Correlation of SARS-CoV-2 Live Virus Neutralization Assay to Bio-Plex™ Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays

Resmi Ravindran,[1] Brett Houser,[2] Vanitha Margan[2]
[1]Department of Pathology and Laboratory Medicine, University of California Davis Medical Center, Sacramento, CA 95817
[2]Bio-Rad Laboratories, Inc., 6000 Alfred Nobel Drive, Hercules, CA 94547

Abstract

In this study we compared and correlated the Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody 11-Plex Panel to the traditional gold standard — live virus neutralization assays — using longitudinal patient samples confirmed positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), by reverse transcription quantitative PCR (RT-qPCR). The results demonstrate that the Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody 11-Plex Panel produces data comparable to the gold standard live virus neutralization assay in less than 3 hours in a standard research laboratory.

Introduction

The determination of neutralizing antibody activity is critical in vaccine development and surveillance, as it shows a direct prophylactic effect of the vaccine and, when measured over time, can provide information on vaccine durability. Although cell-based (live or pseudovirus) assays are the current gold standard for measuring neutralizing antibody activity, there is increasing interest in newer methods, including competitive immunoassays. Conventional live virus neutralization assays, including SARS-CoV-2 neutralization assays, are performed in biosafety level 3 (BSL-3) laboratories for live virus containment, while pseudovirus assays can be performed in BSL-2. Both conventional procedures typically take more than 3 days to generate results. Alternatively, competitive immunoassay methods, such as the Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody 11-Plex Panel, a bead-based multiplex immunoassay, can be performed in a BSL-1 lab and generate results in less than 3 hours (Figure 1). Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays utilize xMAP bead-based technology in a competitive immunoassay format that delivers neutralizing antibody percent inhibition information for multiple SARS-CoV-2 variants simultaneously from a single well in about 70 minutes.

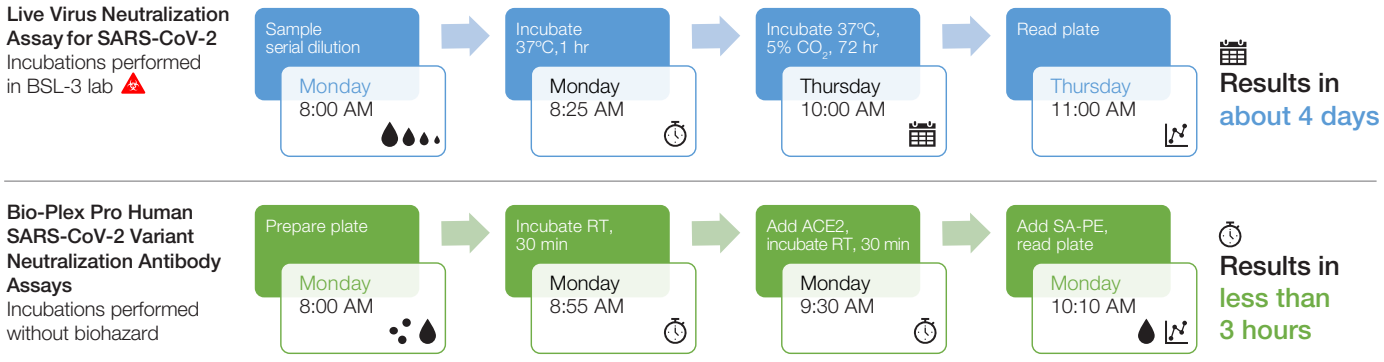

Fig. 1. Comparison of methods. Conventional live virus and Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays. RT, room temperature; ACE2, Bio-Plex Pro Biotinylated Detection ACE2 Receptor; SA-PE, streptavidin-phycoerythrin.

