Seropositivity Testing for SARS-CoV-2 Neutralizing Antibodies

Validation of a Novel Assay for Semi-Quantitative Measurement

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Key Features

- The detection of SARS-CoV-2 neutralizing antibodies offers important information for evaluating immune responses to COVID-19. It has multiple applications such as studies of herd immunity and vaccine development among others.

- Cayman's **SARS-CoV-2 Neutralizing Antibody Detection ELISA Kit** functions as both a simple, qualitative indicator of positive or negative samples and as a semi-quantitative indicator of seropositivity.

- Disease-free positive and negative plasma controls are supplied to confirm the assay is performing as expected.

- Approximating concentrations of neutralizing antibody found in positive samples is achieved using a neutralizing antibody as a reference standard.

- The format of our ELISA (in which neutralizing antibodies must recognize the SARS-CoV-2 spike receptor binding domain to inhibit ACE2 binding) provides an efficient and effective screening tool for determining functional antibody neutralization capabilities.

- The semi-quantitative design is the first of its kind for SARS-CoV-2 seropositivity testing and provides a consistent reference point for neutralizing antibody levels not readily available with standard serological assays.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped positive-stranded RNA virus and the causative agent of COVID-19.[1-5] The SARS-CoV-2 surface glycoprotein, also known as the spike glycoprotein, is located on the outer envelope of the virion and contains the receptor binding domain (RBD), which binds to angiotensin-converting enzyme 2 (ACE2), the functional receptor for SARS-CoV-2.[1,6-10]

SARS-CoV-2 infection can result in the production of neutralizing antibodies, which bind to the SARS-CoV-2 spike RBD preventing further viral entry and infection, starting approximately 4-10 days after symptom onset.[11,12] Plasma levels of SARS-CoV-2 spike glycoprotein-specific IgG antibodies increase for at least four weeks following symptom onset.[11,13] SARS-CoV-2 plasma antibody levels begin to decrease 2-3 months post-infection in both symptomatic and asymptomatic individuals, disappearing completely in some asymptomatic individuals.[14]

The presence of these antibodies can indicate an ability to suppress progression of SARS-CoV-2 infection and is important in evaluating the lifetime and efficacy of specific antibodies in the host. The detection of neutralizing antibodies can provide valuable information for researchers studying immune responses or herd immunity to SARS-CoV-2, as well as for the development of effective vaccines.

Cayman's **SARS-CoV-2 Neutralizing Antibody Detection ELISA Kit** can function as a simple, qualitative indicator of positive or negative samples, but can also be used to determine the approximate amounts of neutralizing antibodies found in positive samples using the included neutralizing antibody reference standard. This semi-quantitative capability is the first of its kind for SARS-CoV-2 seropositivity testing.

