Introduction of a Novel Multiplex Serology Assay to Evaluate the Immune Response to RSV, SARS CoV-2, and Influenza

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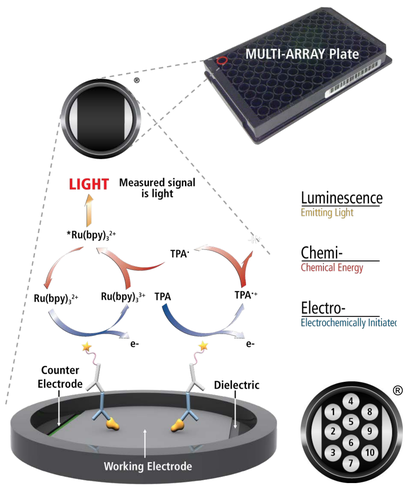
1 Abstract

Respiratory Syncytial Virus (RSV), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), and Influenza are prominent respiratory pathogens that significantly impact global public health. Each of these viruses poses distinct challenges and complexities, contributing to respiratory infections that range from mild illnesses to severe, life-threatening conditions. Understanding the immune responses elicited by RSV, SARS-CoV-2, and Influenza is crucial for effective disease management, preventive strategies, and the development of targeted interventions. Often called a ""tripledemic" pertaining to their seasonal coincidence, these three now have vaccines to prevent severe disease and limit transmission. In light of the emergence of novel SARS-CoV-2 variants, the unpredictable nature of the influenza virus characterized by a circulating strain that seldom replicates from the previous year, and the ongoing anticipation for surveillance data on the new RSV vaccine, the imperative for a reliable and sensitive research tool to concurrently monitor all three infections has become increasingly evident.

In this evaluation, we present compelling evidence of an innovative research tool designed for monitoring serological responses to RSV, SARS-CoV-2, and influenza. This multiplex serological assay offers quantitative measurements of antibodies targeting diverse strains of influenza, select variants of SARS-CoV-2, and the common virus/vaccine component associated with RSV. The development and validation of such a comprehensive research tool are essential steps toward enhancing our capacity to assess and manage the evolving landscape of these respiratory infections. MSD validates the uniformity of the protein coating of the plates, ensures that the calibrator interacts consistently with those immobilized proteins demonstrates that the calibration reagent is robust with multiple logs of dynamic range, and provides evidence of consistent control and sample measurement. This assay holds promise for enhancing our understanding, assessment, and management of the dynamic serological landscape presented by concurrent RSV, SARS-CoV-2, and influenza infections and vaccinations.

2 Platform

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.



Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

3 Method

V-PLEX® COVID-19 Serology Kits are available as panels defined by a set of antigens coated on a 10-spot MULTI-SPOT 96-well plate. A kit includes a reference standard (calibrated against the WHO International Standard for anti-SARS-CoV-2 Immunoglobulin (NIBSC code 20/136) for quantitation), controls, plate(s), a detection antibody (anti-human IgG), and all other reagents necessary to conduct the assay. Nine antigens are arrayed within each well of the V-PLEX Respiratory Panel plate (Table 1).

Table 1. Antigens and Spot Layout for V-PLEX Respiratory Plate 4.

Plate Description	Antigen Type	Respiratory Plate 4	Abbreviation
Spot 1	SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2 Spike Protein	CoV-2 Spike
Spot 2	Influenza	Influenza A Hemagglutinin Protein from A/Wisconsin/588/2019 (H1N1)	H1 A/Wis/19
Spot 3	SARS CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2 Nucleocapsid Protein	CoV-2 N
Spot 4	SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2 Spike Protein Omicron variant XBB.1.5 sublineage	XBB.1.5
Spot 5	Influenza	Influenza A Hemagglutinin Protein from A/Shanghai/2/2013 (H7N9)	H7/Shanghai/13
Spot 6	N/A	BSA	---
Spot 7	RSV	Respiratory Syncytial Virus Pre-Fusion F Protein	RSV Pre-F
Spot 8	Influenza	Influenza A Hemagglutinin Protein from A/Darwin/6/2021 (H3N2)	H3/Darwin/21
Spot 9	Influenza	Influenza B Hemagglutinin Protein from B/Austria/1359417/2021 (B Victoria Lineage)	B/Austria/21
Spot 10	Influenza	Influenza B Hemagglutinin Protein from B/Phuket/3073/2013 (B Yamagata Lineage)	B/Phuket/13

4 Results

a. Precision and Accuracy

Inter- and intra-run precision was assessed by measuring the percent coefficient of variation (%CV) well to well, using a 1:160 dilution of our calibrator across all assays. Accuracy was evaluated through percent recovery analysis of three serology controls.

