cPASS™ SARS-CoV-2 Neutralization Antibody Detection Kit (Cat# L00847)

Vaccine Research and Development
Application Note

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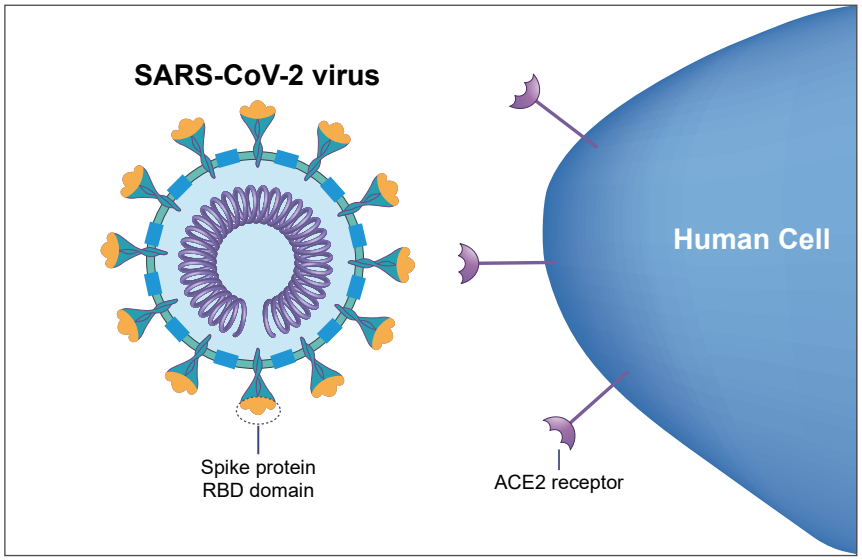
Introduction

COVID-19 caused by SARS-CoV-2 is a zoonotic disease that has already spread globally to a large human population and possibly to domestic and wild animals. There is an urgent need to improve our understanding of the immunology of this disease to contain this pandemic through the development of vaccines and therapeutics.[1] The World Health Organization (WHO) lists seventeen COVID-19 vaccine candidates that are under clinical evaluation globally.[2] There may be an order of magnitude more COVID-19 vaccine candidates in the pre-clinical development phase from a global perspective.

Immune responses to vaccination are routinely measured in blood (cellular immune responses) and serum (humoral immune responses). Cell mediated immune responses are measured by quantifying the number of sub-sets of lymphocyte populations (E.g. flow cytometry analysis of CD4 and CD8 levels) and functional assays (E.g. interferon gamma release assay). Humoral immune responses are measured by immunoassays (E.g. quantifying IgM and IgG antibody levels or titers using ELISA) and functional assays (E.g. neutralizing antibody bioassays).

In the context of SARS-CoV-2, IgA, IgM, and IgG antibody ELISA assays using plasma or serum are being used as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. During early stage of infection, approximately 5-7 days after the onset of symptoms, IgM antibodies are typically detected. IgG antibodies are detected during the active and late phases of infection or during recurrent infection. A small percentage of antibodies bind to sites on the virus that interact with host proteins, masking them, and inhibiting entry of that virus into the host. These are known as neutralizing antibodies. The main target for neutralizing antibodies on coronaviruses is the spike (S) protein, a homo-trimeric glycoprotein that is anchored in the viral membrane (**Fig 1**). Potent neutralizing antibodies often target the receptor interaction site in S1, disabling host receptor interactions and preventing viral entry into the cell [3].



- For the virus to invade human cells, it attaches to the **ACE2 receptor** through the receptor binding domain (**RBD**) of the **Spike protein**.
- If this interaction is blocked with a Neutralizing/blocking antibody, the virus is less likely to invade the cell.

Fig 1. The interaction of the SARS-CoV-2 viral Spike protein with the ACE2 receptor from human cells is needed for the virus to invade human cells.

Neutralizing antibody is one of the most important biomarkers of humoral immunity and vaccine efficacy. Eliciting a neutralizing antibody response is a goal of many vaccine development programs and commonly correlates with protection from disease. There are three types of virus neutralization assays that have been cited in literature specific to SARS associated corona virus. These assays use a dilution series of serum samples (from infected patients or animals) to determine the level (or titer) of neutralizing antibody present. The cytopathogenic effect-based (CPE) virus neutralization assay relies on visual grading of virus infected or uninfected cells to determine the level of neutralization [4]. The plaque reduction neutralization assay (PRNT) relies on counting plaques to quantify virus neutralization, and is the gold standard for neutralizing antibody evaluation [4]. The neutral red staining (NRS) assay measures cell viability upon viral infection to determine neutralization antibody titer [5].

