COVID-19 convalescent plasma therapy donor qualification by SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT)

Application Note

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Introduction

The COVID-19 pandemic has affected almost 30 million people cumulatively and caused nearly a million deaths worldwide as of late September, 2020. Unfortunately, no specific antiviral agents have been proven to be effective. Scientists and healthcare professionals are still looking for treatments that can alleviate symptoms and rescue severe patients, and more and more are now turning to convalescent plasma therapy.

Convalescent plasma therapy has been proven to be successful in multiple infectious disease cases in recent decades when effective antiviral agents are not available. Back in 2009, a prospective H1N1 cohort study showed a significant reduction in viral load in 3-7 days and mortality risk in ICU patients treated with convalescent plasma (Hung IF, 2011) (Hung IFN, 2013). In 2014, convalescent plasma therapy was recommended by the WHO to treat Ebola. (WHO, Ebola convalescent treatment, 2014) In 2015, a convalescent plasma treatment protocol for MERS was established. (Arabi Y, 2015)

Convalescent plasma therapy show effectiveness in severe COVID-19 cases

Recent studies of COVID-19 show the effectiveness of convalescent plasma therapy in patients with severe symptoms. (Mingxiang Ye, 2020) (Kai Duan, 2020) (Chenguang Shen, et al., 2020) Convalescent plasma with a high neutralizing antibody titer shows promising outcomes with clinical symptoms, and paraclinical criteria rapidly improved within 3 days after treatment. In Duan and colleagues' study (Kai Duan, 2020), one dose of 200 ml convalescent plasma with a neutralizing antibody titer above 1:160 from recently recovered COVID-19 patients was transfused to 10 severe patients. Besides the significantly improved clinical symptoms, the level of neutralizing antibodies also increased rapidly to or was maintained at 1:640 in 9 out of 10 cases, indicating the importance of neutralizing antibodies in the effectiveness of convalescent plasma therapy and restoration of immunity against SARS-CoV-2 in COVID-19 patients. (Arabi Y, 2015)

Patient no.	CP transfusion date	Before CP transfusion			After CP transfusion		
		Date	Serum neutralizing antibody titers	Serum SARS-CoV-2 RNA load (Ct value)	Date	Serum neutralizing antibody titers	Serum SARS-CoV-2 RNA load (Ct value)
1	Feb 9	Feb 8	1/160	37.25	Feb 10	1/640	Negative
2	Feb 9	Feb 8	Unavailable	35.08	Feb 11	Unavailable	Negative
3	Feb 13	Feb 12	1/320	38.07	Feb 14	1/640	Negative
4	Feb 13	Feb 12	1/160	37.68	Feb 14	1/640	Negative
5	Feb 12	Feb 11	1/640	Negative	Feb 14	1/640	Negative
6	Feb 12	Feb 11	1/640	Negative	Feb 14	1/640	Negative
7	Feb 12	Feb 11	1/320	34.64	Feb 14	1/640	Negative
8	Feb 12	Feb 11	1/640	35.45	Feb 14	1/640	Negative
9	Feb 12	Feb 11	1/160	Negative	Feb 14	1/640	Negative
10	Feb 9	Feb 8	1/640	38.19	Feb 14	1/640	Negative

Table 1. Comparison of serum neutralizing antibody titers and SARS-CoV-2 RNA load before and after CP therapy (Kai Duan, 2020)

Why do we measure neutralizing antibodies in donor convalescent plasma?

Convalescent plasma from different recovered patients may contain antibodies with different titers and potency. Although we can measure the quantity of SARS-CoV-2 antibodies through many commercially available antibody tests that detect IgG, IgM, or total antibodies, the efficacy of plasma with the same antibody quantity may be different. The effectiveness of binding antibodies relies on the complement system in different hosts, and they have no immediate virus blocking activity. Thus it is hard to predict their efficacy by measuring their quantities.

Different from binding antibodies, neutralizing antibodies block the interaction between virus protein and host receptors (Laura A. VanBlargan, 2016), and thus stop the viral entry and further proliferation. Neutralizing antibodies do not require a complicated immune response pathway and function immediately after transfusion to inhibit viral replication. Their potency can be directly measured by virus neutralization assays. Therefore, neutralizing antibodies' potency becomes a perfect parameter to predict the effectiveness of convalescent plasma therapy and can work as a screening standard to choose donor plasma.

