Homogeneous surrogate virus neutralization assay to rapidly assess neutralization activity of anti-SARS-CoV-2 antibodies

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The COVID-19 pandemic triggered the development of numerous diagnostic tools to monitor infection and to determine immune response. Although assays to measure binding antibodies against SARS-CoV-2 are widely available, more specific tests measuring neutralization activities of antibodies are immediately needed to quantify the extent and duration of protection that results from infection or vaccination. We previously developed a ‘Serological Assay based on a Tri-part split-NanoLuc® (SATiN)’ to detect antibodies that bind to the spike (S) protein of SARS-CoV-2. Here, we expand on our previous work and describe a reconfigured version of the SATiN assay, called Neutralization SATiN (Neu-SATiN), which measures neutralization activity of antibodies directly from convalescent or vaccinated sera. The results obtained with our assay and other neutralization assays are comparable but with significantly shorter preparation and run time for Neu-SATiN. As the assay is modular, we further demonstrate that Neu-SATiN enables rapid assessment of the effectiveness of vaccines and level of protection against existing SARS-CoV-2 variants of concern and can therefore be readily adapted for emerging variants.
ARS-CoV-2 continues to threaten the world’s health as emerging variants of concern have the potential to circumvent deployed vaccines. Simple and rapid SARS-CoV-2 serological tests are needed to accurately measure the level and duration of neutralization activity of antibodies that arise from natural infection or vaccination. Currently, there are several FDA-approved serological tests under Emergency Use Authorizations (EUA), many of which can detect IgM or IgG against SARS-CoV-2, but do not measure their neutralization efficacy specifically1–3. Functional neutralizing antibody titers are often measured with pseudotyped viruses, however, long assay time and discrepancies in published assay protocols have limited their use4–6. Alternatively, surrogate virus neutralization assays have been developed to circumvent the use of pseudovirions7–10. Although some of these assays have shown successful measurement for serosurveillance of clinical samples, they often resemble ELISA, requiring multiple time-consuming binding and washing steps, while others have not yet reported successful measurement of clinical samples, likely due to the instability of recombinant proteins in serum and plasma. Here, we report the development of a homogeneous surrogate virus neutralization assay (hsVNA) called “Neu-SATiN” by reconfiguring our previously designed serological assay (Serological Assay based on split Tri-part NanoLuc®; SATiN)11 to quantify the degree of neutralization from antibodies directly from plasma or serum. SATiN is a serological assay based on a protein-protein interaction approach for detection of IgGs against the Spike protein of SARS-CoV-212.

In our previous report, we developed SATiN as a homogeneous serological assay platform that utilizes a tri-part NanoLuc®, which is split into two small peptide tags, β9 and β10 (each about 1 kDa), and one large fragment, A11S (18 kDa). The SATiN assay utilizes spike protein and Protein G tagged with either β9 or β10. Upon simultaneous binding of the tagged spike protein and Protein G to anti-SARS-CoV-2 antibody, β9 and β10 are brought into proximity which induces refolding of A11S into active luciferase, producing glow-type luminescence11. Using the same tri-part NanoLuc®, we now show the development of a homogeneous neutralization assay version of SATiN (Neu-SATiN). In Neu-SATiN, enzyme fragment peptides, β9 or β10, are fused to the ACE2 receptor, the target of infection, and to the SARS-CoV-2 spike (S) protein. We hypothesized that when the ACE2 and the S proteins interact, the fused split-NanoLuc® fragments are driven to within ~100 Å of each other, allowing A11S to reconstitute into fully functional NanoLuc®. Importantly, when the interaction between the ACE2 and S proteins is blocked by neutralizing antibodies, the tags are prevented from interacting and subsequent complementation of NanoLuc® is blocked (Fig. 1a). Therefore, in Neu-SATiN, the level of neutralization correlates with the decrease of luminescence. As the assay is intentionally modular, full-length ectoderm of the S protein of SARS-CoV-2 variants of concern can be quickly produced and swapped with wild-type S protein to assess immunological protection. Moreover, as Neu-SATiN is designed to be a “mix-and-read” assay that is performed at the conventional lab bench, the actual hands-on time is <30 min, significantly improving turnaround time.

