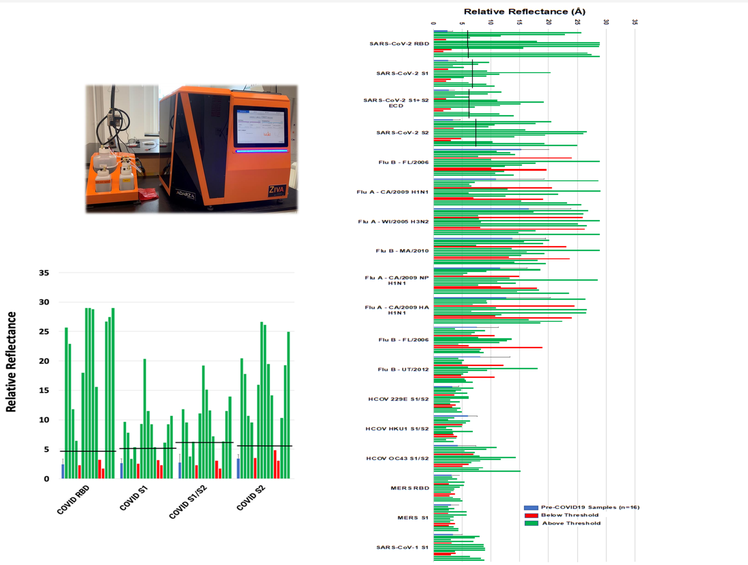

Figure 7: Results from the Adarza ZIVA system for pre-COVID-19 serum samples and single-donor samples from convalescent COVID-19 (PCR-positive) subjects. Pre-COVID-19 single-donor results were averaged (blue bars). Black bars indicate threshold positive values, calculated as two standard deviations above the average negative (pre-COVID-19) signal. Red bars indicate PCR+ individuals yielding signals below the threshold on all SARS-CoV-2 antigens, while green bars indicate signals from single-donor convalescent COVID-19 samples with at least one SARS-CoV-2 antigen response above threshold. Samples were analyzed at a 1:20 dilution, and incubated for 1 hour at room temperature.

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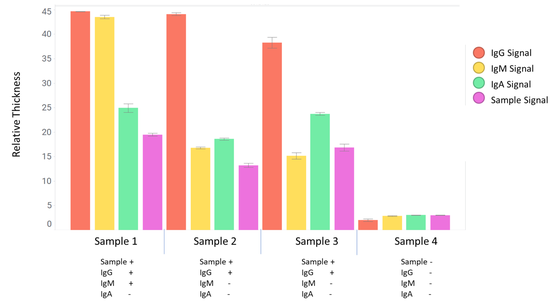

Figure 8: Serological antibody discrimination results from the Adarza ZIVA system for representative pre-COVID-19 serum samples (Sample 4) and single-donor samples from convalescent COVID-19 (PCR-positive, Sample 1-3) subjects. Purple bars indicate SARS-CoV-2 RBD antibody binding levels in Ångstroms of build. The red, yellow, and green represent mass increase from the addition of anti-human IgG, IgM, or IgA respectively.

Conclusion The Adarza® AIR™ technology provides a quick (1 hour), label free assay for serum samples requiring a minimal volume (2 µl sample) to evaluate a serological response to acute respiratory antigens in a simple multiplex platform. The ZIVA™ system is an easy to use instrument performing all the laborious tasks of washing, drying, imaging and calculates values of each assay on the array. In this example, we have used this system to evaluate positive convalescent COVID-19 and negative (pre-COVID-19) serum samples monitoring multiple COVID-19 antigens, while also surveying the response in the context of a broader set of respiratory pathogens.

Adarza BioSystems, Inc. 2184 Welsch Industrial Court, St. Louis, MO 63146
info@adarzabio.com | adarzabio.com