Fast, scalable donor plasma screening by neutralizing antibody test

"Investigational COVID-19 Convalescent Plasma Guidance for Industry" published on May 1, 2020, by the FDA (/media/136798/download) (FDA, 2020) recommended donor eligibility qualifications, which includes at least 1:160 titer of neutralizing antibodies in donor plasma, or 1:80 titer if an alternative matched unit is not available. Due to the lack of supportive data from large-scale clinical studies, convalescent plasma therapy has not yet been approved by the FDA and is regulated as an investigational product. A consistent, high-throughput screening for donor qualification is critical to scale up convalescent plasma therapy as the investigational new drug (IND) to save more lives.

Plaque Reduction Neutralization Tests (PRNT) or Virus Neutralization Tests (VNT), are considered the gold standards to test neutralizing antibody titers. However, these assays require live viruses and cells, biosafety containment facilities, highly skilled operators, they are less sensitive, and take 2-3 days to obtain results. They are thus hard to scale up via automation and distribute widely.

To solve these problems, GenScript has developed SARS-CoV-2 Surrogate Virus Neutralization Test that is based on blocking ELISA methodology and detects the presence of neutralizing/blocking antibodies in a serum or plasma sample. Using purified receptor-binding domain (RBD) protein from the viral spike (S) protein and the host cell receptor ACE2, the test is designed to mimic the virus-host interaction by direct protein-protein interaction in a test tube or an ELISA plate well. During the test, ACE2 protein is plated, and HRP labeled RBD (HRP-RBD) is conjugated to ACE2. When there are neutralizing antibodies present in patient sera, they will block the specific protein-protein interaction between ACE2 and HRP-RBD, and hence reduce the chromogenic reaction, mimicking the virus neutralization process. Serum or plasma samples with more neutralizing antibodies show lower signal intensities. (Figure 1, right)

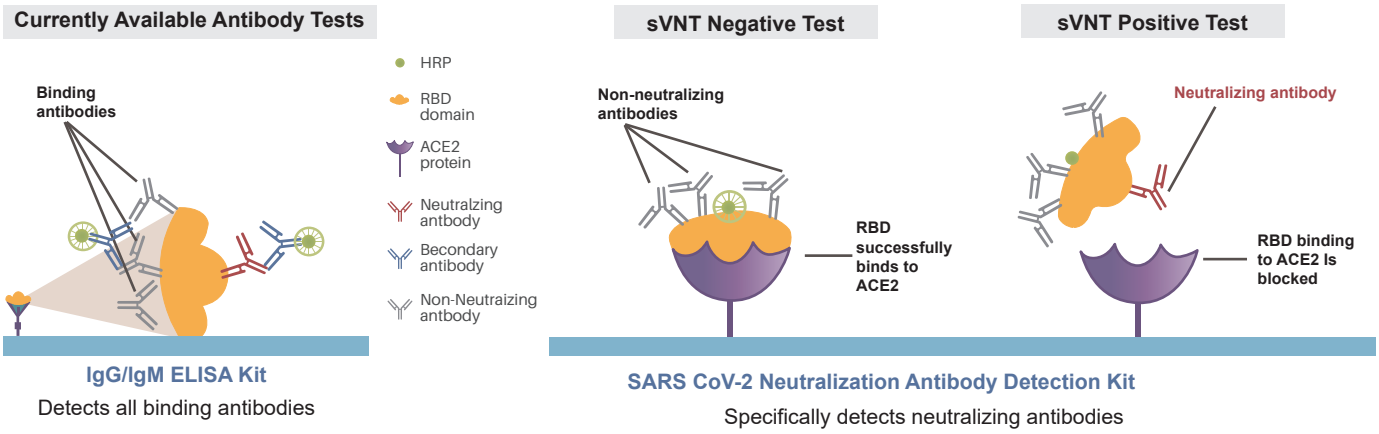


Figure 1. SARS-CoV-2 Surrogate Virus Neutralization Test only detects neutralizing antibodies against SARS-CoV-2 S1-RBD domain (right). Most SARS-CoV-2 antibody tests available on the market detect IgG, IgM or total antibodies, and are not able to tell the neutralizing antibody level (left), which is critical for convalescent plasma therapy donor selection.