BIO-RAD

Materials and Methods

Plasma Samples from Patients with COVID-19

Samples were obtained from patients hospitalized at the University of California Davis (UC Davis) Medical Center (n = 4) with symptomatic illness and who were confirmed positive for SARS-CoV-2 infection by RT-qPCR (Ravindran et al. 2021). The plasma samples were collected upon hospital admission, anonymized, and submitted to the UC Davis Comprehensive Cancer Center shared resources biorepository where samples were aliquoted and stored at –80°C until use. From each patient, longitudinal samples were collected for a period of several days to weeks. The samples were collected between 5 and 26 days after symptom onset as reported by patients (Table 1).

Table 1. Covid-19 patient sample list. Sample IDs for samples collected at various time points after symptom onset.

COVID-19 patient	Sample ID (plasma)	Approximate Days After Symptom Onset
Patient #1	RIB-000016-0	9
	RIB-000016-2	11
	RIB-000016-4	13
Patient #2	RIB-000020-0	5
	RIB-000020-2	7
	RIB-000020-4	9
Patient #3	RIB-00004-6	11
	RIB-00004-12	17
	RIB-00004-21	26
Patient #4	RIB-000012-0	12
	RIB-000012-2	14
	RIB-000012-5	17

Conventional Wild-Type SARS-CoV-2 Live Virus Neutralization Assay

A conventional live virus neutralization assay was performed using plasma samples from COVID-19 patients as previously described (Ravichandran et al. 2020). The plasma samples were heat-inactivated and serially diluted twofold at each time point in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and 100 TCID50 of SARS-CoV-2 isolate (BEI Resources, catalog #WA1/2020) was added to the diluted plasma samples. The plasma-virus mixture was incubated at 37°C for 1 hr and 100 µl of the mixture was transferred into a 96-well plate containing Vero E6 cells (1.4×10^4 cells per well in 20 µl volume). Uninfected Vero E6 cells were used as a negative control and SARS-CoV-2–infected cells were used as a positive control in each plate. Cells were uniformly distributed within the plates by gentle rocking. The plates were incubated at 37°C with 5% CO_2 for 72 hr followed by addition of 50 µl of CellTiter-Glo Reagent (Promega Corporation, USA, #G7572). The plates were rocked for 2 min with an orbital shaker set to 50 rpm followed by incubation at room temperature for 10 min. A Cytation 5 Cell Imaging Multi-Mode Reader (Agilent Technologies, Inc.) was used to measure luminescence. The luminescence from blank wells (120 µl DMEM with 10% fetal bovine serum and 50 µl CellTiter-Glo Reagent) was recorded as baseline values. The reciprocal of the highest dilution at which serum samples showed at least 50% inhibition of SARS-CoV-2 infection or cell death (NT50) was considered as the endpoint neutralization titer, defined as luminescence values higher than or equal to [average of negative control – (average of negative control – average of positive control)/2]. Each experiment was performed twice with samples in duplicates.

Multiplex Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays

The Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody 11-Plex Panel (Bio-Rad™ Laboratories, Inc., #12016897) was used to test the same set of longitudinal SARS-CoV-2–positive plasma samples that were used in the live virus neutralization assay (Table 1). A dilution study was performed to determine the optimal dilution factor that would best correlate to the live virus neutralization data. A dilution series of 1:5, 1:25, 1:37.5, 1:56.3, 1:84.4, and 1:125 was prepared using the Bio-Plex Pro Serology Sample Diluent included with the assay panel. All plasma samples were processed according to the manufacturer's instructions. All assays were run on the Bio-Plex 100 System* with HTF (Bio-Rad, #171000205). The data were generated using Bio-Plex Manager 6.2 Software (Bio-Rad, #171STND01) and percent inhibition [(1 – sample MFI/MFI negative control) x 100] was calculated using Microsoft Excel Software.