Results

Protein fusion design and testing. We considered three options for fusing the split-luciferase fragments to the SARS-CoV-2 spike (S) protein and its target, the human ACE2 receptor: (1) N-terminus, (2) C-terminus, or (3) both termini. Although surface loops are possible fusion points, modifying these domains may interfere with neutralizing antibody binding. Based on our previous experience, we know that reconstitution of split-NanoLuc® is most efficient when the fusion locations are within ~50–100 Å13–15. We used molecular modeling to determine possible fusion points on S protein and ACE2 that place fragments within this proximity (Figs. 2a and 3a).

To test feasibility, we initially built the system using only the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein and ACE2. Based on molecular modeling of RBD and ACE2 (Fig. 2a)16, the distance from the N-terminus of ACE2 to the N-terminus of RBD is ~60 Å and to the C-terminus is ~53 Å (PDB ID: 6M0J). In contrast, the C-terminus of ACE2 is more than 100 Å away from either terminus of RBD and thus was excluded as a potential tag fusion site. The complete list of binders used in this study is listed in Supplementary Table 2. Using purified recombinant proteins, we confirmed that the engineered RBD and ACE2 binders with complementary tags produced detectable luminescent signal when combined with the large fragment of the split luciferase, A11S, in human serum. Each binder combination shows substantial luminescence; however, there are differences in signal-to-background ratios (Supplementary Fig. 1a). We also confirmed substantial decrease in the signal when the binders were incubated with neutralizing antibody (NAb) (Sino Biological, 40592-MM57), indicating inhibition of RBD binding to ACE2 to prevent complementation of the split-luciferase fragments. For any given pair, the fractional decrease in luminescent signal as a function of increasing concentration of NAb displays a typical dose–response curve for inhibition that is specific to the NAb (Supplementary Fig. 1b). This suggests that

Fig. 1 General schematic of the Neu-SATiN COVID-19 neutralization assay and molecular modeling of spike (S) protein and ACE2 interaction. a Tri-part NanoLuc® peptide fragments are individually fused to recombinant S protein (purple) and ACE2 (tan). Interaction of S protein and ACE2 induces complementation of the split-luciferase and ‘turns on’ luminescence (left). In the presence of neutralizing antibodies, the interaction between S protein and ACE2 is blocked, preventing luminescence (right). Figure generated using BioRender. b Molecular model of the predicted refolding of NanoLuc® (PDB ID: 5IBO) is shown (green) after complementation of fragments β10 and β9 is driven by the interaction between full spike protein (trimer) and ACE2 (PDB ID: 7A97).
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or absence of antiviral antibodies and quantify the relative level of

system with luciferase gene reporter (Supplementary Fig. 1e, f).

Pearson

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showed that PS 17 had the highest inhibition activity, and this

to their relative luminescence compared to the control NAb

(b) and convalescent patients (Fig. 2).

Testing 18 patient plasma samples: (S)RBD-β9 and β10-ACE2 binder pair, the signal between 0 μg/mL of NAb (darker color) vs. 100 μg/mL of NAb (lighter color adjacent bar) shows a 10-fold decrease upon neutralization. d) Testing 18 patient plasma samples: (S)RBD-β9 and β10-ACE2 were mixed directly with plasma samples, followed by the addition of the detection solution (ΔT1S and substrate). Samples 16, 17, and 18 (indicated by ↓ above bar) are known to be convalescent whereas other samples are from ICU patients with unknown antibody presence/levels. Source data are provided as a Source data file.

Evaluation of Neu-SATiN on convalescent samples. After validating Neu-SATiN’s ability to detect NAb activity, we validated the assay with clinical plasma samples from actively infected (from ICU) and convalescent patients (n = 18). The plasma samples were tested and binned into 5 different groups in respect to their relative luminescence compared to the control NAb (Fig. 2d). Of the convalescent patient samples (PS 16, 17, 18), patient sample 17 (PS 17) showed inhibition similar to 100 μg/mL of NAb (darker color) vs. 100 μg/mL of NAb (lighter color adjacent bar) shows a 10-fold decrease upon neutralization. Nevertheless, our data confirm that Neu-SATiN is able to distinguish the presence or absence of antiviral antibodies and quantify the relative level of neutralization directly in clinical samples.