Assay Format and Performance

Our competitive assay format measures neutralizing antibodies of the SARS-CoV-2 spike RBD and ACE2 interaction in human plasma and serum. Recombinant SARS-CoV-2 spike RBD is immobilized on the plate, followed by the addition of recombinant ACE2. This interaction is then detected with an antibody conjugated to horseradish peroxidase (HRP), which can easily be quantified by reading absorbance at 450 nm. The interaction between SARS-CoV-2 spike RBD and ACE2 is disrupted in the presence of neutralizing antibodies in the sample, preventing signal emission (**Figure 1**). This assay format allows for the detection of all neutralizing antibodies independent of isotype, including IgA, which is particularly salient to mucosal tissue infections.
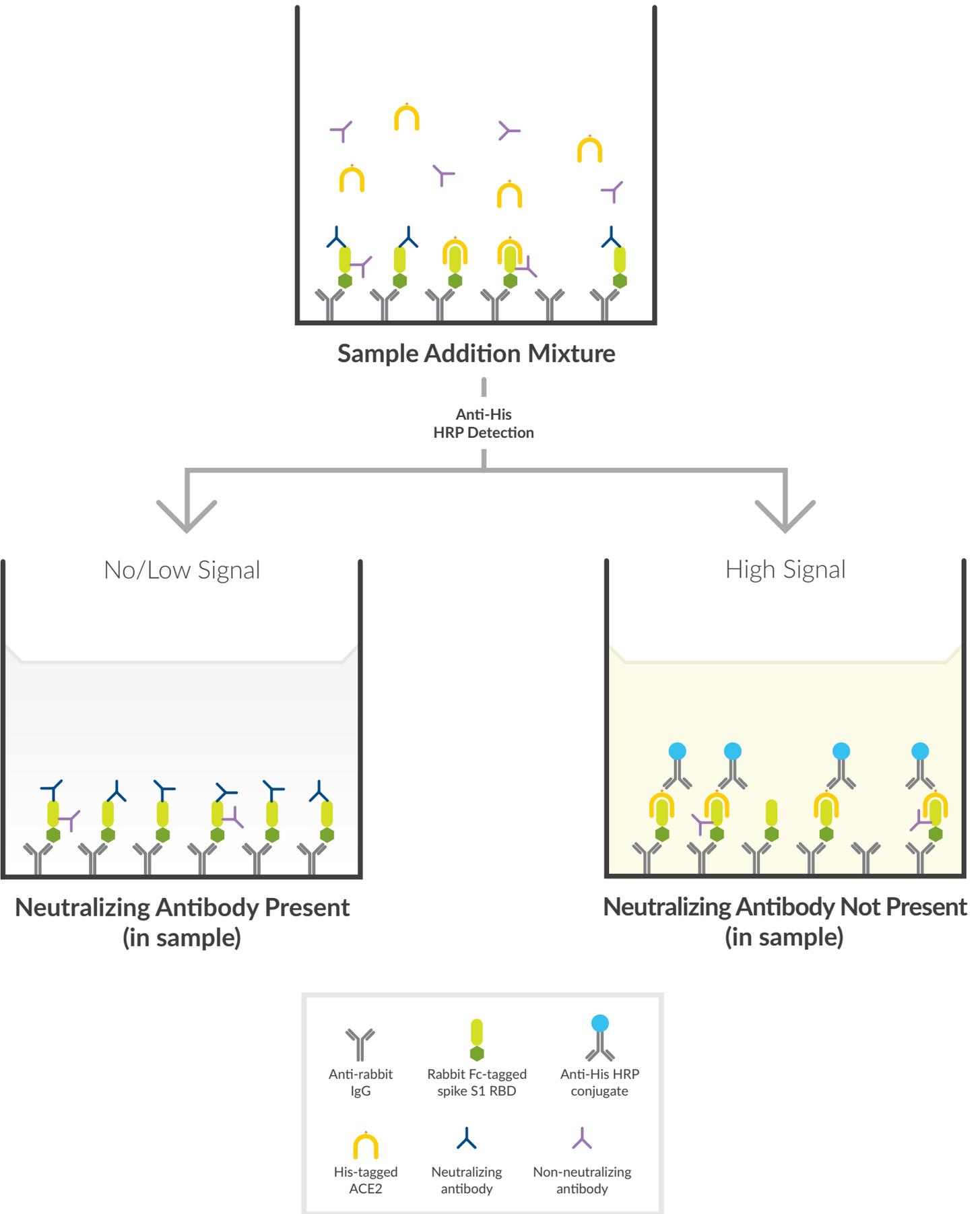
Sample Addition Mixture

Anti-His
HRP Detection

No/Low Signal

High Signal

**Neutralizing Antibody Present
(in sample)**

**Neutralizing Antibody Not Present
(in sample)**

Anti-rabbit
IgG

Rabbit Fc-tagged
spike S1 RBD

Anti-His HRP
conjugate

His-tagged
ACE2

Neutralizing
antibody

Non-neutralizing
antibody

Figure 1. Assay schematic of the SARS-CoV-2 Neutralizing Antibody Detection ELISA Kit.

Simple Qualitative Analysis

For initial testing, samples are screened using the qualitative format of the assay. Positivity of each sample is interpreted by the ratio (B/B_0) of the absorbance value for each sample (B) to the absorbance value of the maximum ACE2 binding wells (B_0). For this assay, a positive *versus* negative cut-off of 70% B/B_0 has been established. This value is based on assaying multiple samples previously determined to be positive or negative in PCR or serological assays. If the B/B_0 of a sample is below 70%, neutralizing antibodies are present and alternatively, if it is above 70%, the sample is considered to be negative (**Figure 2**).
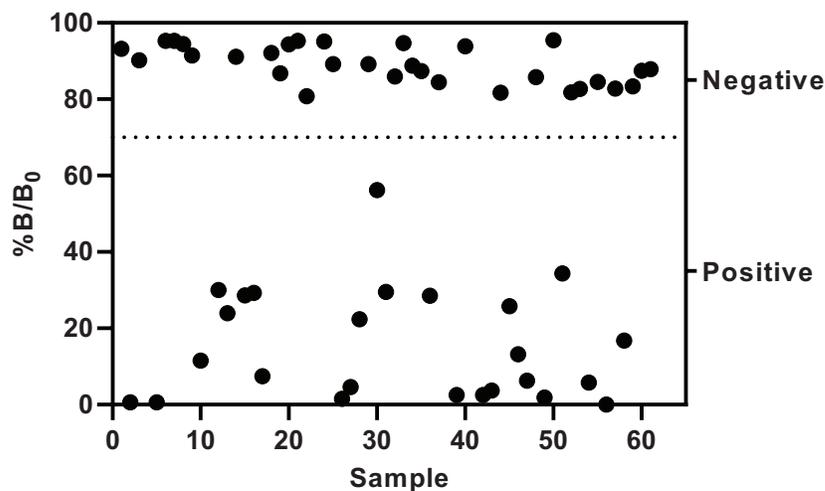