Results

Correlation between Conventional SARS-CoV-2 Live Virus Neutralization Assay and Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays

To establish a correlation between the two techniques, four plasma samples from COVID-19 patients were evaluated for virus titer, each at three different time points after symptom onset. The data were collected from a conventional live virus neutralization assay and the Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays. The live virus neutralization assay titer data (NT50) were then compared to percent inhibition data generated using the Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays. The optimal dilution for correlation was selected based on several factors. First, the dilution series was plotted against the neutralization titer data and the dilutions with high R^2 values were then selected (Figure 2). Next, the corresponding percent inhibition from the Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays for the selected dilution was evaluated to ensure that the percent inhibition followed a trend similar to that of the live virus neutralization assay. This was accomplished by plotting the different time points and selecting the dilution factor that fell within a range that allowed for trending. The dilution of 1:37.5 was selected to ensure that the change in percent inhibition was observable across time points and samples, and did not saturate on either end (Figure 3). A summary of the comparison of all data is shown in Table 2.

*Bio-Plex 200 System (171000205) replaces Bio-Plex 100 System.

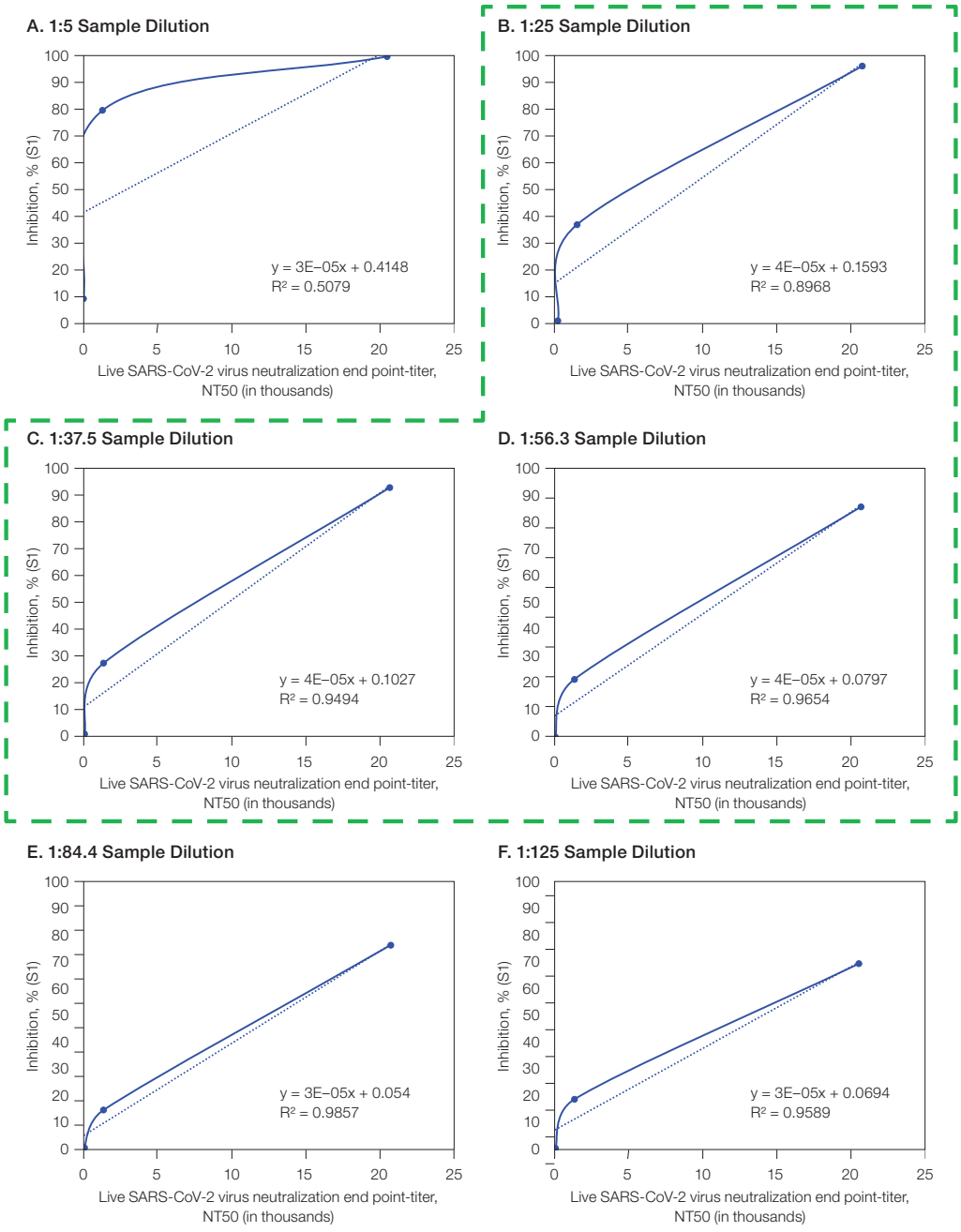

Fig. 2. Correlation between Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays and live virus neutralization assays. Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays were performed for two SARS-CoV-2 wild-type Spike 1 (S1) and receptor binding domain (RBD) and 9 variants (Alpha S1, Beta S1, Gamma RBD, Kappa RBD, Epsilon RBD, D614G S1, N501Y RBD, E484K RBD, and K417N RBD). Data for SARS-CoV-2 S1 are shown. Percent inhibition data obtained using the Bio-Plex Pro Neutralization Antibody Assay for SARS-CoV-2 S1 protein plotted against end-point titer (NT50) determined by conventional live virus neutralization assay for various dilutions of the same COVID-19 plasma samples. Correlation represented as R^2 values. Longitudinal samples from patient #3 (RIB-00004-6, –12,–21) were prepared at the following dilutions: **A**, 1:5; **B**, 1:25; **C**, 1:37.5; **D**, 1:56.3; **E**, 1:84.4; **F**, 1:125. Sample dilutions 1:25 (**B**), 1:37.5 (**C**), and 1:56.3 (**D**) were selected (▬ ▬ ▬) and compared to percent inhibition to determine samples within range (Figure 3). Data for patient #1, 2 and 4 are not shown.