The conventional neutralization assays mentioned above are labor intensive and can take from days to a week to produce results (**Fig 2**). In addition, they are not scalable. Considering the extreme infectivity of SARS-CoV-2 these assays have to be done in BSL-3 facilities with highly trained personnel. In order to overcome drawbacks of the conventional virus neutralization bioassays, GenScript has developed the cPass™ SARS-CoV-2 Neutralization Antibody Detection kit (also known as surrogate virus neutralization assay kit) using an ELISA format (show below) [6]. This ELISA test is scalable, automatable and can be performed by typical lab personnel using a BSL-2 safety level environment. The cPass™ kit can be used to evaluate the neutralizing antibody potency of serum samples from immunized animal models or infected patients.

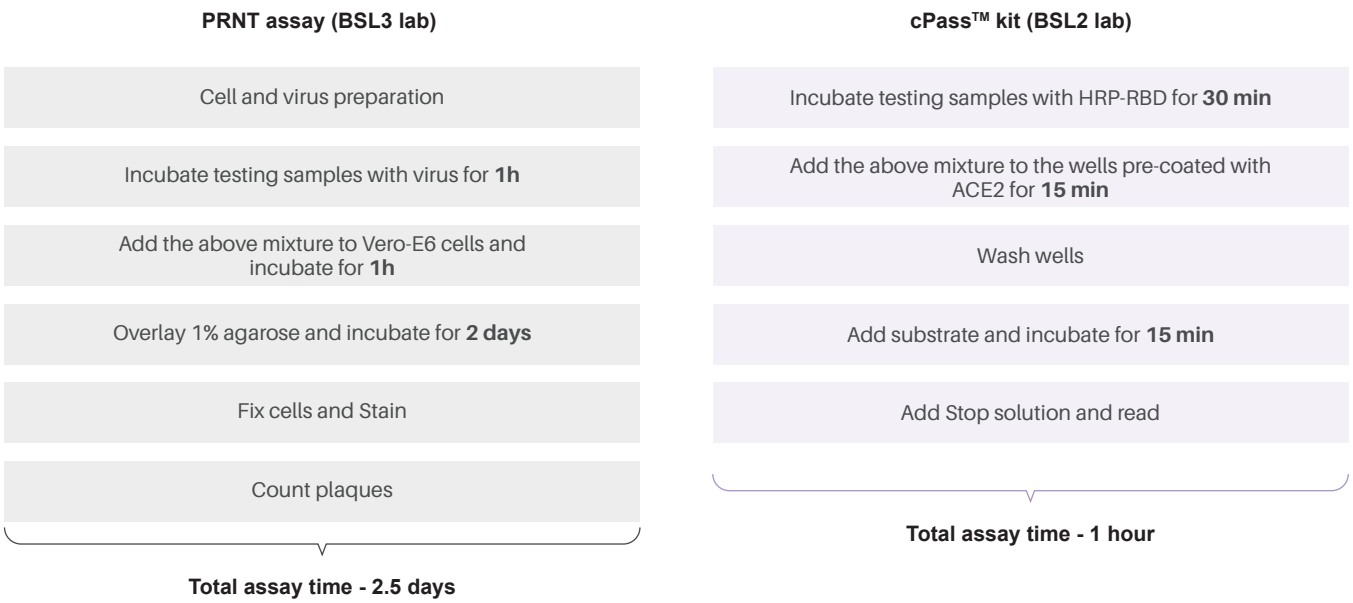


Fig 2. Comparison between the PRNT and cPass kit workflows. The total assay time of cPass kit is an hour and can be performed in a BSL2 lab. The PRNT assay needs both the COVID19 live virus and Vero-E6 cells and takes over 2 days in a BSL3 lab.

A study of 56 samples showed excellent correlation of results with 100% sensitivity and 100% specificity when compared to PRNT. It further confirms that the cPass™ kit is very reliable for evaluation of the antibody potency of serum samples (**Fig 3**).

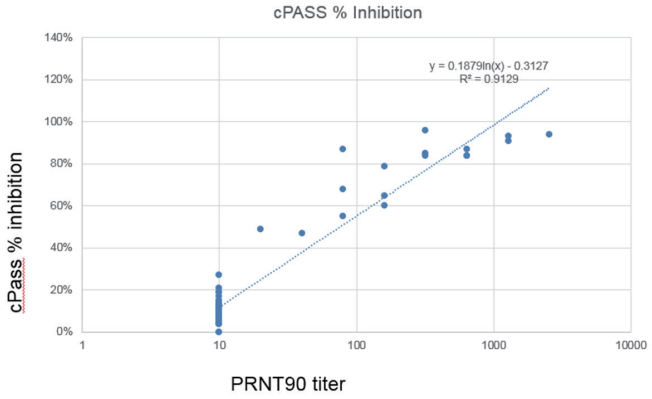


Fig 3. Correlation of the cPass™ kit test with the PRNT test. A study of 56 samples showed excellent correlation of results with 100% sensitivity and 100% specificity when compared to PRNT.