Figure 2. Qualitative assay results (N=62). Samples include a mixture of pre-pandemic, post-pandemic serology positive, and post-pandemic serology negative plasma and serum.

To assure assay performance, positive and negative controls are included as part of the assay kit. The positive control consists of disease-free plasma spiked with a known quantity of recombinant SARS-CoV-2 neutralizing antibody. The use of a recombinant antibody in this way allows for reduced batch-to-batch variability of the positive control and introduces a level of consistency that cannot be obtained with COVID-19-positive plasma or serum controls.

Semi-Quantitative Analysis

Semi-quantitative estimates for antibody levels in samples identified as positive can be determined by comparison to a SARS-CoV-2 neutralizing antibody standard curve. The included standard uses a human recombinant SARS-CoV-2 neutralizing IgG that exhibits a high level of parallelism when compared to positive samples.

It is important to note that although the reference antibody used in our assay performs this task very well, no antibody can function as an absolute quantitative standard and, at best, can only be semi-quantitative. This is because the polyclonal antibody pool within an individual sample will have unique affinity for the target antigen and is certain to be different than any antibody or antiserum one could select as a standard. When using this neutralizing antibody standard to estimate sample concentration, the assay has a range of 7.81-1,000 ng/ml with a midpoint of approximately 107 ng/ml (50% B/B_0) and a sensitivity (80% B/B_0) of approximately 41 ng/ml.

Assay Validations

For assay validation, samples tested in this assay were either serological positive or negative based on the presence or absence of antibodies against a minimum of one viral protein (*i.e.*, nucleocapsid, spike protein, etc.) as determined in an independent assay. Not all seropositive samples will contain neutralizing antibodies to the spike RBD and ACE2 interaction. Although most samples that tested seropositive were also neutralizing positive, one seropositive sample was found to exhibit a negative neutralizing response (**Figure 3**). This result, confirmed in an alternative neutralizing antibody detection assay, is not unexpected as neutralizing antibodies must recognize the SARS-CoV-2 spike RBD to inhibit ACE2 binding in this assay. The strength of neutralization is determined by the RBD epitope to which antibodies bind. Those binding to the top of the RBD strongly compete with ACE2 binding, whereas those directed to the side of the RBD do not compete with ACE2 binding.[15] These results emphasize the importance of effective screening for functional antibody neutralization capabilities rather than testing only for the presence of antibodies against a subset of proteins, such as those against nucleocapsid or spike proteins from convalescent patient samples.
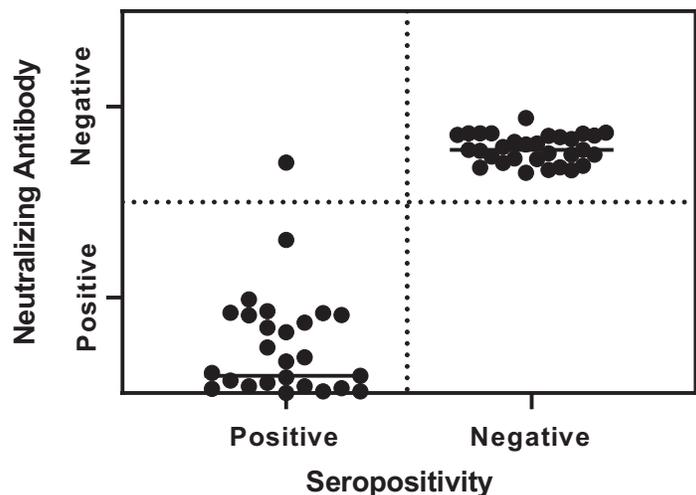

Figure 3. Comparison of seropositivity versus neutralizing antibody positivity.

Samples that tested positive for neutralizing antibodies in the qualitative format were then re-tested using the semi-quantitative format. The data in **Table 1** shows the quantification of the 30 positive samples shown in **Figure 2**. The average concentrations and standard deviation are representative of the combined assessment of concentration at multiple sample dilutions. Significant precision was obtained with coefficient of variation less than 10% for most samples. Concentrations of neutralizing antibody positive samples ranged from 842-89,570 ng/ml.

