Validation of Novel Multiplexed Serology Assays for Detection of IgG Antibodies against SARS-COV-2 Key Variants

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1 Introduction

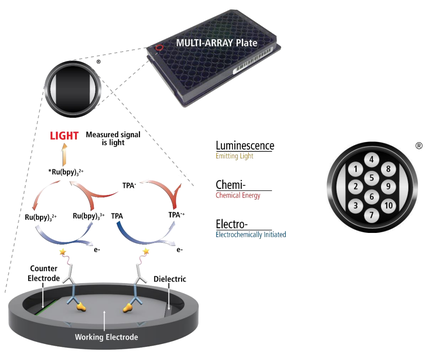
COVID-19 is a viral illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 codes for various structural proteins, including the highly immunogenic Spike protein (S) and the Nucleocapsid protein (N) that plays a key role in virus transcription and assembly. Following the emergence of the virus, a wide range of serological tests were developed to estimate its seroprevalence, guide booster vaccinations, and select patients for anti-SARS-CoV-2 antibody therapies. Serological analysis is also an essential tool for understanding the immune correlates of protection against future COVID-19 waves following natural infection or vaccination. Despite a rapid increase in the number and availability of serology assays that can detect antibodies against SARS-CoV-2, there is limited knowledge about the performance and validation status of these assays. In addition, most are low throughput and only measure response to a single antigen, limiting their utility in capturing the breadth of antibody responses to SARS-CoV-2 and its variants.

Herein, we present validated, quantitative, multiplexed serology assays for the Nucleocapsid protein and key variants of SARS-COV-2 Spike and its receptor-binding domain (RBD) across two panels. The assays utilize 10-spot, 96-well plates coated with SARS-COV-2 antigens and an electrochemiluminescent (ECL) detection system to simultaneously detect IgG antibodies to SARS-CoV-2 Spike and RBD variants, including Alpha, Beta, Delta, and Omicron, in human serum. The panels were evaluated for accuracy, precision, analytical sensitivity, specificity, dilution linearity, and short-term stability. Clinical sensitivity and specificity were assessed using human serum from prepandemic samples. The validated assays accurately quantify IgG antibodies against RBD, Spike protein, Nucleocapsid protein and key variants of Spike protein (BA.2, BA.5, BA.2.12.1, BA.2.75, B.1.1.7, B.1.351, B.1.617.2; AY.4 and B.1.1.529; BA.1) and RBD (Omicron; BA.1, Omicron; BA.2, Alpha, Delta, Beta, BA.2.12.1, BA.2.75 and BA.4; BA.5). The multiplex SARS-CoV-2 ECL serology assays allow for sensitive, high throughput, and simultaneous measurement of IgG levels to multiple antigens in human sera, supporting its use in assessing exploratory endpoints for clinical trials.

2 MSD Platform

MSD Technology

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.

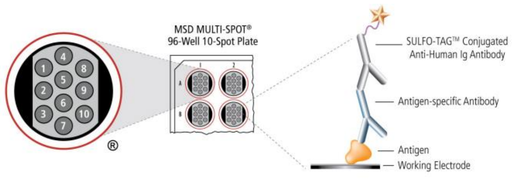


Electrochemiluminescence Technology

- 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), thereby 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 surfaces have 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

3 Method

The V-PLEX® COVID-19 Serology Kits quantitatively measure antibodies to antigens related to SARS-CoV-2 including variants of the SARS-CoV-2 virus, SARS, MERS, circulating Coronaviruses, and other respiratory pathogens. Plates are provided with antigens on spots in the wells of a 96-well plate. Antibodies in the sample bind to the antigens on the spots and anti-human antibodies (IgG, IgM, or IgA) conjugated with MSD SULFO-TAG are used for detection. The plate is read on an MSD® instrument, which measures the light emitted from the MSD® SULFO-TAG.