Assay Principle

The cPASS™ SARS-CoV-2 Neutralization Antibody Detection Kit is based on competitive ELISA methodology and detects the presence of neutralizing/blocking antibodies in a serum or plasma sample. ACE2 receptor protein (the angiotension converting enzyme [2]) is plated and HRP labeled RBD (the receptor binding domain of the SARS-CoV-2 spike protein) (HRP-RBD) is used for detection (**Fig 4**). The serum or plasma samples with more neutralizing antibodies show a lower signal intensity.

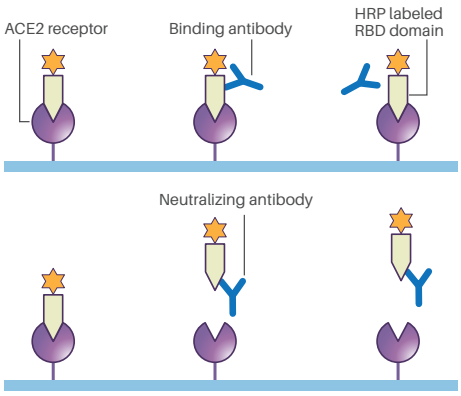


Fig 4. If only binding antibodies are present in the sample, then the HRP labeled RBD still binds to the ACE2 receptor on the plate and generates strong signal.

If the neutralizing/ blocking antibodies are also present in the sample, then they will bind to some of the HRP-RBD and prevent binding to the ACE2 receptor on the plate. During the wash steps, the blocked HRP-RBD is removed, therefore decreasing the signal detected in the well.

Materials

cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (L00847)

Mouse/rabbit antiserum samples: immunized with RBD protein

SARS-CoV-2 patient serum samples

Methods

Serum samples from animal models or human subjects are serially diluted with dilution buffer. The serial dilutions from **Fig 5-7** below can be used as references for the serum samples from mouse, rabbit and infected patients, respectively. The appropriate concentration or dilution range may need to be optimized according to different samples in order to get a sigmoid curve for the IC50 calculation.

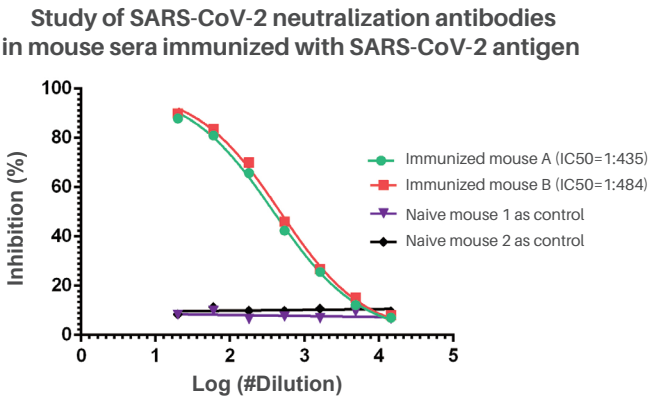


Fig 5. cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit was used to analyze neutralizing antibody levels in mouse serum samples. The mice were immunized with SARS-CoV-2 RBD to generate a neutralizing antibody response, and then compared with unimmunized controls. A representative result showed that the IC50 is 1:435 for mouse A and 1:484 for mouse B.

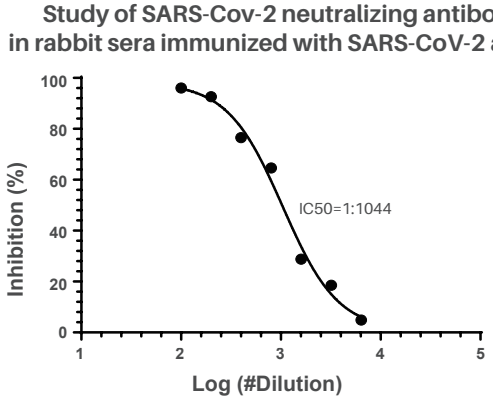


Fig 6. The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit was used to analyze the levels of neutralizing antibody in rabbit serum samples. The rabbits were immunized with SARS-CoV-2 RBD protein to generate a neutralizing antibody response and then compared with control group. A representative result showed that the IC50 was calculated to be 1:1044.

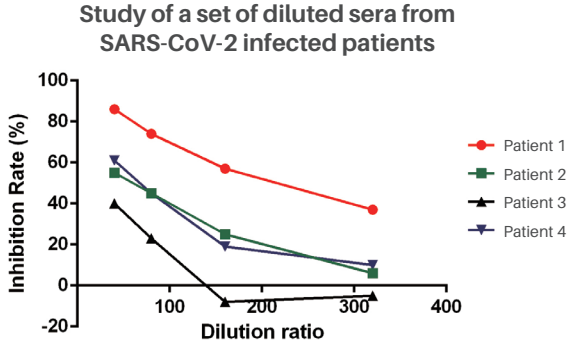


Fig 7. The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit was used to analyze a set of diluted sera from SARS-CoV-2 infected patients. A representative result demonstrated that cPass™ kit has a good dose-dependent response with human serum.

