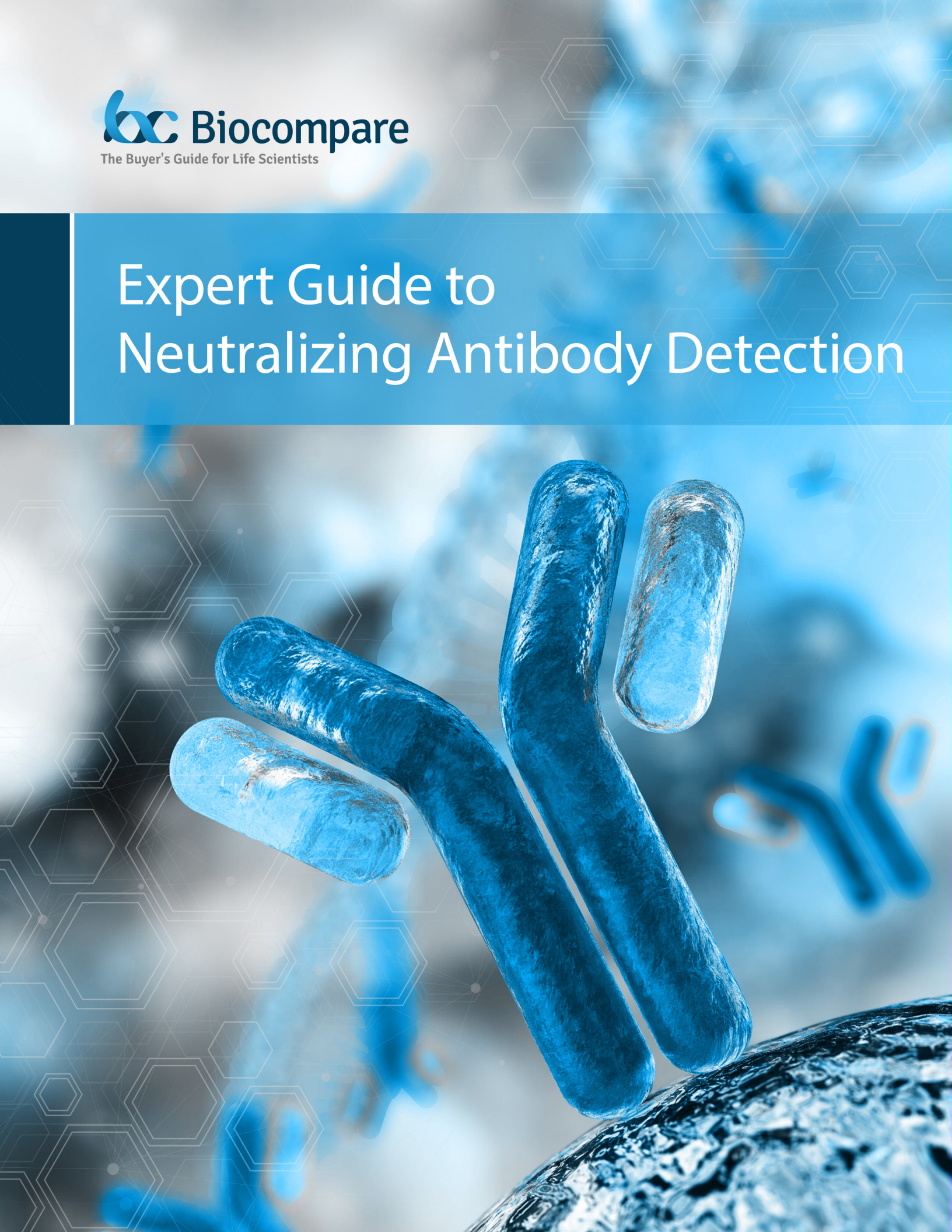
Biocompare
The Buyer's Guide for Life Scientists

Expert Guide to Neutralizing Antibody Detection

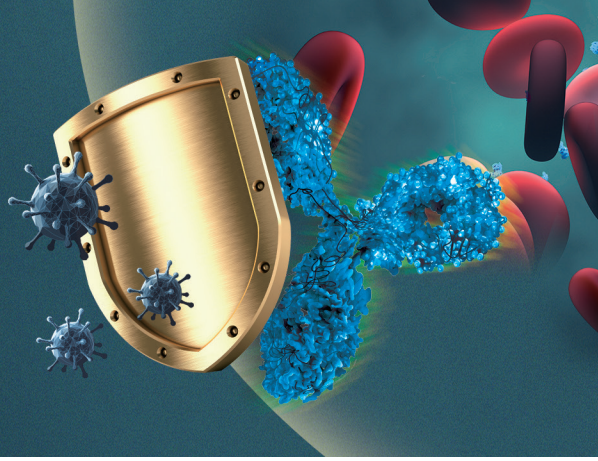


Contents

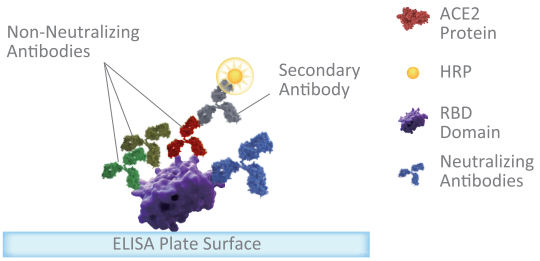
Detect COVID-19 Neutralizing Antibodies in 1 hour!

Not all antibodies confer immunity!

Test with cPass™.

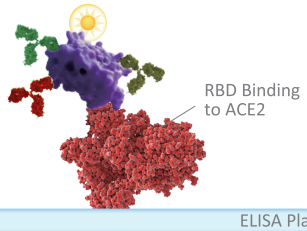


Currently Available Antibody Tests

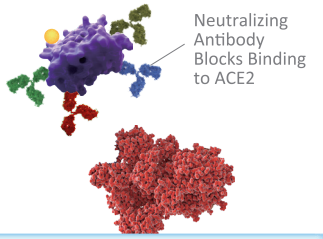


Detect **all** binding antibodies

cPass™ Negative Test



cPass™ Positive Test



cPass™ **specifically** detects **neutralizing antibodies**

cPass™ First-in-the World Neutralizing Antibody Test


Herd Immunity

Immunity Passport


Therapy Validation


Vaccine Efficacy

cPass™ Key Benefits


Species Independent

Not limited to human samples, test is applicable to any species


Safe & Accessible

No biohazard facility required making it highly accessible to global communities


Scalable

Can be easily adapted for high throughput automated testing

GenScript
Make Research Easy

Toward a New Gold Standard for COVID-19 Neutralizing Antibody Detection

cPass™ has become an essential assay in workflows involving surveillance, diagnostics, and vaccine development.

Angelo DePalma, Ph.D.

In May 2020, while public health officials were scrambling for therapies and diagnostics to address the SARS CoV-2 pandemic, a **paper** in *Nature Communications* described a significant breakthrough: a human monoclonal antibody (mAb), from the blood of previously infected patients, that neutralized SARS-CoV-2. With this target in hand, efforts at discovering novel treatments and diagnostics, and even basic coronavirus research, took on new perspectives.