Table 1. Quantification of neutralizing antibody in samples found positive in qualitative assay.

Sample	Average concentration (ng/ml)	Sample	Average concentration (ng/ml)	Sample	Average concentration (ng/ml)
1	4,681 ± 331.8	11	30,938 ± 1,851.5	21	9,253 ± 310.3
2	842 ± 52.1	12	56,192 ± 2,970.0	22	55,357 ± 6,627.6
3	14,522 ± 502.0	13	1,867 ± 93.8	23	6,298 ± 388.0
4	1,657 ± 181.9	14	12,142 ± 807.0	24	4,829 ± 522.7
5	3,599 ± 354.6	15	8,588 ± 482.9	25	61,406 ± 5,942.7
6	2,177 ± 94.5	16	89,570 ± 7,215.0	26	19,861 ± 1,753.5
7	1,858 ± 126.1	17	1,882 ± 232.9	27	2,164 ± 211.1
8	2,881 ± 39.2	18	8,146 ± 468.4	28	6,978 ± 388.4
9	1,861 ± 63.8	19	6,567 ± 606.5	29	7,508 ± 386.0
10	2,299 ± 163.4	20	2,096 ± 368.6	30	12,395 ± 1,009.0

It has been shown in several studies that antibody titers against SARS-CoV-2 proteins drop off significantly in the weeks following infection.[14] Such antibody titers are typically subjective in nature and values can vary widely depending on the assay used. Estimating concentrations of neutralizing antibody is an effective way to determine post-infection efficacy of convalescent plasma or serum samples. The semi-quantitative principle of Cayman's **SARS-CoV-2 Neutralizing Antibody Detection ELISA Kit** provides a solid reference point for antibody levels that is generally absent with standard serological assays. **Figure 4** shows semi-quantitative assay results for neutralizing antibody in plasma from a recovered COVID-19-infected individual collected at the indicated timepoints post-onset of symptoms. These data confirm that the timing of sample collection is a key factor when determining SARS-CoV-2 neutralizing antibody concentrations. These findings, along with the well-accepted variance of response within a population, will mandate the practice of careful data collection and analysis.
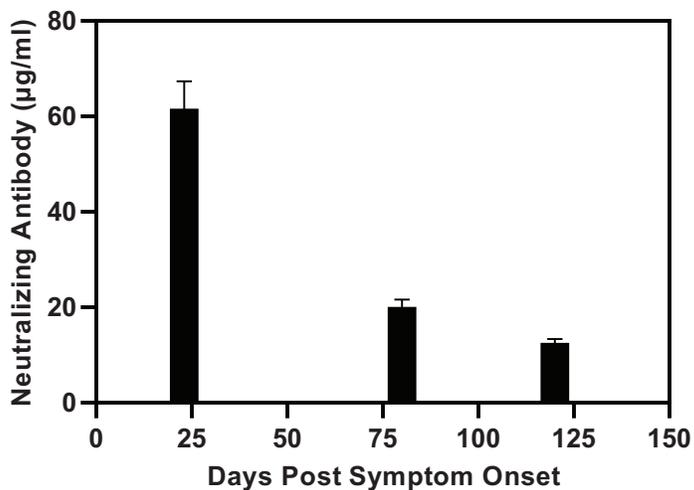

Figure 4. Concentration of neutralizing antibody in COVID-19 convalescent plasma over time.

Conclusion

This assay is an essential tool for studying the host's immune response to SARS-CoV-2 infection. The measurement of SARS-CoV-2 neutralization antibodies can be used to effectively assess vaccination regimens, convalescent patient antibody presence, and the development of neutralizing antibodies against the SARS-CoV-2 spike glycoprotein *in vitro*. In addition, the semi-quantitative nature of the assay lends itself to reproducible measurement of neutralizing antibody concentrations, in relation to a fixed reference antibody, over long periods of time.

Related Cayman products

Item No.	Product Name
502070	SARS-CoV-2 Neutralizing Antibody Detection ELISA Kit
502080	SARS-CoV-2 Neutralizing Antibody Human Plasma Control Set
502090	SARS-CoV-2 Neutralizing Antibody Human Serum Control Set
31585	SARS-CoV-2 Neutralizing Antibody (*coming soon*)
31568	SARS-CoV-2 Neutralizing Antibody-Positive Human Serum
31567	SARS-CoV-2 Neutralizing Antibody-Negative Human Serum
31569	SARS-CoV-2 Neutralizing Antibody-Negative Pre-pandemic Human Serum
502050	SARS-CoV-2 Spike-ACE2 Interaction Inhibitor Screening Assay Kit

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