SARS-CoV-2 Surrogate Virus Neutralization Test also shows strong correlation to PRNT, which is the gold standard to measure neutralizing antibody titers (Figure 2.) and shows the equivalence of plasma vs. serum samples (Figure 3.). Considering PRNT is very time consuming and laborious to scale up, the SARS-CoV-2 Surrogate Virus Neutralization Test could provide a much faster, easier, safer alternative and becomes an ideal solution for high-throughput, standardized donor QC in convalescent plasma therapy.

Compared to PRNT, the SARS-CoV-2 surrogate Virus Neutralization Test is a lot easier to use and scale-up. It can be performed in BSL-2 laboratories by technicians familiar with ELISA since it does not require any live virus. Whereas, PRNT can only be performed in BSL-3 labs by highly trained technicians because it requires live viruses. Since SARS-CoV-2 Surrogate Virus Neutralization Test uses blocking ELISA based format, it is relatively easier to standardize, less labor-intensive, scalable, and compatible with most of the automated ELISA systems. PRNT relies on counting plaques to quantify virus neutralization, thus is more difficult to scale up. The turnaround time of SARS-CoV-2 Surrogate Virus Neutralization Test is only 1-2 hours, only a small fraction of 2-3 days turnaround time of PRNT.

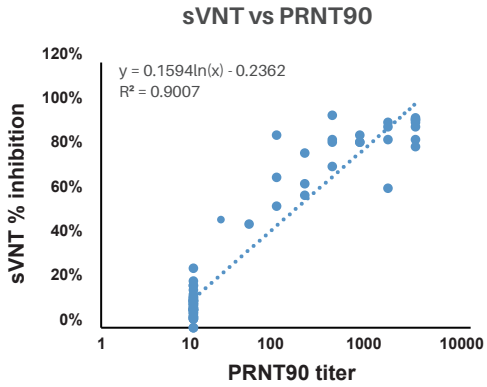


Figure 2. A study of 40 samples showed excellent correlation of results with 100% sensitivity and 100% specificity when compared to PRNT.

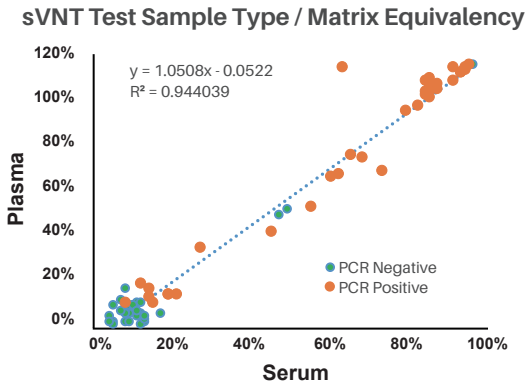
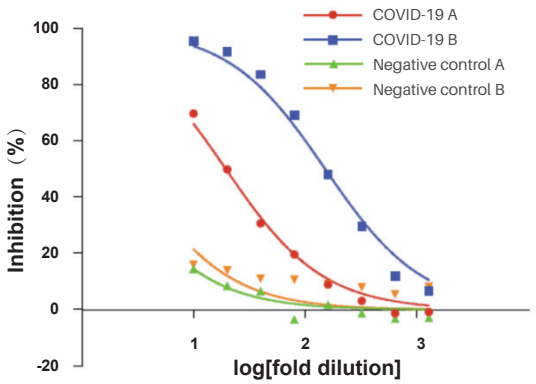


Figure 3. Sample equivalency test of SARS-CoV-2 Surrogate Virus Neutralization Test from 35 PCR positive and 35 PCR negative patients.

In addition, SARS-CoV-2 Surrogate Virus Neutralization Test generates dose-dependent data. Dr. Linfa Wang, the Director of the Program in Emerging Infectious Diseases at Duke-NUS Medical School, Singapore, who is the inventor of SARS-CoV-2 Surrogate Virus Neutralization Test, showed dose-dependent results from COVID-19 patient sera (Figure 4.). Similar results were observed in our clinical validation (Figure 5.). The relative inhibition potency of the neutralizing antibodies in the serum can be compared via IC50 or titer, and used as a scalable QC standard for donor plasma or serum.

