Development of New SARS-CoV-2 Variant Neutralization Antibody Assays Using the Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Custom Assay Developer Kit

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Abstract

In this study, we demonstrate the utilization of the Bio-Plex Pro Human SARS-CoV-2 Neutralization Custom Assay Developer Kit to efficiently develop a new variant assay for the detection and determination of percentage inhibition of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralization antibodies with affinity to the SARS-CoV-2 Lambda variant.

Introduction

The first recorded cases of coronavirus disease 2019 (COVID-19) were in December 2019, and the infectious disease was determined to be caused by the SARS-CoV-2 virus. This pandemic has since infected over 270 million people globally and caused more than 5 million deaths as of December 15, 2021, according to the WHO Coronavirus (COVID-19) Dashboard (World Health Organization 2021). A major concern for researchers is the virus's ability to mutate. Point mutations are identified by amino acid modification or deletion and location. Certain combinations of mutations can result in increased transmissibility and virulence, changes in disease presentation, or a decrease in effectiveness of public health and social measures or therapeutics, diagnostics, or vaccines. Viral variants are defined by these point mutations and associated characteristics. Variants that have shown evidence of these types of characteristics are classified as variants of concern (VOC). As of November 29, 2021, the WHO has identified five variants of concern (Alpha, Beta, Gamma, Delta, and Omicron). Variants of interest (VOI) are classified based on projected expression of the characteristics of VOCs. Currently there are two variants of interest (Lambda and Mu). Since variants of concern and interest play a critical role in managing global public health during this pandemic, researching the effectiveness of the vaccines and therapies with regard to the evolving variants is critical.

Neutralization antibodies are the main defense against infectious disease, blocking viral entry into host cells. SARS-CoV-2 gains entry into the cell via the ACE2 receptor and viral spike trimer protein, consisting of three monomers of spike 1 (S1), which includes a receptor binding domain (RBD) and spike 2 (S2) proteins. Here we measure the antibody effectiveness of neutralization on the binding between the ACE2 receptor and viral spike trimer protein by using a competitive assay (Figure 1).

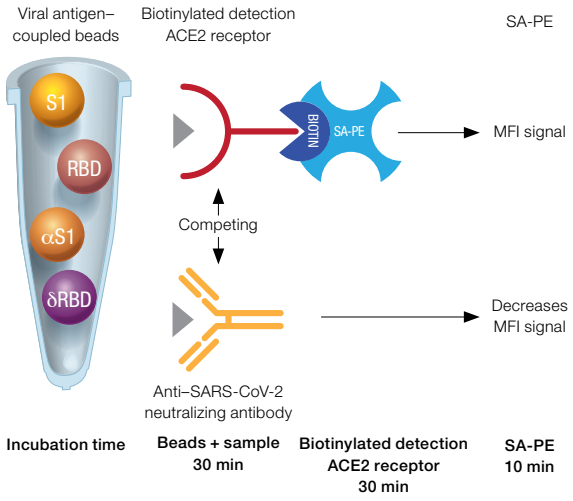

Fig. 1. Competitive assay format and incubation times for Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Assays. MFI, median fluorescence intensity; δRBD, Delta receptor binding domain; αS1, Alpha spike 1; SA-PE, streptavidin-phycoerythrin.

Materials and Methods

The Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Custom Assay Developer Kit (Bio-Rad Laboratories, Inc., catalog #17007632), SARS-CoV-2 Spike RBD Protein (with L452Q and F490S mutations) (Sino Biological Inc., #40592-V08H113), SARS-CoV-2 Spike Trimer (with G75V, T76I, SYLTPGD 247–253 deletion, L452Q, F490S, D614G, and T859N mutations) (ACROBiosystems, #SPN-C52Hs), and Bio-Plex Pro SARS-CoV-2 Neutralization Antibody Standard (Bio-Rad, #12016945) were used to develop the Lambda variant assay. The assay was run on the Bio-Plex 200 System with HTF (Bio-Rad, #171000205) using a 96-well plate. The Bio-Plex Pro Human Serology Sample Diluent was used as the negative control. All required reagents to run the assay after

coupling are included as part of the Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody 2-Plex Panel (Bio-Rad, #12016848) that comes with the Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Custom Assay Developer Kit (Bio-Rad, #17007632).