Neutralizing antibodies (NAbs) are monoclonal antibodies (mAbs) that defend cells from disease-causing viruses and bacteria. They work by first identifying pathogens, then recruiting immune system cells to eliminate them. NAbs work through one of several mechanisms, depending on the virus. In enveloped viruses, NAbs block the attachment to and entry into cells; in non-enveloped viruses, they bind to the capsid protein. Some NAbs block bacterial toxins directly, as in diphtheria. By binding to the coronavirus spike protein, NAbs prevent the pathogen from attaching to and entering human cells.

Researchers had been hunting for NAbs long before the *Nature* paper appeared. An **earlier study** showed the effectiveness of NAbs in targeting four viruses, including a coronavirus, while **another** demonstrated the feasibility of treatments based on anti-Ebola NAbs. In July 2020, a German academic group announced a **collaboration** with Boehringer Ingelheim on a coronavirus NAb treatment, and three months later the U.S. Food and Drug Administration approved Eli Lilly's COVID-19 convalescent plasma–derived COVID Nab. LY-CoV555 clears the virus from the bloodstream with **no apparent side effects** while lowering viral loads by 99.97% and **reducing hospitalizations by 75%**. Most recently, the U.S. Food and Drug Administration approved Eli Lily's bamlanivimab, a biomanufactured analog of coronavirus NAbs.

Enter the assay

Coronavirus NAb buzz was not just about therapeutics. In November 2020, FDA approved, through

an Emergency Use Authorization (EUA), the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit from GenScript. cPass is the first serologic test for detecting COVID NAbs in individuals with prior SARS-CoV-2 infections. Because it broadly supports investigations into coronavirus immunology, cPass has become an essential assay in workflows involving surveillance, diagnostics, and through its application to studies on vaccine-mediated antibody-based immunity, vaccine development.

FDA had previously issued EUAs to more than 50 antibody serology tests, but those assays only detect binding antibodies that attach to SARS-CoV-2 but do not appreciably affect the virus's infectivity or virulence.

cPass targets spike glycoproteins, the structures residing on the coronavirus surface that are required for the virus to infect cells. The spike protein is also the virus's major immunodominant antigen, and has been central to both therapeutic and diagnostic R&D.

Previous assays for coronavirus NAbs relied on cell culture based infection assays using either wild-type SARS-CoV-2 or pseudotyped viruses. **Infectivity as-**

says are labor-intensive, require biosafety facilities, and are difficult to standardize.

Wild-type or pseudotyped viruses require biosafety levels 3 and 2, respectively. The assays use cell-based infectivity as their readouts but are difficult to standardize due to a lack of reference materials and standardized protocols. Significant assay characteristics will vary depending on the selected culture conditions, virus strains (for model viruses), and cell lines. Assays based on culturing cells are complex, prone to variability, require trained personnel, take two to three days to run, and are inherently low-throughput.

Assay protocol

The cPass Neutralization Antibody Detection kit identifies circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the receptor binding domain (RBD) of the viral spike glycoprotein and the ACE2 cell surface receptor. Using human serum or plasma samples, the qualitative test identifies individuals possessing an adaptive immune response to SARS-CoV-2.

cPass is a blocking ELISA detection assay that mimics *in vivo* virus neutralization. The assay consists of a horseradish peroxidase- (HRP) conjugated recombinant SARS-CoV-2 RBD fragment (HRP-RBD) and the human ACE2 receptor protein (hACE2). When concentrations of neutralizing antibodies against SARS-CoV-2 RBD are high enough, the protein-protein interaction between HRP-RBD and hACE2 is blocked and reported as the readout.

The cPass assay is initiated by incubating samples or controls with the HRP-RBD conjugate to allow binding to circulating neutralization antibodies to form HRP-RBD/neutralizing antibody complexes. The mixture of unbound and newly complexed HRP-RBD is then added to a capture plate pre-coated with SARS-CoV-2 receptor protein, hACE2, which captures unbound HRP-RBD while the complexes between circulating neutralization antibodies and HRP-RBD remain in the supernatant, from which they are removed by simple washing.

Addition of 3,3',5,5'-tetramethylbenzidine turns the assay solution blue, and after quenching the solution turns yellow. The optical density of the final solution is then read at 450 nm. Samples with inhibition ≥ 30% are considered positive for neutralizing antibodies, while values below 30% are considered negative.

Actionable connections

cPass uncovers scientifically actionable relationships between NAbs and the immunologic status of individuals and populations.

A recent study analyzed blood from 49 patients recently recovered from coronavirus infection using cPass. Researchers found that neutralizing antibody titers can be used to assess reinfection risk, and correlates with certain aspects of disease severity. For example, NAb titers were higher in older patients, those with symptomatic infection, and in patients who had experienced severe pneumonia. Interestingly, two children who had experienced asymptomatic infection showed no NAbs.

Researchers at Duke University recently published data on validation of cPass using cohorts of subjects from two different countries. They reported 100% specificity, 95–100% sensitivity, and the ability to distinguish SARS-CoV-2 NAbs from those originating from other coronaviruses.

In a preliminary internal **study** on 56 samples, Genscript scientists demonstrated 100% sensitivity and

100% specificity for the cPass assay, and excellent correlation with the gold standard plaque reduction neutralization assay. cPass detected NAbs in both animal and human serum in a dose-dependent manner, and the assay is not isotype-dependent. Additionally, cPass is safer, requires less expertise, is scalable, and gives results in an hour as opposed to 2–3 days for the plaque reduction protocol.

Advancing SARS-CoV-2 science requires timely application of the right tools for addressing such questions as who is infected, their immunologic status, and how well vaccines protect the public. By quantifying levels of NAbs that are central to answering these questions cPass supports all these critical functions and more.

About the author

Angelo DePalma earned his Ph.D. in organic chemistry from Stony Brook University and was previously senior scientist at Schering-Plough. He has written extensively on biotechnology, biomanufacturing, medical devices, pharmaceutical commerce, laboratory instrumentation, and advanced materials.

COVID-19 Convalescent Plasma Therapy Donor Qualification by cPass SARS-CoV-2 Surrogate Virus Neutralization Test

New test may help improve manufacturing consistency and therapeutic outcomes.