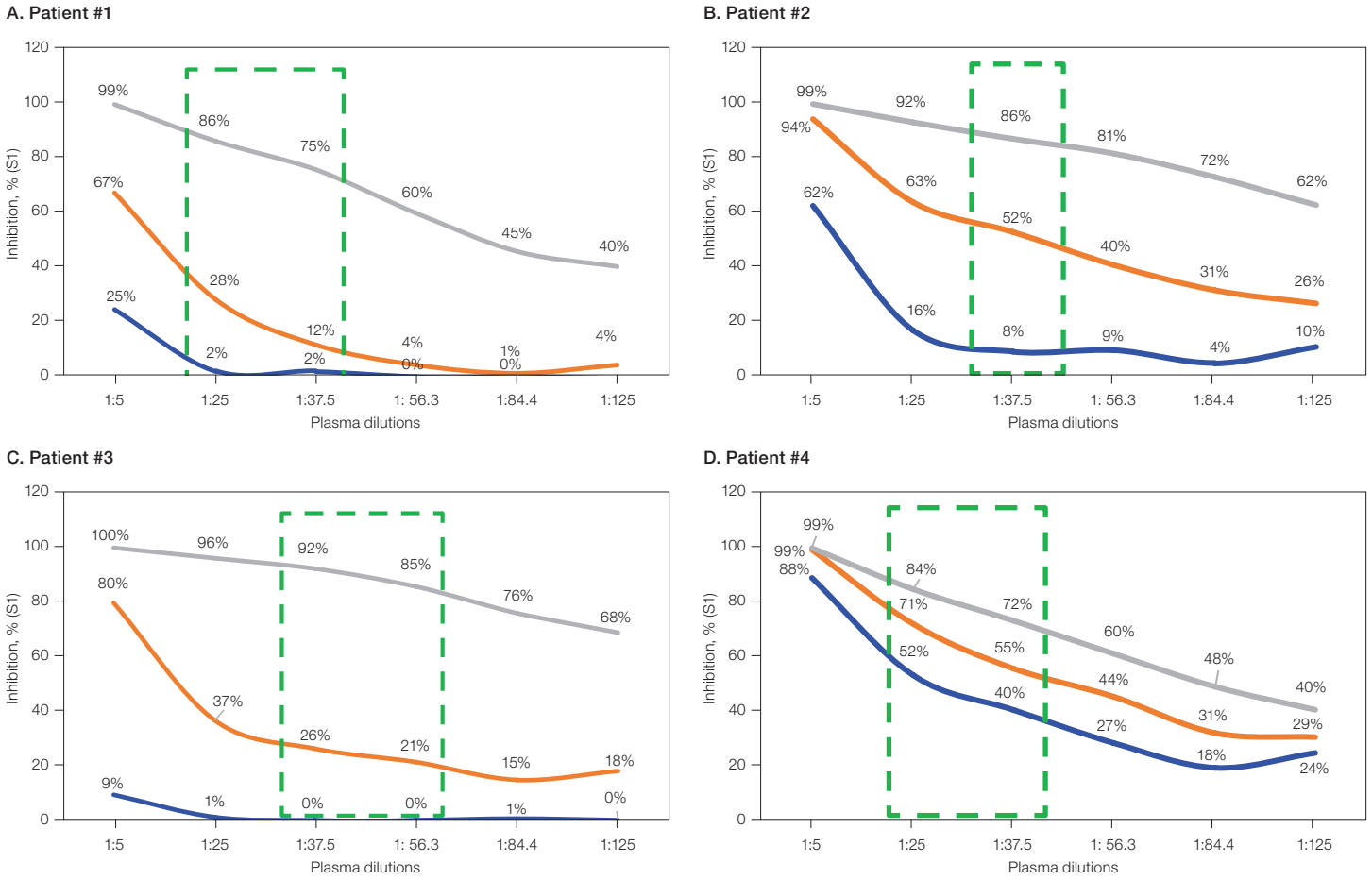

Fig. 3. Determination of sample dilutions within assay range. Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays were performed for two SARS-CoV-2 wild-type (S1 and RBD) and 9 variants (Alpha S1, Beta S1, Gamma RBD, Kappa RBD, Epsilon RBD, D614G S1, N501Y RBD, E484K RBD, and K417N RBD). Data for SARS-CoV-2 S1 are shown. Percent inhibition data from the Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays for S1 protein against the dilution series for patient plasma samples, with three sample collection time points from symptom onset. **A**, patient #1, ( ) 9 days,( ) 11 days, ( ) 13 days; **B**, patient #2, ( ) 5 days, ( ) 7 days, ( ) 9 days; **C**, patient #3, ( ) 11 days, ( ) 17 days, ( ) 26 days; **D**, patient #4, ( ) 12 days, ( ) 14 days, ( ) 17 days. Optimal dilutions ( ) for all three samples were selected to ensure other similar samples would be predicted to fall within range and not report as saturated percent inhibition.