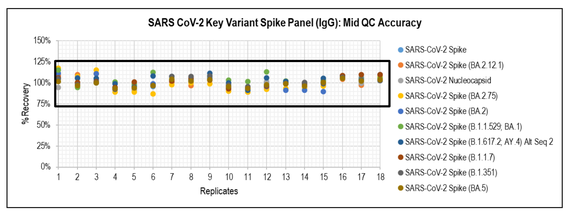
V-PLEX COVID-19 Serology Kits are available as panels defined by a set of antigens coated on a 10-spot MULTI-SPOT 96-well plate. A kit includes a reference standard calibrated against the WHO International Standard (NIBSC code 20/136) for quantitation, controls, plate(s), one of the available detection antibodies (anti-human IgG), and all other reagents necessary to conduct the assay. The table below outlines the antigens coated on SARS-CoV-2 Key Variant Spike and RBD Panel 1 plates.

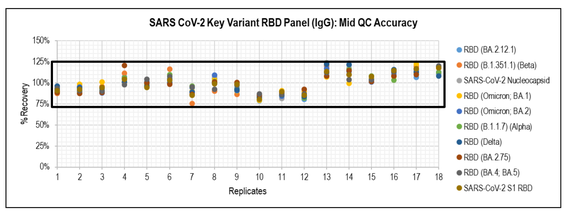
Plate Description	SARS-CoV-2 Key Variant Spike Panel 1	SARS-CoV-2 Key Variant RBD Panel 1
Spot 1	SARS-CoV-2 Spike	RBD (BA.2.12.1)
Spot 2	SARS-CoV-2 Spike (BA.2.12.1)	RBD (B.1.351.1) (Beta)
Spot 3	SARS-CoV-2 Nucleocapsid	SARS-CoV-2 Nucleocapsid
Spot 4	SARS-CoV-2 Spike (BA.2.75)	RBD (Omicron; BA.1)
Spot 5	SARS-CoV-2 Spike (BA.2)	RBD (Omicron; BA.2)
Spot 6	SARS-CoV-2 Spike (B.1.1.529; BA.1)	RBD (B.1.1.7) (Alpha)
Spot 7	SARS-CoV-2 Spike (B.1.617.2; AY.4) Alt Seq 2	RBD (Delta)
Spot 8	SARS-CoV-2 Spike (B.1.1.7)	RBD (BA.2.75)
Spot 9	SARS-CoV-2 Spike (B.1.351)	RBD (BA.4; BA.5)
Spot 10	SARS-CoV-2 Spike (BA.5)	SARS-CoV-2 S1 RBD

4 Results

a. Accuracy, Precision & Sensitivity

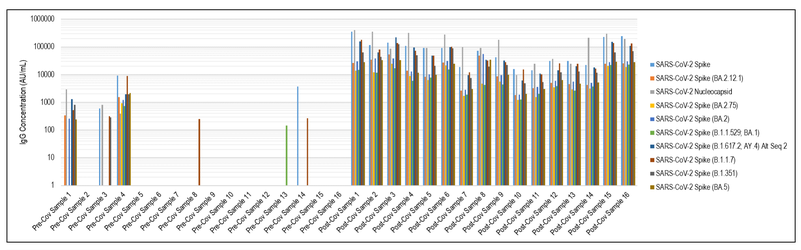
Inter-assay (between runs) and intra-assay (within runs) accuracy and precision were assessed by performing 6 runs on 3 different days by 2 different analysts. Each run consisted of standard curves, quality control (QC) samples, Upper Limit of Quantification (ULOQ), and Lower Limit of Quantification (LLOQ) samples. SARS-CoV-2 Key Variant Spike and RBD panels demonstrated good accuracy and precision. Inter-assay and intra-assay QC accuracy were ±25% and precision <20%, respectively (representative data for Mid QC is shown). The ULOQ and LLOQ of the assay are the highest and lowest known concentrations, at which precision was ≤25%, accuracy of 70%-130%, and total error of ≤40% are achieved within and between the established runs.