We compared Neu-SATiN to a pseudovirus neutralization (PSV) assay based on a human immunodeficiency virus (HIV) system with luciferase gene reporter (Supplementary Fig. 1e, f). The results from Neu-SATiN and PSV assay showed good correlation at highest antibody concentrations (Extended Fig. 1e, f) with a Pearson’s r value of 0.86. Correlation is slightly lower across all IgG concentrations (Supplementary Fig. 1e) with a Pearson’s r value of 0.81. Both PSV assay and Neu-SATiN showed that PS 17 had the highest inhibition activity, and this was similar to the positive control (human serum spiked with 100 μg/mL NAb).

Neu-SATiN full-length S protein evaluation of serum samples with differing levels of virus protection. Even though many of the antibodies produced by vaccines target the RBD of S protein17,18, the more pressing concern is protection against variants of concern which often have mutations outside of the RBD19,20. In fact, all currently approved vaccines in US and Canada are based on full-length S protein sequences and have been generated before the onset of variants in 2021. As such, we expanded Neu-SATiN using ACE2 and the full ectodomain of S protein, trimerized through the foldon domain, including known amino acid substitutions to stabilize conformation21. We hypothesized that using the full, trimerized S protein should provide a more comprehensive measurement of the neutralization effect. Molecular modeling was used again to examine the distances between the termini of two proteins (Fig. 3a) using PDB ID: 7A9722. Based on this analysis, we decided to fuse β10 to the N-terminus of S protein and β9 to the N-terminus of ACE2. The length of linker connecting the proteins to the tags was also doubled from 9 to 18 amino acids to increase the flexibility of the tags and to encompass a broader range of potential binding conformations. Consistent with previous studies23, we found the interaction between S ectodomain and ACE2 monomer is relatively low, likely due to ACE2 instability, and decided to use a dimerized ACE2 binder created by fusing the ACE2 N-terminal domain (a.a. 16–614) with the human IgG Fc fragment (Supplementary Table 1). These two binders, denoted as β10-(S)-WT and β9-ACE2-Fc, were validated for binding-induced luminescence (Fig. 3b). The full wild-type spike protein (WT) version of the binder pair shows average luminescence signal of 1.6 × 10⁶ RLU indicating successful complementation of the split-NanoLuc® fragments and shows a robust 3000-fold signal-to-background ratio. We produced mutated S proteins for each of the major SARS-CoV-2 variants of concern: Alpha, Beta, Gamma, Delta, and Omicron19,20 (mutation sequences in Supplementary Table 2). The mutations were made throughout the ectodomain of S protein and not just limited to the RBD. Additional mutations were made to the furin cleavage site to prevent unwanted degradation of the binders by cell culture proteases and to enable purification of recombinant proteins24,25. The orientation of the tags on each protein was kept the same: β10-(S)-Variant and β9-ACE2-Fc. All five variants (Alpha, Beta, Gamma, Delta, and Omicron) produced distinct luminescence signals with high signal-to-background (Fig. 3b). The neutralization of the WT pair (WT S protein with ACE2) corresponds well with concentrations
of a NAb (Sino Biological, 40592-R001) (Fig. 3c); however, there was limited neutralization of most of the variants using this NAb (Supplementary Fig. 2).

We obtained another set of serum samples \((n = 43)\) that have been tested with two different Emergency Use Authorized COVID-19 S protein-binding antibody assays: (1) COV2G Siemens 1st Gen (target antigen: RBD, cutoff: \(\geq 1.0\)), and (2) EUROIMMUN (target antigen: S1, cutoff: \(\geq 1.1\)). The samples can be further categorized into three different groups: (1) SARS-CoV-2 exposed with no vaccination history (post-infection, Samples 1–18), (2) SARS-CoV-2 exposed with vaccination history (post-infection and post-vaccination, Samples 19–28), and (3) neither exposed nor vaccinated (no infection and no vaccination, Samples 29–43). All of the samples were screened with our full-length-WT S protein Neu-SATiN for the presence of neutralizing antibodies. Commercial human serum spiked with NAB (Sino Biological, 40592-R001) was used as a positive control and to determine relative neutralization activity of the patient samples.