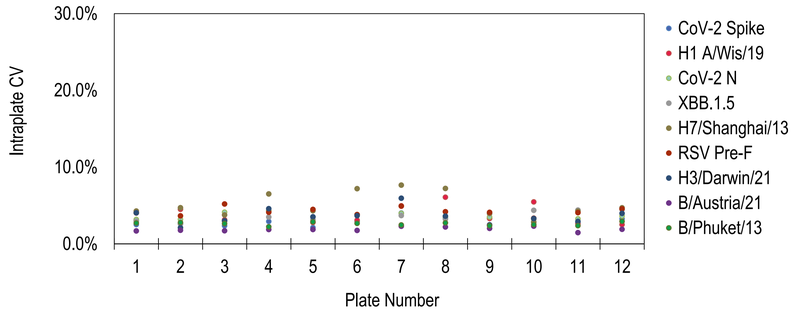


Figure 1. Precision Analysis by CV evaluation. Data points are representative of 12 plates from 3 different batches. For all 9 assays on the plate, %CVs did not exceed 8%.

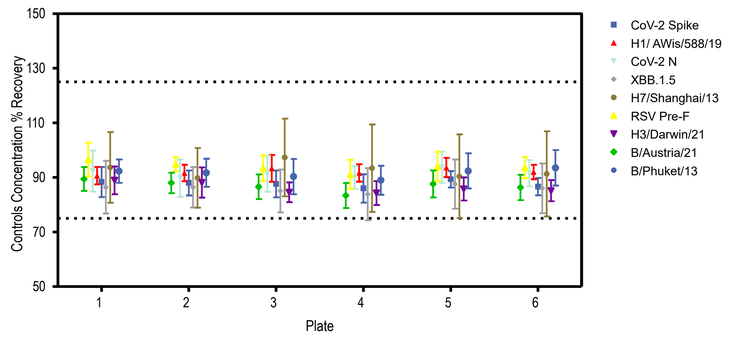


Figure 2. Accuracy Evaluation using Percent Recovery. Three controls per plate in duplicate across 6 plates were evaluated for the percent recovery of their concentration. Dotted lines represent 75% and 125% recovery. Percent recoveries did not exceed those bounds.

b. Sample Measurement Consistency

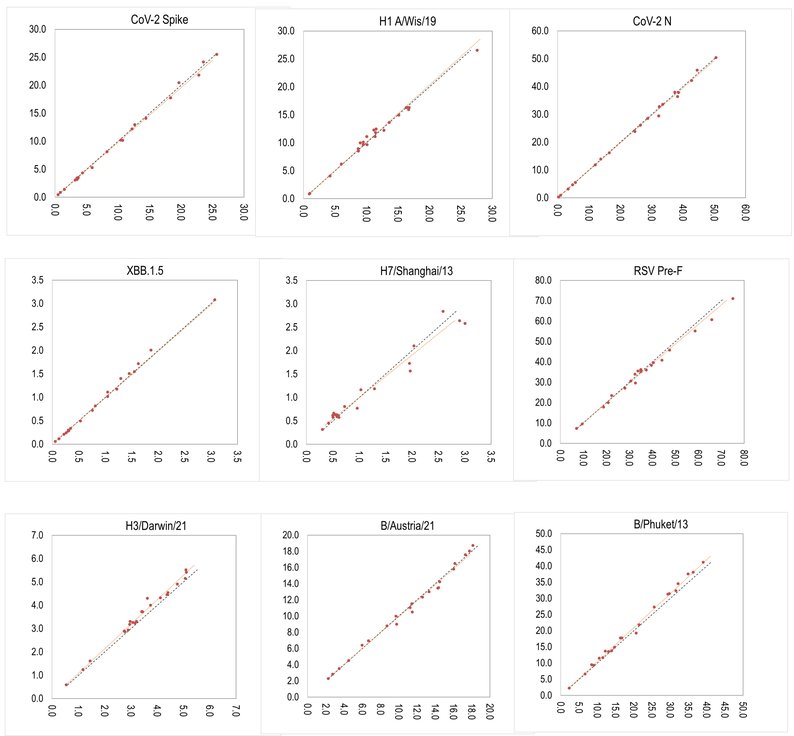


Figure 3. Sample Measurement Consistency. Representative graphs are the visual representation of all 22 tested samples within the limits of quantitation. The slope (dashed black line) versus the weighted Deming regression (dotted orange-red) lines showcase the limited variability between measured concentrations from two different plate lots.