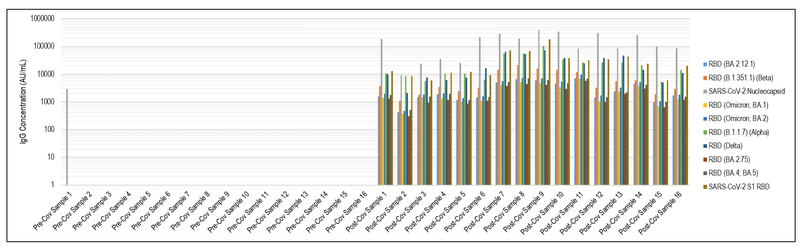




Panel			SARS-CoV-2 Key Variant Spike Panel (IgG)										SARS-CoV-2 Key Variant RBD Panel (IgG)									
Assay			SARS-CoV-2 Spike	SARS-CoV-2 Spike (BA.2.12.1)	SARS-CoV-2 Nucleocapsid	SARS-CoV-2 Spike (BA.2.75)	SARS-CoV-2 Spike (BA.2)	SARS-CoV-2 Spike (B.1.1.529; BA.1)	SARS-CoV-2 Spike (B.1.617.2; AY.4) Alt Seq 2	SARS-CoV-2 Spike (B.1.1.7)	SARS-CoV-2 Spike (B.1.351)	SARS-CoV-2 Spike (BA.5)	RBD (BA.2.12.1)	RBD (B.1.351.1) (Beta)	SARS-CoV-2 Nucleocapsid	RBD (Omicron; BA.1)	RBD (Omicron; BA.2)	RBD (B.1.1.7) (Alpha)	RBD (Delta)	RBD (BA.2.75)	RBD (BA.4; BA.5)	SARS-CoV-2 S1 RBD
Calibration Range (AU/mL)			72 - 0.0176	11 - 0.0027	70 - 0.0171	5.7 - 0.0014	12 - 0.0029	5.9 - 0.0014	40 - 0.0098	44 - 0.0011	25 - 0.0061	11 - 0.0027	2.2 - 0.0005	4.4 - 0.0011	70 - 0.0171	1.7 - 0.0004	2.6 - 0.0006	18 - 0.0044	14 - 0.0034	1.4 - 0.0003	2.1 - 0.0005	29 - 0.0071
ULOQ	Concentration (AU/mL)		**72**	**11**	**70**	**5.7**	**12**	**5.9**	**40**	**44**	**25**	**11**	**2.2**	**4.4**	**70**	**1.7**	**2.6**	**18**	**14**	**1.4**	**2.1**	**29**
	Inter-assay	Accuracy	96	94	99	93	97	94	94	93	92	92	91	98	95	92	93	100	95	96	91	94
		Precision	5.6	10.6	5.0	7.7	5.9	9.4	10.9	7.3	7.4	10.2	7.9	12.1	6.3	7.6	9.8	9.1	6.4	12.7	9.0	8.2
	Intra-assay	Accuracy	88-106	77-113	88-107	84-109	85-106	78-114	79-113	83-105	81-107	78-115	77-107	78-120	85-106	76-104	83-113	88-121	83-105	78-126	79-108	82-106
		Precision	0.2-14.5	0.3-13.8	0.0-18.3	0.8-15.5	0.3-16.3	0.0-24.7	0.0-17.7	0.0-12.8	0.5-15	81-116	0.1-5.9	0.013.7	0.1-9.2	0.5-13.2	0.6-9.9	0.9-11.2	0.2-9.8	0.1-17.1	0.2-10.7	0.2-12.6
LLOQ	Concentration (AU/mL)		**0.115**	**0.033**	**0.111**	**0.039**	**0.037**	**0.029**	**0.064**	**0.03**	**0.04**	**0.032**	**0.019**	**0.037**	**0.592**	**0.023**	**0.022**	**0.037**	**0.118**	**0.026**	**0.025**	**0.035**
	Inter-assay	Accuracy	108	105	109	101	102	98	95	97	98	100	99	101	100	104	101	102	100	100	103	102
		Precision	5.8	11.7	4.8	12.1	8.3	12.8	6.8	9.6	11.5	9.2	18.3	14.1	12.8	17.8	12.5	14.4	15.0	17.0	21.0	13.7
	Intra-assay	Accuracy	96-120	85-124	100-118	82-126	84-116	79-117	84-111	87-120	73-125	81-116	79-121	81-130	82-124	78-130	86-127	89-130	86-119	73-127	80-128	83-129
		Precision	0.1-13.5	0.3-13.8	0.0-19.2	0.3-24.7	0.3-22.7	1.2-18.2	0.1-18.2	0.5-23.7	0.3-21.9	0.6-16.9	0.8-14.8	0.0-20.2	0.1-10.1	0.0-19	0.6-14	0.2-12	0.5-14.1	1.3-23.6	0.2-16.2	0.7-17.9

b. Assay Specificity





Specificity was evaluated by testing human serum samples collected pre- and post-COVID. Sixteen pre-COVID samples (collected in 2017) and sixteen post-COVID samples (collected in 2022) were tested at 5000-fold dilution.