Samples with previous infection history (Samples 1–28, \(n = 28\)) showed normalized activity score <10% (Fig. 3d—indicated by red dotted line) in Neu-SATiN, with the exception of Sample 2 (frequency = 27/28, 96%). These were also the samples that scored \(>20.00\) in Siemens 1st Gen and \(>8\) in EUROIMMUN EIA, both indicating the presence of anti-SARS-CoV-2 antibodies against the WT S protein (Supplementary Table 3). Intriguingly, when the patients were previously infected and then vaccinated...
Fig. 3 Validation of full spike proteins (wild type and variants) and ACE2 binders in the serosurveillance of clinical samples. a Molecular modeling of the distances between N-terminus of ACE2 to the N-termini of nearest S protein is ~48 Å and ~88 Å, respectively (PDB ID: 7A97). b Comparison of signal and background for the full spike (wild type and variants) and ACE2 pairs. c Serial dilution of a commercially available NAb (Sino Biological, 40592-R001) in the presence of β10-(S)WT and β9-ACE2-Fc pair. d Serum samples that have been tested previously on two different COVID-19 detection assays were also tested using Neu-SATiN. Wild type full spike protein with β10 tag (β10-(S)WT) and ACE2 with β9 tag (β9-ACE2-Fc) was used as the binders. Signals from patient samples were normalized to the signal from normal human serum with the binders alone (no neutralizing antibodies). The red dotted line at 10% activity (i.e., 90% neutralization) indicates the cutoff line to distinguish neutralizing samples from the non-neutralizing samples; ‘+’ and ‘−’ signs below the sample number indicate the result from two prior tests detecting anti-SARS-CoV-2 antibodies (COV2G Siemens 1st Gen and EUROIMMUN EIA). e Activity measured in protein-based surrogate neutralization assay (pB5NA) versus neu-SATiN for the wild-type (WT) variant shows high correlation with a Pearson’s correlation test r value of 0.88. N = 66. f Neutralization efficacy of patient samples against WT and variant S proteins. Samples used in (d) (n = 43) were tested with an additional n = 35 patient samples that were positive for anti-SARS-CoV-2 antibodies. Luminescence signals from each patient serum were measured and normalized to human serum (no NAb). Known post-vaccination, post-infection, and negative (no known infection or vaccination) samples were plotted separately. Red dotted line at 10% indicates the cutoff between the negative samples and the positive samples for distinction of neutralization. g Comparison of serum data from patients that were vaccinated to patients that were vaccinated after a documented infection (n = 40). All patient samples were collected before November 2021, prior to any known Omicron infections. Source data are provided as a Source data file.
Fig. 4 Assessing the potency of neutralizing antibodies. 

a) Neutralizing capabilities of FDA EUA-approved therapeutic antibodies Regn10933 (left, casirivimab), Regn10987 (center, imdevimab), and JS016 (right, etesevimab) were evaluated using Neu-SATiN against different S variants. 

b) Evaluation of neutralizing antibodies in individuals with unvaccinated sera (n = 2), with one vaccination dose (n = 4), with two vaccination doses and collected within 50 days after the second shot (n = 13, intervals between shots were 21–37 days), and with two vaccination doses collected more than 50 days after the second shot (n = 5, intervals between shots were 21–36 days). 

c) Aggregated data showing titers at 50% neutralization (NT50) for each group. Source data are provided as a Source data file.
Discussion

Since the outbreak of the COVID-19 pandemic, the importance of a virus neutralization assay has risen. Virus neutralization assays are the main tools for developing vaccine and therapeutic strategies. Although the PSV assay is effective in measuring the degree of infection, maintaining cell cultures and making pseudovirus particles are labor intensive with potential safety concerns. Moreover, batch-to-batch variability in virus production and cell transfection efficiency limit standardization and robust assay results. Numerous immunosassays for rapid detection of anti-SARS-CoV-2 antibodies have been developed; however, these assays mainly focus on capturing and detecting antibodies binding to virus proteins (e.g., spike or nucleocapsid). Since it is well known that mere binding does not necessarily imply neutralization, a true neutralization assay for the better understanding of protective immunity against SARS-CoV-2 is needed. To circumvent the use of virus particles and cells, surrogate versions of neutralization assays have been developed. These assays often use ELISA or similar platforms, with multiple time-consuming binding and washing steps, preventing high-throughput screening. In need of an accurate yet more rapid virus neutralization assay, we developed Neu-SATiN as a homogeneous surrogate virus neutralization assay (hsVNA), using a split-luciferase system.