Table 2. Summary of data from all patient samples. Summary data used to determine optimum plasma dilution for correlation of conventional live virus neutralization assay to Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays. Data for selected 1:37.5 dilution ( ).

	Days After Symptom Onset	Live Virus Neutralization End-point Titer, NT50	Inhibition at 1:5 Dilution, % (S1)	Inhibition at 1:25 Dilution, % (S1)	Inhibition at 1:37.5 Dilution, % (S1)	R^2 Value at 1:37.5 Dilution	Inhibition at 1:56.3 Dilution, % (S1)	Inhibition at 1:84.4 Dilution, % (S1)	Inhibition at 1:125 Dilution, % (S1)
Patient #1	9	64	25	2	2	0.9771	0	0	0
	11	1,024	67	28	12		4	1	4
	13	5,120	99	86	75		60	45	40
Patient #2	5	64	62	16	8	0.8662	9	4	10
	7	640	94	63	52		40	31	26
	9	2,560	99	92	86		81	72	62
Patient #3	11	0	9	1	0	0.9494	0	1	0
	17	1,280	80	37	26		21	15	18
	26	20,480	100	96	92		85	76	68
Patient #4	12	1,280	88	52	40	0.7833	27	18	24
	14	1,280	99	71	55		44	31	29
	17	10,240	99	84	72		60	48	40