IgG levels in most pre-COVID samples were BLQ. All post-COVID samples had precision <20% and quantifiable levels of IgG for all antigens. Higher levels of IgG were observed in post-COVID compared to pre-COVID samples indicating good specificity of the assays.

c. Parallelism & Prozone Effect

Parallelism and the presence of a prozone effect in human sera were assessed in 8 individual donor samples. Each sample was tested in serial dilutions to yield an 8-point titration (i.e., 100, 625, 1,250, 2,500, 5,000, 10,000, 20,000, and 40,000-fold dilution). The dilution-adjusted concentrations across the dilution series were assessed to evaluate SARS-CoV-2 IgG detectability and to characterize matrix interferences. Percent recovery was measured as the mean concentration at each dilution relative to the mean concentration at 5000-fold dilution. Good dilution linearity was observed between 625 to 40,000-fold dilutions in the Spike panel and 2,500 to 20,000-fold dilution in the RBD panel (%Recovery range ±30% is highlighted in green). The Hook effect was observed in a few serum samples at 100-fold dilution.

SARS-CoV-2 Key Variant Spike Panel (IgG)																						
Assay		SARS-CoV-2 Spike		SARS-CoV-2 Spike (BA.2.12.1)		SARS-CoV-2 Nucleocapsid		SARS-CoV-2 Spike (BA.2.75)		SARS-CoV-2 Spike (BA.2)		SARS-CoV-2 Spike (B.1.1.529; BA.1)		SARS-CoV-2 Spike (B.1.617.2; AY.4) Alt Seq 2		SARS-CoV-2 Spike (B.1.1.7)		SARS-CoV-2 Spike (B.1.351)		SARS-CoV-2 Spike (BA.5)	
%Recovery		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Dilution Factor (Serum, n=8)	**100**	66	19-109	97	65-113	44	12-97	162	81-402	135	97-191	158	103-268	158	33-506	82	33-134	121	76-211	99	75-112
	625	96	74-112	102	89-112	91	69-107	102	93-115	106	98-122	114	100-125	113	82-143	104	89-119	102	97-110	104	90-112
	1,250	103	94-108	102	90-120	99	93-105	102	99-111	103	99-114	127	100-232	107	93-119	105	95-117	102	98-108	104	100-116
	2,500	101	97-104	101	94-115	98	95-100	101	98-113	100	96-108	103	95-114	101	98-106	100	92-114	98	94-101	97	94-105
	5,000	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	10,000	100	97-102	99	90-106	100	98-102	101	92-105	101	97-107	100	90-108	99	94-110	100	91-111	101	93-105	100	93-116
	20,000	99	90-106	99	92-105	99	94-103	103	95-106	100	97-103	99	89-107	95	85-102	99	91-112	102	96-108	100	93-112
	40,000	103	85-108	107	91-116	100	73-109	112	96-115	104	98-119	107	95-142	100	77-122	100	90-111	106	85-113	101	85-112