There have been several reports describing efforts to develop surrogate virus neutralization assays (sVNA) for the detection of antibodies against SARS-CoV-2. These reports show capabilities as surrogate assays; however, many of these platforms require chemical modification and conjugation of proteins to beads or other solid surfaces, requiring intermediate purification and/or washing steps to remove unbound reagents and analytes. In contrast, Huang et al. reported the development of an hsVNA using the binary version of split-NanoLuc. Although the approach is similar to the platform we report here, the authors used a monomeric or dimeric form of S1 domain of spike protein tagged with β10 (SmBiT), paired with ACE2 (LgBiT, NanoLuc) beta strands 1 through 9, which was shown to have limited detection of non-RBD targeting antibodies. The importance of conformational changes in spike protein–altering ACE2 binding has been emphasized in many studies. Also, with new variants of concern emerging, many of the deployed vaccines and second-wave vaccines in development are targeting full spike protein. We initially designed our assay by investigating the interactions between ACE2 and RBD. The result is in agreement with reports that neutralization activities of NAB mostly come from anti-RBD immunoglobulins; however, immunologic response can also produce antibodies against epitopes outside of the RBD and may require the trimeric structure of the full spike protein for binding. This compelled us to develop stabilized, trimeric, full-length spike (both WT and major variants of concern) versions of the binders and stable, dimerized ACE2 receptor. The data presented in Fig. 3 demonstrates that β10–(S)WT/β9–ACE2-Fc can differentiate the degree of neutralization directly from patient sera and that variant versions of the spike binders can distinguish anti-SARS-CoV-2 NAB positive serum samples from negative samples (Fig. 3e, f). In addition, Neu-SATiN can provide quantitative analysis of NT50 and thus enables the measurement of potency of anti-SARS-CoV-2 antibodies against different strains (Fig. 4). Combined, this provides a comprehensive screen of a patient’s level of protection against the current variants of concern. As the assay is modular, emerging variants of interest can be quickly produced and incorporated as we have demonstrated with the inclusion of the Omicron variant that become prominent during the initial review of this manuscript. As all the patient samples were collected before November 2021, prior to any known wide-spread Omicron infections, our results also suggest that prior infection/vaccination does not provide significant neutralizing protection against the variant.

The results obtained with Neu-SATiN correlate with PSV assays and other antigen-based assays in detecting the neutralization potential of antibodies in clinical samples. It is important to note that natural immunity can produce antibodies that bind several antigens and function through alternate mechanisms, including antibody-dependent phagocytosis (ADP) or antibody-dependent cellular cytotoxicity (ADCC). Although the current format of Neu-SATiN cannot measure these types of antiviral activity, the majority of vaccines use Spike domains; therefore, this assay can be used to assess protection developed from immunization. One advantage of Neu-SATiN is that it can be performed homogenously and directly using plasma or serum, which significantly reduces hands-on assay time to <50 min and can be run under standard lab conditions. Importantly, we have demonstrated that the split-NanoLuc based Neu-SATiN can be applied to full-length spike proteins of the original strain and variants to test neutralization levels of convalescent patient sera. Having a modular technology as a surrogate assay that can be easily adopted as a point-of-care tool is important in tracing and adapting to the evolution of the current pandemic.

Methods

Ethical statement. Studies were approved by relevant ethical boards: ARUP/University of Utah Institutional Review Board (IRB-approved protocol 0007740) and Office of Environmental Health and Safety at the University of Toronto (REB-approved protocol REB-20-107). Informed consent was obtained for participants and no compensation was provided.