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

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)

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)

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 cPass™ 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)

cPass™ 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 cPass™ SARS-CoV-2 surrogate Virus Neu-

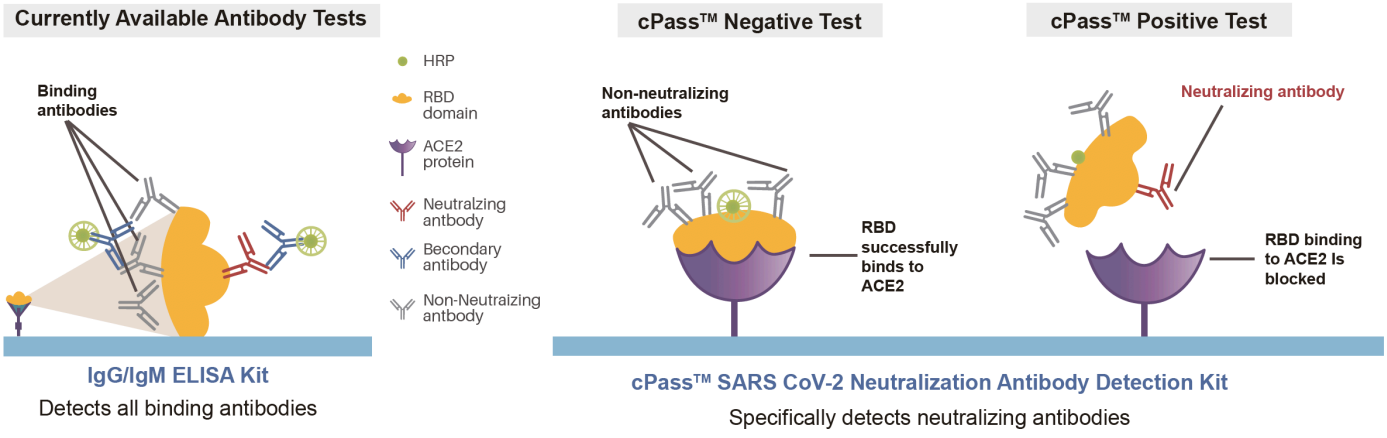


Figure 1. cPass™ SARS-CoV-2 surrogate Virus Neutralization Test only detects neutralizing antibodies against SARS-CoV-2 S1-RBD domain (right). Most SARS-CoV-2 antibody tests available on the market detect IgG, IgM or total antibodies, and are not able to tell the neutralizing antibody level (left), which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

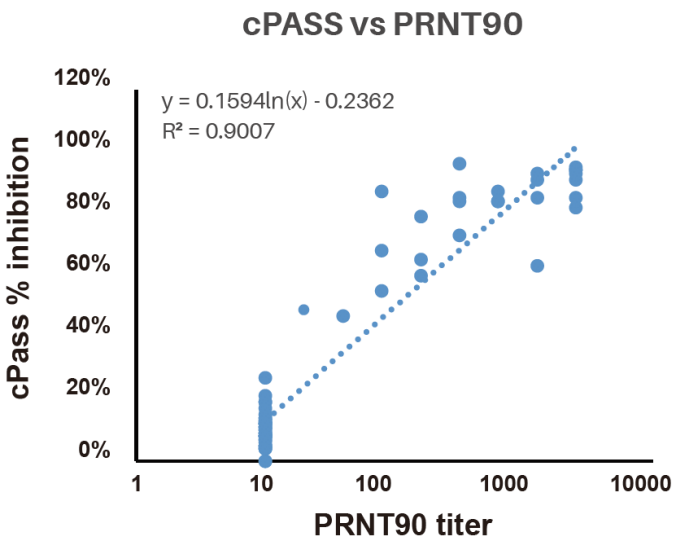


Figure 2. A study of 40 samples showed excellent correlation of results with 100% sensitivity and 100% specificity when compared to PRNT.

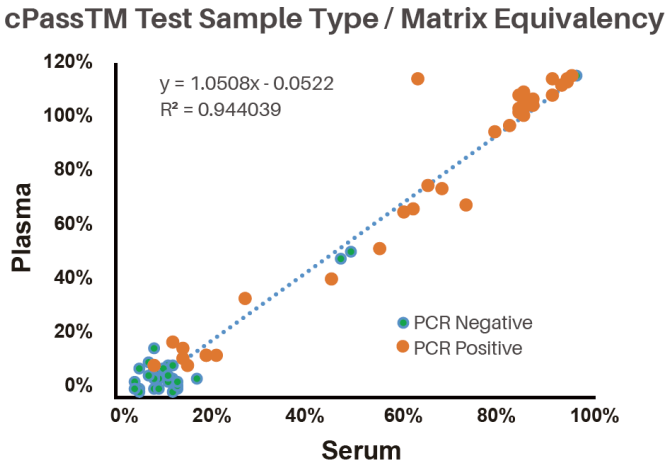


Figure 3. Sample equivalency test of cPass™ SARS-CoV-2 surrogate Virus Neutralization Test from 35 PCR positive and 35 PCR negative patients

tralization 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 cPass™ 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 cPass™ 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 cPass™ SARS-CoV-2 surrogate Virus Neutralization Test is only 1-2 hours, only a small fraction of 2-3 days turnaround time of PRNT.

In addition, cPass™ 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 cPass™ SARS-CoV-2 surrogate Virus Neutralization Test, showed dose-de-

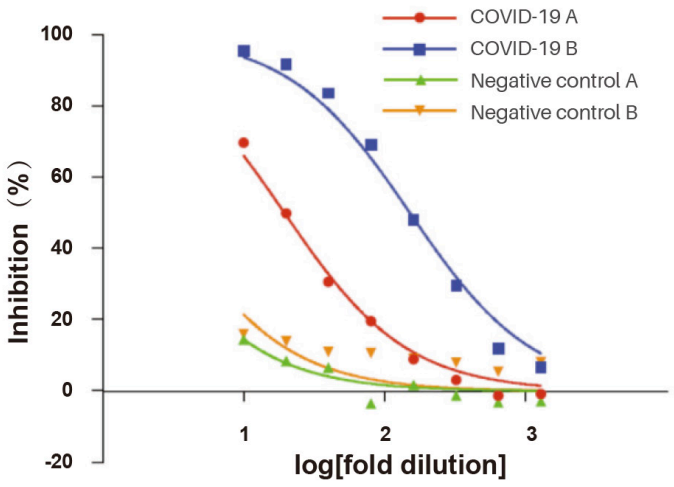


Figure 4. Inhibition of SARS-CoV-2 RBD–hACE2 interaction by sera from patients with COVID-19 in surrogate virus neutralization test (Chee Wah Tan, 2020)

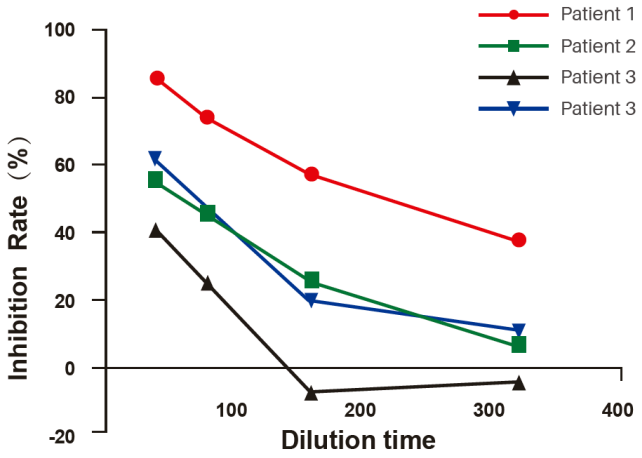


Figure 5. cPass™ SARS-CoV-2 Surrogate Virus Neutralization Test Kit was used to analyze a set of diluted sera from SARS-CoV-2 infected patients, demonstrating that the kit had good linearity and potential usage of semi-quantitative analysis of SARS-CoV-2 neutralizing antibodies

pendent 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.