The Lambda variant assay was developed as a qualitative assay (results reported as percentage inhibition) and no standard curve was generated. Replicates of the negative control, positive control, blanks, and serum samples were run. For this assay, 23 SARS-CoV-2–positive serum samples confirmed by PCR in May 2020 and 22 serum samples from healthy subjects collected before December 2019 were used. Bead conjugation and assays were prepared and tested as described in the Bio-Plex Amine Coupling Kit Instruction Manual (10000148774). Different coupling concentrations were prepared (1 µg/scale, 3 µg/scale, and 5 µg/scale) and used to determine the optimal antigen load for the bead conjugation. The standard included in the Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody 2-Plex Panel was used to show the dose response of the standard. An antigen load of 3 µg for 1.25 x 10^6 beads was selected to be the bead conjugation condition. Selection was based on a comparison between a standard dilution series generated for each of the bead-antigen conjugations where the percentage inhibition was calculated for each point and the performance characteristics (percentage CV and recovery) were optimal at an estimated 50% inhibition. Figure 2 shows the plate layout that was used to find the optimal antigen concentration. Bead regions 20 and 38 were used for RBD Lambda and spike trimer Lambda, respectively. Note that any bead region can be used. One vial of bead region 27 is included in the developer kit.

Data generated on the Bio-Plex 200 System for the Lambda variant assay were acquired at the low photomultiplier tube (PMT) setting. All data were analyzed using Bio-Plex Manager 6.2 Software (Bio-Rad, #171STND05).

Results

Data collected from both positive and negative samples were analyzed using Bio-Plex Manager 6.2 Software. The resulting percentage inhibition was calculated using the following formula and was compared between two groups of serum samples: SARS-CoV-2–positive samples and samples collected before 2019 and presumed to be negative for SARS-CoV-2 (Figure 3).

Percentage inhibition = (1 – [MFI of sample/MFI of negative control]) x 100

Example of Plate Layout for Determining Coupling Concentration

	1 µg/scale		3 µg/scale		5 µg/scale							
	1	2	3	4	5	6	7	8	9	10	11	12
A	Negative control	Negative control	Negative control	Negative control	Negative control	Negative control	X	X	X	X	X	X
B	Diluted standard 1	Diluted standard 1	Diluted standard 1	Diluted standard 1	Diluted standard 1	Diluted standard 1	X	X	X	X	X	X
C	Diluted standard 2	Diluted standard 2	Diluted standard 2	Diluted standard 2	Diluted standard 2	Diluted standard 2	X	X	X	X	X	X
D	Diluted standard 3	Diluted standard 3	Diluted standard 3	Diluted standard 3	Diluted standard 3	Diluted standard 3	X	X	X	X	X	X
E	Diluted standard 4	Diluted standard 4	Diluted standard 4	Diluted standard 4	Diluted standard 4	Diluted standard 4	X	X	X	X	X	X
F	Diluted standard 5	Diluted standard 5	Diluted standard 5	Diluted standard 5	Diluted standard 5	Diluted standard 5	X	X	X	X	X	X
G	Diluted standard 6	Diluted standard 6	Diluted standard 6	Diluted standard 6	Diluted standard 6	Diluted standard 6	X	X	X	X	X	X
H	Blank	Blank	X	X	X	X	X	X	X	X	X	X

X, free wells for 52 samples or 26 duplicates.

Fig. 2. Suggested plate layout.