Cell lines, cell culture media and antibodies, and cloning reagents. The HEK293 cell line was provided by Prof. Jason Moffat at University of Toronto and was originally purchased from ATCC (CRL-1573). Calu-3 cells were purchased from ATCC (HTB-55). HEK293 cell culture reagents were purchased from Thermo Fisher Scientific. Cloning reagents were purchased from NEB. Two neutralizing antibodies: 40592-MM57 (used for RBD pairwise screening) and 40592-R001 (used for WT pair screening) were purchased from Sino Biological. Regn10933 (CPC511A), Regn10987 (CPC512A), and JS016 (CPC516A) were purchased from Cell Sciences. Various antibody dilutions were used in this work, with final concentrations in assays ranging from 0 to 600 μg/mL. Specific concentrations can be found within the Figures and corresponding Figure legends.

Clinical samples. Samples were obtained from either the University of Utah School of Medicine, ARUP Laboratories, or from Unity Health. University of Utah School of Medicine (total n = 16) were obtained from infected patients within 48 h of admission to the ICU (n = 13) or within 3–5 weeks of positive PCR test for convalescent patients (n = 3). Samples for determining neutralization (total n = 63) in uninfected, infected, and/or vaccinated were generously provided by ARUP Laboratories (IRB-approved protocol 0007740). Samples for antibody titer and serosurveillance studies (total n = 24) were collected from Unity Health employees, enrolled through REB-approved protocol REB-20-107, Toronto. All samples were deidentified.

Vector construction and transient transfection in HEK293 cells. Vector cloning for the binder expression was performed as previously reported. All cDNA were cloned into an in-house mammalian expression vector derived from pCMV5 with a signal peptide sequence appended at the N-termini and an octa-histidine stretch at the C-termini. For the constructs that had the tag at the N-termini, either β9 or β10 sequence was placed after the signal peptide, followed by the binder sequence. Likewise, the constructs with C-terminus tag had β9 or β10 sequence right before octa-histidine (Supplementary Table 1). The final products were transfected into HEK293 cells using polyethylenimine Max (Polysciences).

Production and purification of binders. HEK293 cells transfected with binder constructs were cultured in DMEM supplemented with 10% FBS and 1X antimycotic-antibiotic mixture. Typically, cells were seeded at 50% confluency, and the media was collected every day until the cells became fully confluent. The collected media was filtered through 0.22 μm PES filter before purification. Purification was done on AKTA FPLC using HisPureTM Cobalt Resin. Tween 20 (0.01%), rathexol (0.1%), and glycerol (10%) were added to the final product and kept at ~80°C before use.
Reconstitution of split-NanoLuc® driven by (S)RBD and ACE2 interaction. For the screening of (S)RBD binders with ACE2 binders, 10 µL of commercial human serum (Abclonal® S-1000-M1) spiked with titrating concentrations from 0 µg/mL to 100 µg/mL of the neutralizing antibody (Sino Biological, 40592-MM57) was combined with 5 µL of (S)RBD binders (10 pmol) and 5 µL of ACE2 binders (10 pmol), and incubated for 30 min. The incubation was done using white, round bottom 96-well plates at room temperature with vigorous shaking. Then, the “detection solution” which consists of coelenterazine (substrate) and Δ11S was premixed and 80 µL of the detection solution was added to each well. The final concentrations of the substrate, coelenterazine, was 10 µM and the large enzyme fragment, Δ11S, was 500 nM in well in a total volume of 100 µL. Luminescence signal was measured using TECAN Infinite M1000pro in a kinetic cycle. The results reported here are from the 30-min timepoint.

Split NanoLuc®-based virus neutralization assay: testing spiked samples and convalescent samples. Measuring neutralization activity of clinical samples was done in a similar fashion as described above: 10 µL of clinical samples were mixed with 10 pmol of spike protein (5 µL; full S or (S)Variant) and 10 pmol of ACE2 fusion (5 µL). All three components were incubated together for 30 min with vigorous shaking. Then, 80 µL of the detection solution (defined above) was added (final concentration of coelenterazine fragment, Δ11S, was 500 nM per well and the kinetic cycle of luminescence was measured. For serial dilutions to quantify activity of low titer samples, the assays were performed in a buffer containing 20 mM Tris (pH 7.5), 0.1% Tween-20, 2 mM TCEP, 2 mM EDTA, 25 mM NaCl, and 0.05% BSA. Serum samples (12.5 µL) were first diluted in a volume of 50 µL of buffer, followed by additional one-half serial dilutions down to 1:64 dilutions. An aliquot (5 µL) of the diluted sample was added with 5 µL of β10 modified S binder (20 nM; full S or (S)Variant) and incubated for 30 min. An aliquot (5 µL) of the reaction mixture was further mixed with 45 µL of substrate mixture containing 555.6 nM β9-ACE2-Fc, 100 nM Δ11S, 22.2 µM furimazine (substrate). After 1 h incubation, luminescence signals were measured using a complete plate reader. The inhibition curve of a sample against S protein variant was obtained by fitting the readings at different dilutions into the normalized response model with variable slope in GraphPad. The titer of 50% neutralization was calculated according to each model.