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.)

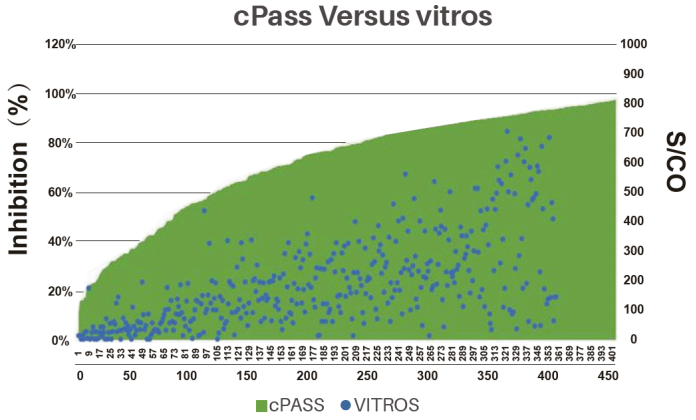


Figure 6. cPass™ SARS-CoV-2 surrogate Virus Neutralization Test vs. total antibody Anti-SARS-CoV-2 Test, shows no correlation between total antibodies and neutralizing antibody. Relying on tests measuring total antibodies or antibodies based on their isotypes for donor screening may lead to the ineffectiveness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

Future outlook: How we can help more people?

In conclusion, cPass™ 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 cPass™ test are listed as follows:

- Safe and accessible: no biohazard facility required, making it highly accessible to global communities
- Fast and scalable: <1hr 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 cPass™ 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.

References

1. Arabi Y, B. H. (2015). Feasibility, safety, clinical, and laboratory effects of convalescent plasma therapy for patients with Middle East respiratory syndrome coronavirus infection: a study protocol. Springerplus, 4: 709.
2. Chee Wah Tan, W. N.-C.-W.-J.-F. (2020). A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nature Biotechnology, 10.1038/s41587-020-0631-z.
3. Chenguang Shen, P., ZhaoqinWang, P., Fang Zhao, P., Yang Yang, M., Jinxiu Li, M., Jing Yuan, M., & FuxiangWang, M. (2020). Treatment of 5 Critically Ill Patients WithCOVID-19 With Convalescent Plasma. JAMA, 1582-1589.
4. Fan Wu, A. W. (2020). Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. medRxiv, 03.30.20047365.
5. FDA. (1 de May de 2020). Investigational COVID-19 Convalescent Plasma Guidance for Industry. Obtenido de FDA: https://www.fda.gov/media/136798/download
6. Hung IF, T. K. (2011). Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. Clin Infect Dis, 52: 447–56.
7. Hung IFN, T. K. (2013). Hyperimmune IV immunoglobulin treatment: a multicenter double-blind randomized controlled trial for patients with severe 2009 influenza A(H1N1) infection. Chest, 144: 464–73.
8. Jianhui Nie, Q. L. (2020). Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. Emerging Microbes & Infections, 9(1):680-686.
9. Kai Duan, B. L. (2020). Effectiveness of convalescent plasma therapy in severe COVID-19 patients. PNAS, 9490–9496.
10. Laura A. VanBlargan, L. G. (2016). Deconstructing the Antiviral Neutralizing-Antibody Response: Implications for Vaccine Development and Immunity. Microbiology and Molecular Biology Reviews , 80: 989-1010.
11. Mingxiang Ye, D. F. (2020). Treatment With Convalescent Plasma for COVID-19 Patients in Wuhan, China. J Med Virol, 10.1002/jmv.25882. doi: 10.1002/jmv.25882.
12. Thomas F. Rogers, F. Z.-T. (2020). Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. Science, Online, 0.1126/science.abc7520.
13. WHO. (Aug de 2014). Ebola convalescent treatment. Obtenido de Use of convalescent whole blood or plasma collected from patients recovered from Ebola virus disease for transfusion, as an empirical treatment during outbreaks: http://apps.who.int/iris/rest/bitstreams/604045/retrieve
14. WHO. (Feb de 2020). Clinical management of COVID-19. Obtenido de Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected: https://www.who.int/docs/default-source/coronaviruse/clinical-management-of-novel-cov.pdf

cPass SARS-CoV-2 Neutralization Antibody Detection Kit for Vaccine Research and Development

Solution is applicable from COVID-19 vaccine development to efficacy assessment and also for monitoring durability of NAb responses.

Introduction

SARS-CoV-2 (COVID-19) 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.

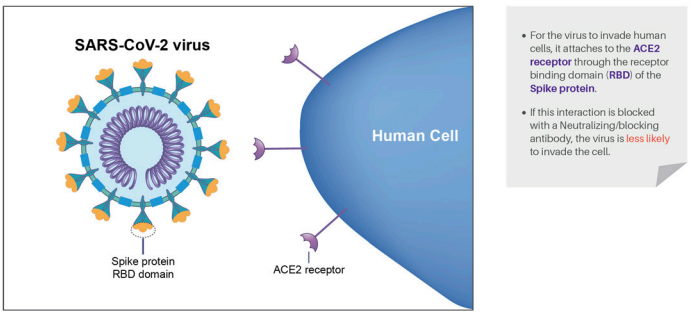


Figure 1. The interaction of the SARS-CoV-2 viral Spike protein with the ACE2 receptor from human cells is needed for the virus to invade human cells.

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 (Figure 1). Potent neutralizing antibodies often target the receptor interaction site in S1, disabling host receptor interactions and preventing viral entry into the cell.[3]

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 (Figure 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