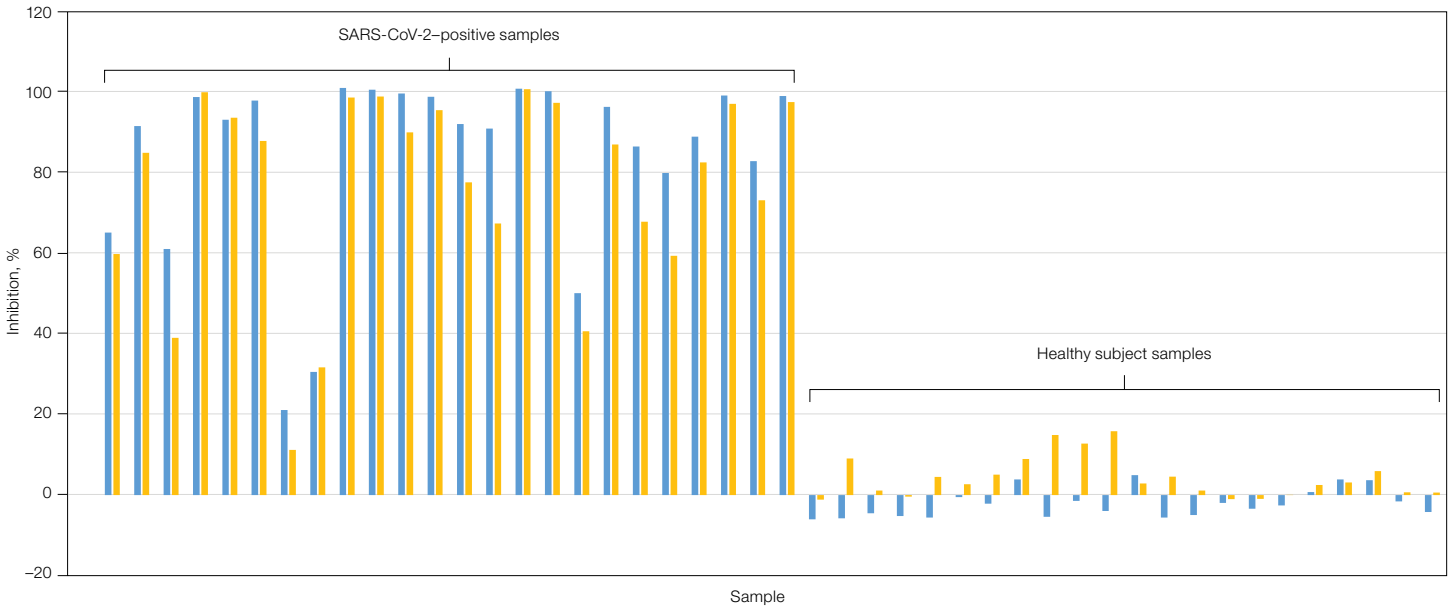

Fig. 3. Percentage inhibition of the Lambda variant. Serum samples were assayed using theBio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Custom Assay Developer Kit. MFI was measured in the Bio-Plex 200 System and the results of the percentage inhibition were calculated in an Excel sheet. **Left**, samples positive for SARS-CoV-2 from 23 patients; **Right**, 22 samples collected before the COVID-19 outbreak. RBD Lambda (■); spike trimer Lambda (■).

Conclusions

The calculated percentage inhibition exhibited in Figure 3 represents the successful development of a Lambda variant assay that can be useful in determining the amount of neutralizing antibodies against the Lambda variant present in the SARS-CoV-2–positive samples. We have demonstrated that the Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Custom Assay Developer Kit can be effectively used to rapidly create new competitive assays for the determination of the antibody neutralization activity on current SARS-CoV-2 variants and any future variant that may arise.

Reference

World Health Organization (2021). WHO Coronavirus (COVID-19) Dashboard. covid19.who.int, accessed December 15, 2021.

Visit **bio-rad.com/Bio-PlexAssayDeveloperKit** for more information.

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