Testing spiked samples and convalescent samples with pseudovirus neutralization (PSV) assay. The active sera (from patients in ICU) and convalescent sera were purified using Protein G magnetic beads (Promega Corporation, G7471) as per manufacturer’s instructions. The concentration of purified IgG was assured using NanoDrop 2000. Pseudovirions were produced by co-transfecting 293T human embryonic kidney cells using PEI transfection reagent (Polysciences, Warrington, PA) with NL4-3 HIV-1 genome (pNL4-3.LucR-E, including the firefly luciferase gene inserted into the nef coding sequence and frame shift mutation in Env and Vpr) and a plasmid encoding the desired virus fusion protein (pCAGGS-SARS2-S-FlagD614G, kind gift of M. Farzan for SARS-CoV-2 S or pMDG VSV-G for VSV as a specificity control). The pseudovirions were purified using an affinity capture column with a nickel-chelating resin (Ni-NTA magnetic beads, Qiagen, Valencia, CA) and then washed to remove unbound ACE2 and 20 µL of Streptavidin-Peroxidase (NRCoV2-20-Fc, NRC) was added alongside a set of pooled negative/positive control sera. The plates were then washed to remove unbound ACE2 and 20 µL of Streptavidin-Peroxidase Polymer (Sigma #S2438) was added to each well (25 ng/well) and incubated for 1 h with shaking. Plates were washed one last time and developed by adding 20 µL of ELISA Pico Chemiluminescent Substrate (diluted 1:2 in MilliQ H2O) was dispensed into each well. After a 5-min incubation, plates were read on an En200 plate reader (BioTek Instruments) at 20 ms/well and a read height of 1.0 mm. Luminescence signal was blank adjusted, and percentage of ACE2-Spike interaction determined by dividing the luminescence values by the maximal signal (no serum control; maximal ACE2-spike binding; 0% inhibition).

Statistical and reproducibility. All data are presented as mean ± standard deviation (SD) and the number of biological repeats, as well as sample size, are reported. No statistical method was performed to predetermine sample size. Statistical analyses were performed using GraphPad Prism 9. Correlation between the NeuSATIN assay and other assays (PSV or pbSNA) was analyzed using Pearson product–moment correlation.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability Source data are provided with this paper.

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Author contributions

S.J.K. conceptualized the Neu-SATiN assay, was actively involved in experiments and data analysis, and wrote the bulk of the manuscript. Z.Y. was actively involved in designing and preparing probes, conducted experiments and data analysis, and assisted in manuscript editing. M.C.M. was actively involved in many experiments and data analysis and assisted in manuscript writing. D.M.E. and M.S.K. conceptualized and performed the pseudovirus assay and contributed to manuscript editing. A.L. was involved in probe preparation. M.P., A.B., C.B., and P.J. contributed to providing patient samples. Y.G. and M.L. coordinated the protein-based serum neutralization assay. J.C.D. and M.G. coordinated patient samples and performed ELISA neutralization assays. R.A.C. and E.A.M. contributed to providing patient samples. I.S. and S.C.O. guided and supervised the work, contributed to assay development and manuscript writing as well as coordinated the preparation of the manuscript. All authors approved its content.

Competing interests

A patent covering all the main aspects/keys of the Neu-SATiN assay has been filed by the governing council of the University of Toronto with the US Patent Office. The application is currently pending. I.S., S.C.O., S.J.K., and Z.Y. are named as coinventor on the patent application. The remaining authors declare no competing interests.

Additional information

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