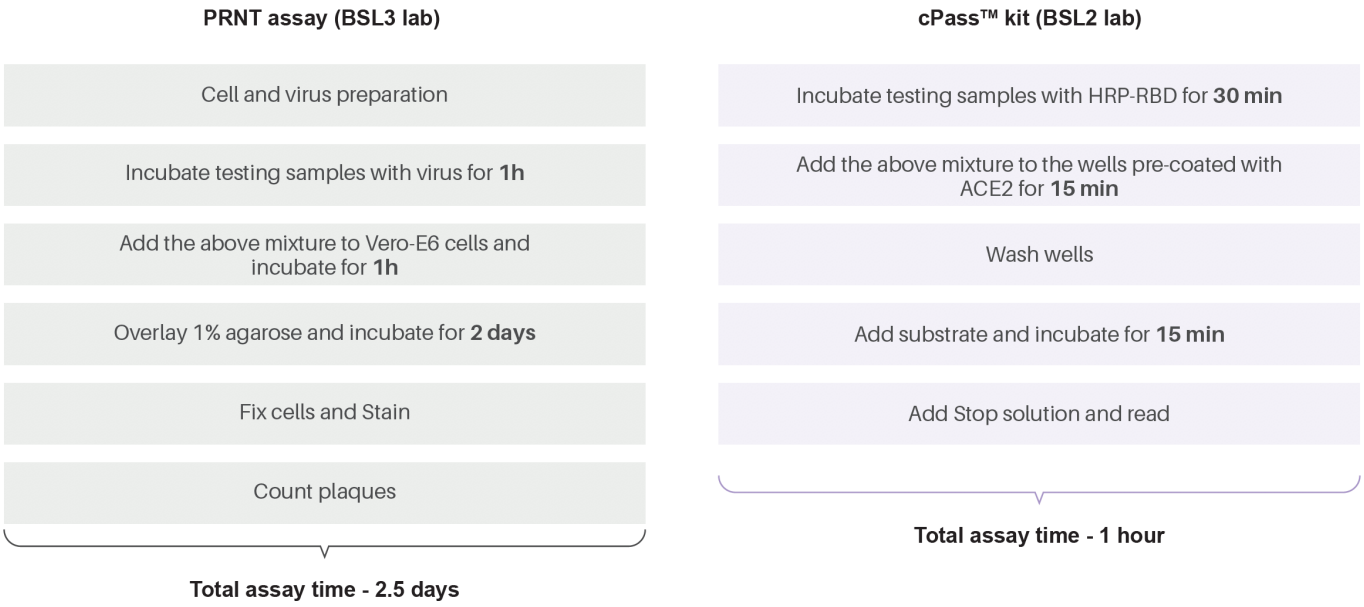


Figure 2. Comparison between the PRNT and cPass™ kit workflows. The total assay time of cPass™ kit is an hour and can be performed in a BSL2 lab. The PRNT assay needs both the COVID-19 live virus and Vero-E6 cells and takes over 2 days in a BSL3 lab.

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.

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 (Figure 3).

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)

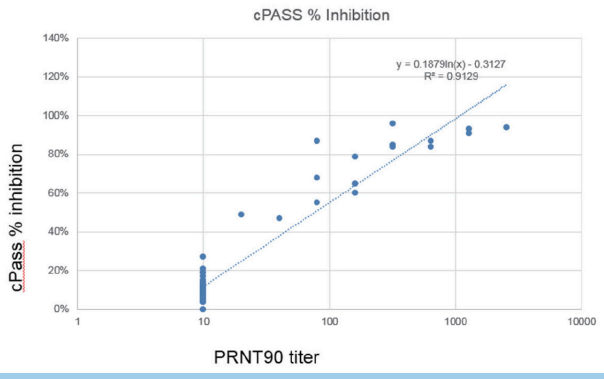


Figure 3. Correlation of the cPass™ kit test with the PRNT test. A study of 56 samples showed excellent correlation of results with 100% sensitivity and 100% specificity when compared to PRNT.

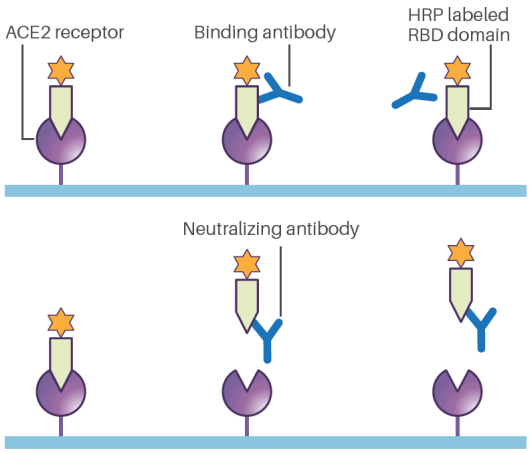


Figure 4. If only binding antibodies are present in the sample, then the HRP labeled RBD still binds to the ACE2 receptor on the plate and generates strong signal.

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.

(HRP-RBD) is used for detection (Figure 4). The serum or plasma samples with more neutralizing antibodies show a lower signal intensity.

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 Figures 5-7 below can be used as

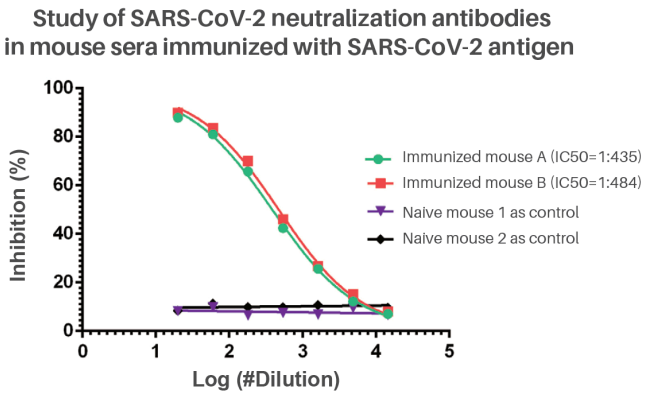


Figure 5. cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit was used to analyze neutralizing antibody levels in mouse serum samples. The mice were immunized with SARS-CoV-2 RBD to generate a neutralizing antibody response, and then compared with unimmunized controls. A representative result showed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

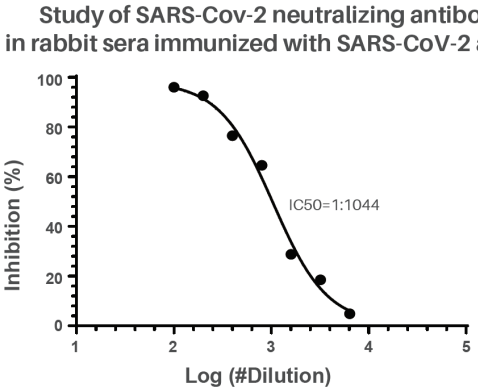


Figure 6. The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit was used to analyze the levels of neutralizing antibody in rabbit serum samples. The rabbits were immunized with SARS-CoV-2 RBD protein to generate a neutralizing antibody response and then compared with control group. A representative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

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.

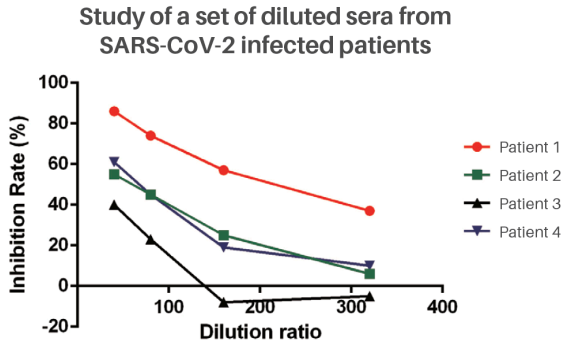


Figure 7. The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit was used to analyze a set of diluted sera from SARS-CoV-2 infected patients. A representative result demonstrated that cPassTM kit has a good dose-dependent response with human serum.

Application note

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 (Figure 5), rabbit (Figure 6) and human serum (Figure 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 (Figures 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-CoV-2 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 (Figure 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 (Figure 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-CoV-2 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 (Figures 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 COVID-19 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 COVID-19 since it can be used across the entire spectrum of COVID-19 vaccine development to vaccine efficacy assessment and monitoring durability of neutralizing antibody responses.