c . Calibration Curves

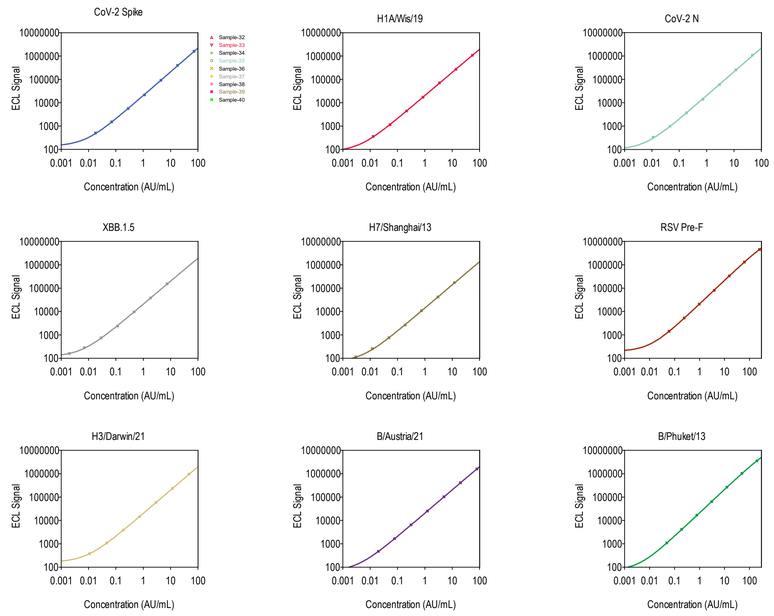


Figure 4. Calibration Curves. These representative graphs show the wide dynamic range of the serology assays. The squares represent 7 dilution points of the calibrator (4x dilutions), and the line represents fitting the signals from the calibrator to a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting. Slopes range between 0.9 and 1.1, showcasing the linear dilution of the calibrator for all assays.

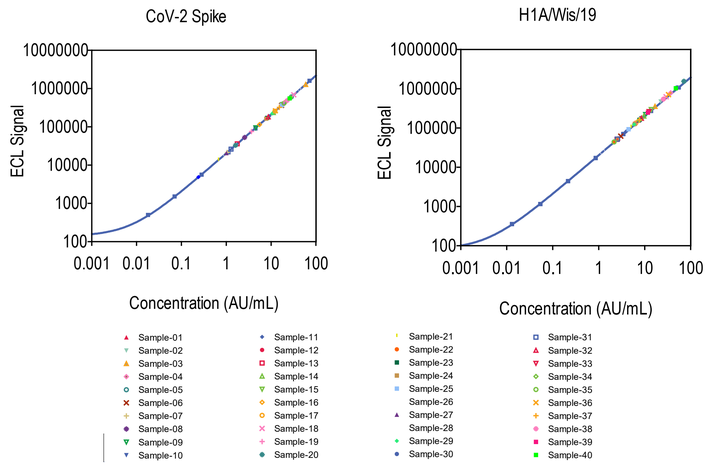


Figure 5. Representative Sample Data. Convalescent SARS-CoV-2 and influenza samples were commercially obtained for forty individuals. Most were quantifiable at 1:5000 dilution, within the linear portion of the calibration curves, and above the limits of quantification, allowing for simultaneous screening of all assays.

5 Conclusion

Here we have presented compelling evidence of an innovative research tool, MSD's V-PLEX Respiratory Panel 4 with a calibrator and controls, which has been designed for monitoring serological responses to RSV, SARS-CoV-2, and influenza. This multiplex serological assay offers quantitative measurements of antibodies targeting diverse strains of influenza, SARS-CoV-2 variants of concern, and the common virus/vaccine component associated with RSV. The development and validation of such a comprehensive assay is an essential step toward enhancing our capacity to assess and manage the evolving landscape of these respiratory infections. MSD validates the uniformity of the protein coating of the plates, ensures that the calibrator interacts consistently with those immobilized proteins demonstrates that the calibration reagent is robust across multiple logs of dynamic range, and provides evidence of consistent control and sample recovery. This tool will augment our understanding, assessment, and management of the dynamic serological landscape presented by concurrent RSV, SARS-CoV-2, and influenza infections and vaccinations.

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