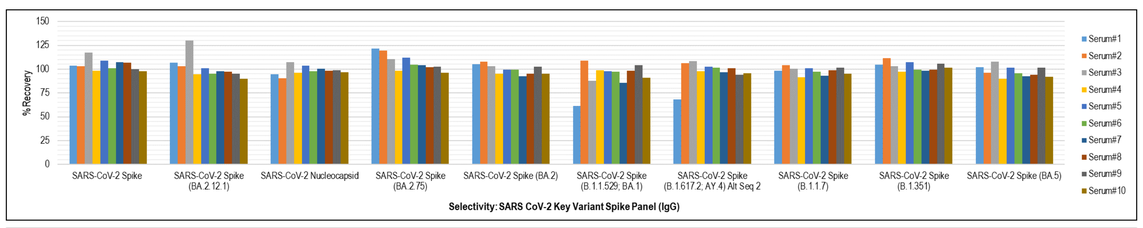
SARS-CoV-2 Key Variant RBD Panel (IgG)																						
Assay		RBD (BA.2.12.1)		RBD (B.1.351.1) (Beta)		SARS-CoV-2 Nucleocapsid		RBD (Omicron; BA.1)		RBD (Omicron; BA.2)		RBD (B.1.1.7) (Alpha)		RBD (Delta)		RBD (BA.2.75)		RBD (BA.4; BA.5)		SARS-CoV-2 S1 RBD	
%Recovery		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Dilution Factor (Serum, n=8)	**100**	117	59-193	268	222-343	48	10-172	125	61-173	142	66-179	88	36-211	78	25-199	111	80-134	133	69-210	75	20-124
	625	99	60-138	160	62-339	87	48-144	153	63-297	105	66-145	110	68-164	101	63-154	95	77-111	101	64-141	100	71-161
	1,250	87	59-118	100	62-141	86	60-108	103	64-204	90	61-123	96	68-126	92	61-125	93	71-126	87	65-127	94	63-135
	2,500	88	69-105	86	66-107	87	73-114	89	71-108	90	71-108	90	79-105	87	71-116	87	71-107	85	62-113	87	74-108
	5,000	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	10,000	106	84-127	107	80-119	103	81-119	111	93-129	107	85-129	98	71-127	100	76-121	105	83-130	103	78-124	98	74-118
	20,000	97	89-111	94	77-102	95	85-109	106	91-123	98	92-110	87	68-95	88	69-110	102	91-112	94	82-113	87	75-100
	40,000	143	87-224	139	100-203	124	84-157	164	108-287	148	85-244	127	93-150	123	84-164	170	119-273	142	90-198	123	93-153

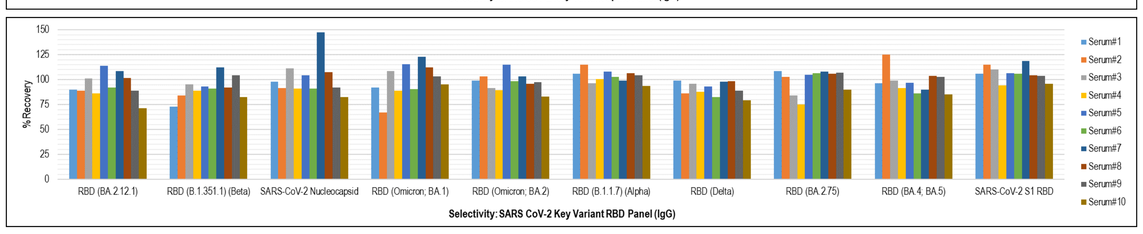
d. Selectivity

Selectivity was assessed in 10 individual human serum samples to evaluate matrix interference. In addition, 3 individual hemolyzed and lipemic human serum samples were tested to evaluate impact of hemolysis and lipemia on the assay, respectively. Each sample was diluted 5,000-fold and spiked with a reference standard. Spiked and unspiked samples were tested in duplicate. Spike recovery was calculated as measured concentration divided by nominal (endogenous and spike) concentration.