References

1. Guidelines on clinical evaluation of vaccines: regulatory expectations – WHO, 2016.
2. Draft landscape of COVID-19 candidate vaccines – 29 June 2020.
3. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Azkur, A.K et al. European Journal of Allergy and Clinical Immunology, May 2020.
4. Potent neutralizing antibodies against SAR-CoV-2 identified by high through-put single-cell sequencing of convalescent patients's B cells. Cao Y et al. Cell, July 2020.
5. Assays for the assessment of neutralizing antibody activities against Severe Acute Respiratory Syndrome (SARS) associated coronavirus (SCV) Wang S et al. Journal of Immunological methods, March 2005.
6. cPASS™ SARS-CoV-2 Neutralization Antibody Detection Kit Manual (Cat# L00847)
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.

Neutralizing a Threat: Sailors Spared from Coronavirus Reinfection

An outbreak on a fishing vessel could help determine if infection with the virus protects against later reinfection.

Sailors become accidental research subjects

The **coronavirus pandemic** has left researchers quickly scrambling to understand the SARS-CoV-2 virus causing COVID-19. But ethical concerns make it difficult to experiment on human subjects and retrospective analyses of real-life outbreaks often remain as a last resort instead.

One commercial fishing crew recently became the accidental focus of coronavirus research when the virus went rampant on their vessel. Some of the sailors remained healthy, and **researchers now suspect** that the neutralizing antibodies they had developed during prior infection with SARS-CoV-2 may have protected them from re-infection.

The 18-day journey of the American Dynasty

The American Dynasty left Seattle, Washington in May 2020. In an effort to prevent a COVID-19 outbreak on the ship, most of the crew members underwent rigorous testing prior to departure.

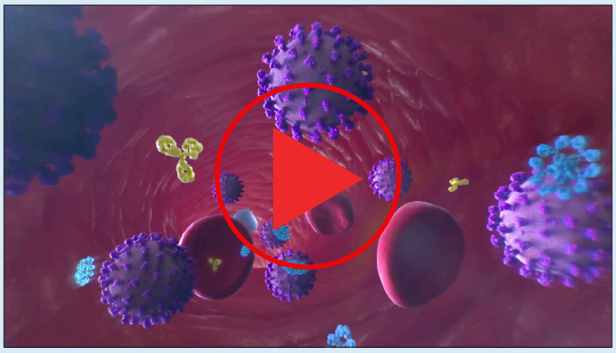
Out of 122 crew members, 120 were tested for SARS-CoV-2 nucleic acids by reverse transcription PCR (RT-PCR) and for viral antibodies using a **serology test.** All of the RT-PCR tests gave negative results; six of the serology tests were positive, suggesting these sailors had previously been infected with the virus. Presumed non-infectious on the day of departure, the crew set sail.

But after 18 days at sea, one person became sick and needed medical intervention, so the ship returned to port. The remaining crew underwent regular RT-PCR and antibody testing for another 32 days upon arrival.

This time, testing was carried out with a more sophisticated test produced by GenScript that uses a new technology called cPass to detect neutralizing antibodies. In the end, 104 crew members fell ill with COVID-19, while three sailors remained healthy. These were found to carry neutralizing antibodies against SARS-CoV-2.

Video: Antibodies attacking the virus

Why does the neutralizing antibody (NAb) matter, and how does the cPass™ SARS-CoV-2 NAb Detection Kit by GenScript works to detect the presence of the neutralizing antibodies.



What is the importance of neutralizing antibodies?

Infection with SARS-CoV-2 or other viruses causes the body to produce **two different kinds of antibodies**. Neutralizing antibodies are able to bind a particular protein on the virus, and in the process, render the virus incapable of infecting cells.

Binding antibodies also bind to viral proteins, but they do not neutralize the virus or prevent infection. Neutralizing antibodies are of particular interest to researchers because their presence usually correlates with protection from infection. Production of neutralizing antibodies is also one component of the **immune response** sought by researchers working on vaccine development.

"Testing for neutralizing antibodies will play an important role in advancing SARS-CoV-2 research, from assessing seroprevalence in populations, tracking infections in animals, to vaccine development, and protective immunity studies," Jungsoo Park, Marketing Director at GenScript said in an interview.

Antibody tests can show if an individual is currently infected with a virus or has previously been infected. Initially, the crew members of the American Dynasty were tested for SARS-CoV-2 binding antibodies.

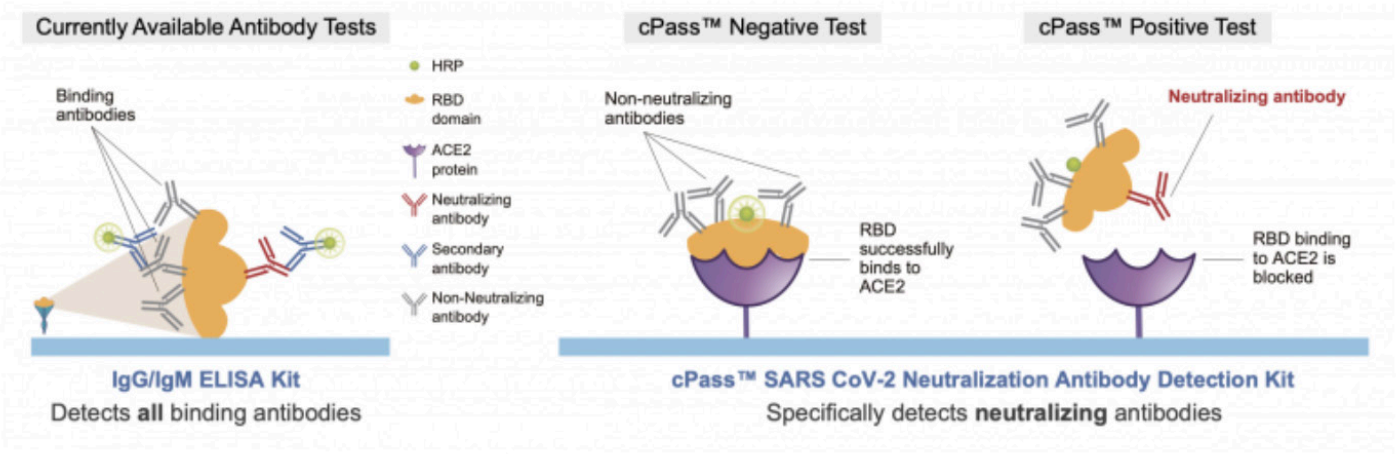
But upon their return to shore, the pre-departure samples were re-tested alongside fresh samples using the GenScript test for neutralizing antibodies, to help researchers better understand the sailors' immune profiles.

It turned out that three of the original six sailors who tested positive did not have any SARS-CoV-2 neutralizing antibodies after all; the researchers concluded that initial antibody tests for these individuals were most likely false positives.

The other three sailors who had neutralizing antibodies before setting sail stayed healthy during the outbreak. Researchers can't be certain that the neutralizing antibodies protected these three from re-infection, but so far, the evidence suggests this could be the case.