*NAb: Neutralizing Antibodies



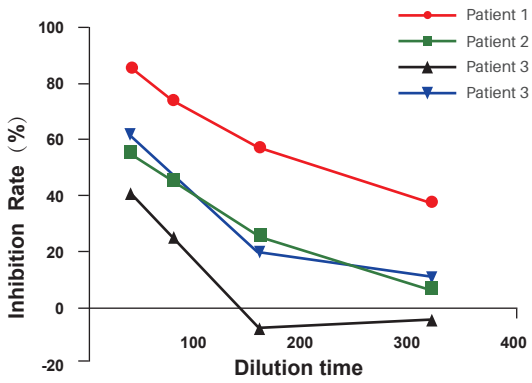


Figure 4. Inhibition of SARS-CoV-2 RBD–hACE2 interaction by sera from patients with COVID-19 in surrogate virus neutralization test (Chee Wah Tan, 2020)

Figure 5. SARS-CoV-2 Surrogate Virus Neutralization Test Kit was used to analyze a set of diluted sera from SARS-CoV-2 infected patients, demonstrating that the kit had good linearity and potential usage of semi-quantitative analysis of SARS-CoV-2 neutralizing antibodies

Most antibody tests for SARS-CoV-2 on the market detect IgG, IgM, or total antibodies, which are not correlated with neutralizing antibody levels. Compared with the FDA EUA COVID-19 Total Antibody Test that detects total antibodies, it is evident that there is no correlation between the presence of neutralizing antibodies with total IgG, IgM, IgA, and other isotypes. Using tests to measure total antibodies or antibodies based on their isotypes may lead to poor selection of reliable donors and thus result in the ineffectiveness of the convalescent plasma therapy. (Figure 6.)

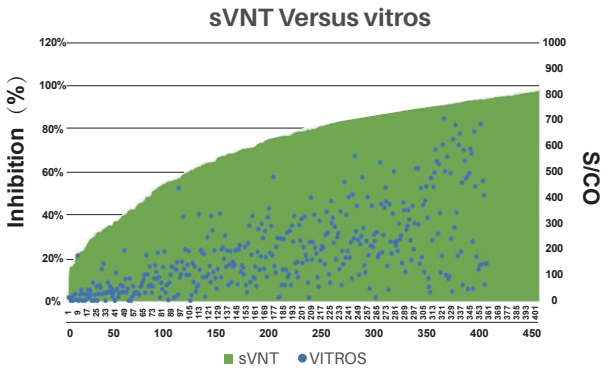


Figure 6. SARS-CoV-2 Surrogate Virus Neutralization Test vs. total antibody Anti-SARS-CoV-2 Test, shows no correlation between total antibodies and neutralizing antibody. Relying on tests measuring total antibodies or antibodies based on their isotypes for donor screening may lead to the ineffectiveness of the convalescent plasma therapy.

Future outlook: How we can help more people?

In conclusion, SARS-CoV-2 Surrogate Virus Neutralization Test may help to standardize the donor convalescent plasma qualification, giving better consistency of the manufacturing and the outcome of the therapy. The key benefits of sVNT test are listed as follows:

- Safe and accessible: no biohazard facility required, making it highly accessible to global communities
- Fast and scalable: <1 hr to run and can be easily adapted for high throughput automated testing
- Efficacious and reliable: results directly indicate the virus blocking efficacy and correlate perfectly with Plaque Reduction Neutralization Test (PRNT)

As convalescent plasma therapy lines up as the first choice for COVID-19 treatment, a group of researchers from 40 institutions encouraged recovered COVID-19 patients to donate plasma to treat active COVID-19 cases across the country. These institutions include the Mayo Clinic, Johns Hopkins University, Washington University, Einstein Medical Center and the Icahn School of Medicine at Mount Sinai, and many others working closely with the US Food and Drug Administration (FDA) and industry partners. With the rapid growth of donor plasma and large needs of convalescent plasma therapy from numerous severe COVID-19 cases, establishing a standard, nation-wide donor qualification mechanism becomes urgent. To benefit more people and streamline the treatment, plasma fractionation then further manufacturing of hyperimmune globulin (H-Ig) purified from the pooled convalescent plasma may be more feasible to fulfill the large demands of COVID-19 treatment, and SARS-CoV-2 Surrogate Virus Neutralization Test can not only provide a reliable and scalable qualification for donor plasma but also monitor the plasma fractionation as quality control for the whole process.

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