Reagents are prepared according to the kit manual [6]. Incubate HRP-RBD with testing samples (or positive /negative control) with appropriate dilution for 30 min. The mixture is then added to the wells pre-coated with ACE2 protein in the 96 well plates for 15 min. The wells are washed with wash solution for a total of four cycles. HRP substrate TMB is added to each well and incubated in the dark for 15 min. Stop solution is added to each well and absorbance is read at 450 nm on a microplate reader.

The average optical density (OD) of the negative control is used to calculate the inhibition %. Results of each individual samples can be calculated using the formula here:

$$\text{Inhibition} = \left(1 - \frac{\text{OD value of Sample}}{\text{OD value of Negative Control}}\right) \times 100\%$$

IC50 (50% inhibitory concentration) is the common metric used to evaluate the neutralizing antibody potency. Here we use the IC50 to represent the serum titer at which the neutralizing antibody exerts half of its maximal inhibitory effect. IC50 values are calculated according to the dose response curves by using the Hill equation [7].

Results

Inhibition curves were generated by using cPass™ kit to analyze the neutralizing antibody levels in mouse (**Fig 5**), rabbit (**Fig 6**) and human serum (**Fig 7**) samples. Different serum dilutions are plotted against the corresponding inhibition rate to generate dose response curves.

Both mice and rabbits immunized with SARS-CoV-2 RBD generate functional neutralizing antibodies (**Fig 5 and 6**) with inhibition of RBD and ACE2 binding. The cPass™ kit has been designed to generate IC50s that are species-independent and can be compared across species. According to the IC50, the relative inhibition potency of neutralizing antibodies in the serum can be compared. This result shows that mice that were immunized with a SARS-CoV2 antigen produced a high percent inhibition at low dilutions, and that the percent inhibition decreased as the dilution factor increased. This indicates the measurable presence of neutralizing antibodies in the animals' sera.

Serum samples from a cohort of SARS-CoV-2 infected patients were tested using the cPass™ kit (**Fig 7**). The inhibition rate of the generated neutralizing antibodies showed a dose-dependent response with serial dilutions of the serum samples. Patient 1 showed an overall better inhibition rate than other patients. This indicates better neutralizing antibody activity against the SARS-Cov-2 virus than the other patients (**Fig 7**). Those data demonstrate that cPass™ kit can be used to assess neutralizing antibody responses to human and veterinary vaccines.

Conclusions

Our results demonstrate that the cPass™ kit is an excellent assay for detection of the SARS-Cov2 neutralizing antibodies in the serum of different animal species and humans.

- The cPass™ kit showed a strong correlation with the PRNT assay (the gold standard for neutralizing antibody quantification).
- The cPass™ kit can be used to detect the neutralizing antibodies in animal and human serum with dose-dependent result across a wide-range of concentrations.
- The cPass™ kit is not isotype dependent and hence can detect neutralizing antibodies of different isotypes (data not shown).
- The cPass™ kit was able to detect mouse, rabbit and human antibodies from serum samples without any changes to the assay reagents or format (Fig 5, 6, 7). Both plasma and serum samples show equivalent results (data not shown).

The features of cPass™ kit confer the following benefits:

- The cPass™ kit is safer to use than the PRNT assay. It can be performed in BSL-2 laboratories by technicians familiar with ELISA, since it does not require any live virus.
- The cPass™ kit is easier to standardize, less labor-intensive, and scalable because it uses a standard blocking ELISA format that is compatible with most automated microplate washers and readers. The turnaround time is only 1-2 hours.
- The cPass™ kit is not species dependent, hence it can be used across the spectrum of pre-clinical and clinical COVID19 vaccine development studies.
- The cPass™ kit can be used to assess immune response to COVID 19 vaccines.
- The cPass™ kit can also be applied to population-wide studies to assess durability of neutralizing antibody response.

In conclusion, the cPass™ kit is a breakthrough solution in the fight against COVID19 since it can be used across the entire spectrum of COVID19 vaccine development to vaccine efficacy assessment and monitoring durability of neutralizing antibody responses.

References

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7. Biphasic Mathematical Model of Cell–Drug Interaction That Separates Target-Specific and Off-Target Inhibition and Suggests Potent Targeted Drug Combinations for Multi-Driver Colorectal Cancer Cells. Jinyan Shen *et al*. Cancers, 13 Feb 2020.

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