A neutralizing antibody test that stands out from the rest

Currently, the gold standard method for detecting neutralizing antibodies is the virus neutralization test (VNT), a cumbersome procedure that involves infecting cells with live viruses in highly contained biosafety facilities. It takes several days to complete, and it is not amenable to large-scale testing.



GenScript has developed a surrogate VNT, providing a unique alternative for neutralizing antibodies testing: the cPass SARS-CoV-2 Neutralization Antibody Detection kit. cPass uses specially engineered proteins to **biochemically simulate** the VNT procedure.

"This is an extremely useful, first-of-its-kind test for detecting neutralizing antibodies," said Park. "The cPass SARS-CoV-2 Neutralization Antibody Detection is not only fast, safe, and accessible, but it can be easily scaled-up for high-throughput, automated testing, and it works on any species."

What's next in SARS-CoV-2 research?

There is still so much to learn about SARS-CoV-2, and in the absence of controlled human studies, much new knowledge will come from retrospective analyses of COVID-19 outbreaks like the one on the American Dynasty.

Novel technologies such as GenScript's cPass SARS-CoV-2 Neutralization Antibody Detection kit will be central to helping scientists better understand the role of neutralizing antibodies in SARS-CoV-2 immunity.

This article was originally published in Labiotech.eu and can be viewed in its entirety **here**.

Antibodies: A Beacon of Hope During the COVID-19 Pandemic Dark Days?

Tools and technologies that accelerate the development of research procedures are of critical importance.

The pharmaceutical industry has been stung into action to develop both preventative and therapeutic interventions: presently **3,438 clinical** studies are underway worldwide and **625** drugs and vaccines are in development.

Clearly, time is not our friend in the fight against the pandemic, which is why methods that accelerate the development of research procedures are of critical importance. In light of this, global biotech company GenScript, which specializes in **custom monoclonal antibody generation,** has utilized the cutting-edge optofluidic technology of the Beacon® B cell sorting platform and the power of rabbit monoclonal antibodies (mAbs) to develop effective solutions.

Using this technology, GenScript discovered several COVID-19 spike protein antibodies that target the receptor-binding domain of SARS-CoV-2 Spike protein, the mode of action of which enables the virus to infect cells.

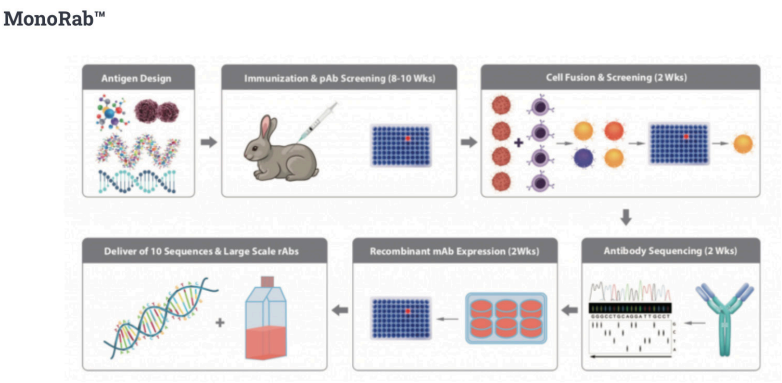


Figure 1. Process of generating rabbit mAbs using the MonoRab platform

Two technologies combined in the fight against COVID-19

Increasingly, the distinct advantages of rabbit mAbs have led to their greater use in all areas of life sciences, from basic research to diagnostics and therapeutics.

Consequently, GenScript developed MonoRab™, a proprietary rabbit antibody generation platform,

which can produce mAbs with high specificity, affinity, and sensitivity against a wide range of antigens.

The company has an extensive selection of SARS-CoV-2 control antibodies available on-demand to accelerate the research and development of COVID-19 vaccines, diagnostics, and neutralizing antibody therapies. This makes them well suited for mAb controls for virus neutralization assays, in vitro diagnostic development, as well as vaccine and infectious disease research.

The Beacon® platform

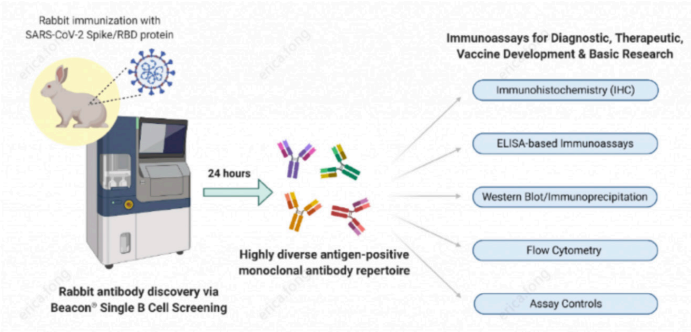


Figure 2. Process of generating rabbit mAbs using the MonoRab platform

Beacon® is an optofluidics system, meaning it uses light to control fluid flow at a micrometer level, to rapidly process and analyze cells.

It has two major advantages over its competitors. First is its speed, which can perform mAb screening in just 24 hours compared with two to three months using hybridoma/display library screening methods. The second is its capacity to discover a diverse antibody repertoire – where other antibody generation platforms traditionally fall short.

Other notable features include cutting-edge technology to maximize the chances of locating the desired antibody; fast-track antibody discovery to shorten time investment; a streamlined automated workflow and platform; and high-diversity, high-specificity reagent antibodies.

The value of MonoRab allied with the Beacon® platform

The distinct natural features of rabbits mean that they are the preferred species for generating mAbs. Rabbits are able to generate highly diverse antibodies against antigens that are normally nonimmunogenic in mice. Indeed, rabbit products can have up to 100-fold higher affinity for their target antigen than mice.