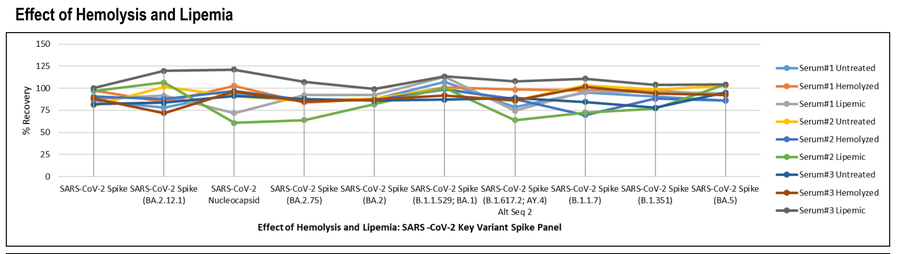
Matrix effect

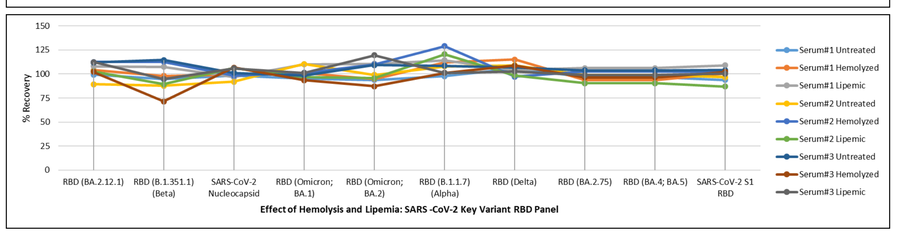
No discernable matrix effect: >80% of samples recovered within ±25% across both panels.





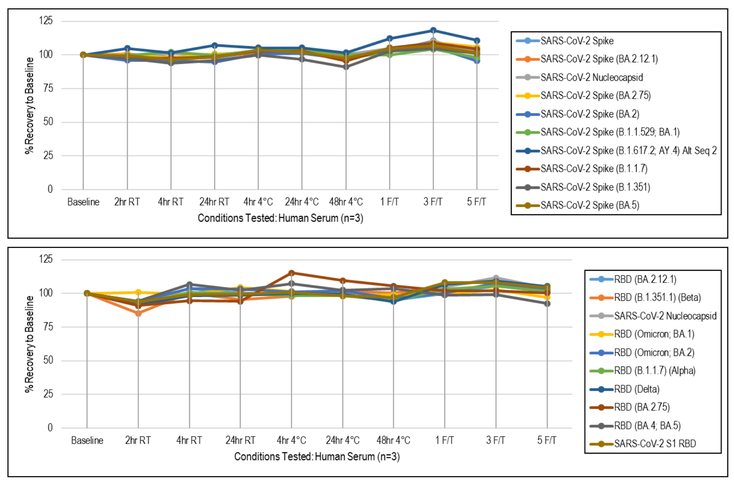
Effect of Hemolysis and Lipemia





To evaluate the impact of lipemia and hemolysis on assay selectivity, three samples were tested after spiking with triglyceride-rich lipoprotein (1,500 mg/dL) and hemolysate (1,000 mg/dL). The samples were further spiked with a reference standard. Data indicates that most assays are tolerant to hemolysis and lipemia.

e. Short Term Stability



Experiments were performed to assess the stability of samples throughout the course of laboratory procedures. Human serum samples (n=3) were analyzed after being subjected to storage at RT (23°C ± 2°C) for 0, 2, 4, and 24 hours or 2-8°C for 0, 4, 24, and 48 hours. Freeze/Thaw Stability was assessed through 0, 1, 3, and 5 freeze-thaw cycles.

The data indicates that the samples are stable through storage at RT for 24 hours and at 2-8°C for 48 hours The samples were stable for up to 5 freeze-thaw cycles. Percent recovery was ±25% of baseline sample concentration and precision was ≤20%.

5 Conclusions

We validated quantitative, multiplexed serology assays that accurately and precisely quantify IgG antibodies against the antigens in SARS-CoV-2 Key Variant Spike Panel 1 (BA.2, BA.5, BA.2.12.1, BA.2.75, B.1.1.7, B.1.351, B.1.617.2; AY.4, B.1.1.529; BA.1, COV-2 S and COV-2 N) and SARS-CoV-2 Key Variant RBD Panel 1 (Omicron; BA.1, Omicron; BA.2, Alpha, Delta, Beta, BA.2.12.1, BA.2.75, BA.4; BA.5, COV-2 S1 RBD, and COV-2 N). The multiplex SARS-CoV-2 ECL serology assays allow for sensitive, high-throughput, and simultaneous measurement of IgG levels to multiple antigens in human sera, supporting its use in assessing exploratory endpoints for clinical trials. The assays were validated in MSD's Bioanalytical Laboratory, which is GLP-compliant and provides preclinical and clinical sample testing services.

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