Conclusion

In these correlation experiments, we demonstrated that the percent inhibition data from the Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays were found to correlate and were comparable to the gold standard conventional live virus neutralization assay's NT50 values at 1:37.5 dilution of COVID-19 plasma samples in Bio-Plex Pro Serology Sample Diluent. The Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody Assays enabled the processing of 38 samples in less than 3 hours in a BSL-1 laboratory rather than the 4 or more days in either BSL-3 or BSL-2 settings required for the conventional virus neutralization assay methods. Sample dilution factors should be determined for various sample cohorts to optimize the correlation.

Reference

Ravichandran S et al. (2020). Antibody signature induced by SARS-CoV-2 spike protein immunogens in rabbits. Sci Transl Med 12, eabc3539.

Ravindran et al. (2021). Immune response dynamics in COVID-19 patients to SARS-CoV-2 and other human coronaviruses. PLoS One 16(7), e0254367.

Ordering Information

Catalog #	Description
Multiplex Kits	
12016897	**Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody 11-Plex Panel**, 1 x 96-well
12016848	**Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody 2-Plex Panel**, 1 x 96-well
Singleplex Variants*	
12016868	**Bio-Plex Pro SARS-CoV-2 Alpha S1 Coupled Beads**
12016849	**Bio-Plex Pro SARS-CoV-2 Beta S1 Coupled Beads**
12017225**	**Bio-Plex Pro SARS-CoV-2 Delta RBD and Spike Trimer 2-Plex Coupled Beads**
12016875	**Bio-Plex Pro SARS-CoV-2 Epsilon RBD Coupled Beads**
12016898	**Bio-Plex Pro SARS-CoV-2 Gamma RBD Coupled Beads**
12016850	**Bio-Plex Pro SARS-CoV-2 Kappa RBD Coupled Beads**
12016838	**Bio-Plex Pro SARS-CoV-2 D614G S1 Coupled Beads**
12016943	**Bio-Plex Pro SARS-CoV-2 E484K RBD Coupled Beads**
12016942	**Bio-Plex Pro SARS-CoV-2 K417N RBD Coupled Beads**
12016869	**Bio-Plex Pro SARS-CoV-2 N501Y RBD Coupled Beads**
Reagents	
12016944	**Bio-Plex Pro Biotinylated Detection ACE2 Receptor**
12016945	**Bio-Plex Pro SARS-CoV-2 Neutralization Antibody Standard**
12016837	**Bio-Plex Pro Serology Beads Storage Buffer**
12017037	**Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Reagent Kit**
Custom Variant Neutralization Antibody Assay Developer Kit	
17007632	**Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Custom Assay Developer Kit**

* Use any mixture of singleplex variant coupled beads in combination with the Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody 2-Plex Panel or combine the coupled beads with Bio-Plex Pro Biotinylated Detection ACE2 Receptor, SARS-CoV-2 Neutralization Antibody Standard, and Human SARS-CoV-2 Neutralization Antibody Reagent Kit.

** 12017225 comes with a vial of premixed beads with Delta RBD and Delta Spike Trimer plus a Delta positive control.

Visit bio-rad.com/nAbSARSCoV2 for more information.

Visit cdc.gov/training/quicklearns/biosafety for information on biosafety levels.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23* ***Finland*** *00 800 00 24 67 23*
France *00 800 00 24 67 23* ***Germany*** *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300* ***Hungary*** *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050*
Italy *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Luxembourg*** *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670*
The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23*
Russian Federation *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
Switzerland *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150* ***United Kingdom*** *00 800 00 24 67 23*

Bulletin 3345 Ver A US/EG *22-0366 0722 Sig 0122*