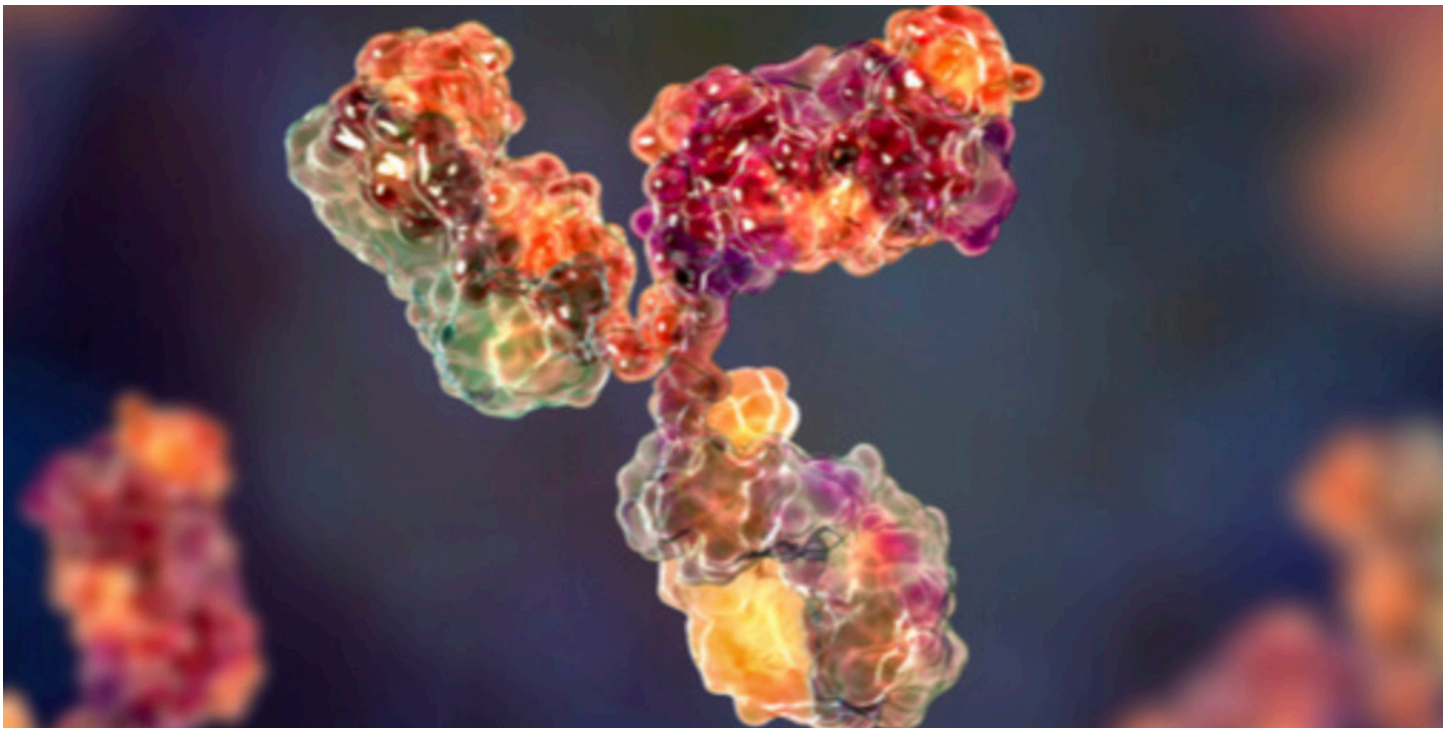
Using GenScript's MonoRab™, in combination with Beacon®, the research process for high-quality rabbit antibody generation, which would have otherwise taken three months using the traditional hybridoma method, can take just 24 hours.

An experienced player in the antibodies field

GenScript's sector expertise means it is well placed to confront COVID-19. GenScript's marketing director, Jungsoo Park, said "Our antibody service is cited in a lot of research papers, in other words, our antibodies successfully contributed to many research projects."

In the pandemic's early days, many of GenScript's scientists set their usual research aside and got together to focus on the development of a COVID-19 solution. Hence, less than two weeks after the viral genome sequence was released by the Chinese Centers for Disease Control, GenScript released a SARS-CoV-2 detection assay for initial screening.

The company credits this rapid development to the **cPass detection kit,** which enables the detec-



tion of neutralizing antibodies against SARS-CoV-2. Compared with existing real-time quantitative polymerase chain reaction and enzyme-linked immunosorbent assay tests, cPass is able to tell a person's immunity (chance of future reinfection) by measuring the presence of neutralizing antibodies.

The scientists performed research to characterize the structure, function, and antigenicity of proteins in the SARS-CoV-2 virus. Consequently, GenScript successfully expressed antibodies against the SARS-CoV-2 proteins to expedite the development of therapies or further study viruses.

GenScript's custom generation antibody service represents a one-stop-shop for high-quality antibody production. It has over 14 years of experience in generating over 50,000 custom antibodies for its customers, producing high-specificity monoclonal and polyclonal antibodies for applications ranging from basic research to in vitro diagnostics and therapeutics.

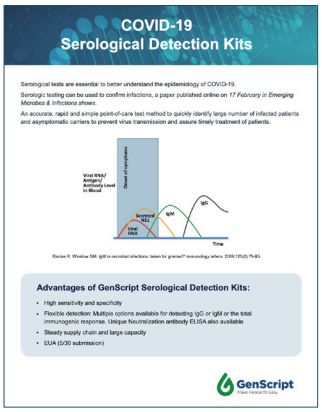
Park stated "We offer flexibility in our highly tailored services for our antibody generation services to ensure we meet the research needs of our customers. We also make sure we deliver the antibodies within the guaranteed turnaround time. We produce all the off-the-shelf products in house, so we can ensure the quality of our products."

COVID-19 is testing the whole planet. Healthcare, biotechnology, and pharmaceutical companies continue to strive to find a cure. GenScript – the only contract research organization to perform antibody discovery using rabbit antibodies and the Beacon® system – will endeavor to find a breakthrough.

This article was originally published in Labiotech.eu and can be viewed in its entirety **here**.

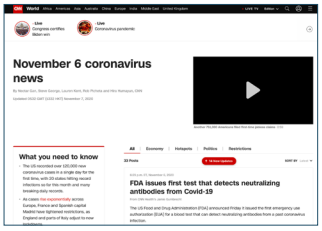
Resources

Detect COVID-19 Neutralizing Antibodies in 1 Hour

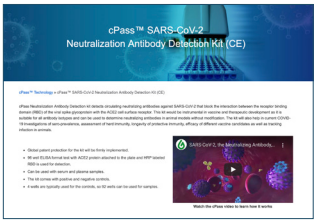


COVID-19 Serological Detection Kits

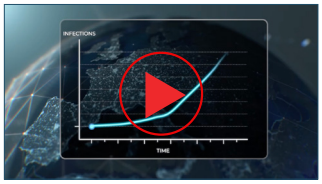
cPass™ Technology: A Safer Method to Detect Neutralizing Antibodies



FDA Issues First Test that Detects Neutralizing Antibodies from COVID-19



cPass™ SARS-CoV-2 Neutralizaton Antibody Detection Kit (CE)



SARS-CoV-2, the Neutralizing Antibody and cPass™ SARS-CoV-2 NAb Detection Kit

Clinical Testing

cPass™ Test		
N= 219	**Confirmed Positive**	**Confirmed Negative**
cPass™ Test Positive	125	3
cPass™ Test Negative	8	83

Specificity= 97 % , Sensitivity= 94 %

Specificity testing included samples from 86 normal healthy individuals. Reported specificity of this group was 96.5% (83/86). Sensitivity testing of samples from 133 clinically diagnosed, PCR positive COVID-19 positive patients. Reported sensitivity of this group was 94 % (125/133). Additional data available on request*.

cPass™
SARS-CoV-2 Neutralization Antibody Detection Kit

- ✓ First to Market
- ✓ CE-IVD Mark
- ✓ Provisional Authorization by HSA**

Format	ELISA
Test/Kit	96 Wells/Plate
Sample Type	Serum or Plasma
Protocol Time	1 Hour

GenScript Diagnostics

860 Centennial Ave. Piscataway, NJ 08854 USA

(732) 317-5055

CovidKits@genscript.com

*Since antibody response is delayed after infection, we recommend collecting samples from patients 8 days post symptom onset!

** Health Sciences Authority (HAS) in Singapore has provisionally authorized the use of cPass™ to detect and diagnose COVID-19 infection.

GenScript
Make Research Easy