Supporting information for:

Cross-validation of ELISA and a portable surface plasmon resonance instrument for IgG antibodies serology with SARS-CoV-2 positive individuals

Abdelhadi Djaileb[a,b]⊥, Maryam Hojjat Jodaylami[c]⊥, Julien Coutu[c], Pierre Ricard[c], Mathieu Lamarre[d], Léa Rochet[a], Stella Cellier-Goetghebeur[a], Devin Macaulay[d], Benjamin Charron[c], Étienne Lavallée[a], Vincent Thibault[c], Keisean Stevenson[c], Simon Forest[c], Ludovic S. Live[b], Nanouk Abonnenc[e], Anthony Guedon[e], Patrik Quessy[e], Jean-Francois Lemay[e], Omar Farnós [f], Amine Kamen[f], Matthew Stuible[g], Christian Gervais[g], Yves Durocher[g], François Cholette[h,i], Christine Mesa[h], John Kim[h], Marie-Pierre Cayer[j], Marie Joëlle de Grandmont[j], Danny Brouard[j], Sylvie Trottier[k], Denis Boudreau[d], Joelle N. Pelletier*[a] and Jean-Francois Masson*[c]

[a] Department of Chemistry, Department of Biochemistry and PROTEO, The Québec Network for Research on Protein Function, Engineering and Applications, Université de Montréal, CP 6128 Succ. Centre-Ville, Montreal, Québec, Canada, H3C 3J7

[b] Affinité Instruments, 1250 rue Guy, Suite 600, Montréal, Québec, H3H 2L3

[c] Department of Chemistry, Quebec Centre for Advanced Materials (QCAM), Regroupement Québécois sur les Matériaux de Pointe (RQMP), and Centre interdisciplinaire de recherche sur le cerveau et l'apprentissage (CIRCA), Université de Montréal, CP 6128 Succ. Centre-Ville, Montreal, Québec, Canada, H3C 3J7

[d] Department of Chemistry and Centre for Optics, Photonics and Lasers (COPL), Université Laval, 1045, av. de la Médecine, Québec City, Québec, Canada, G1V 0A6

[e] CNETE and PROTEO, The Québec Network for Research on Protein Function, Engineering and Applications, Cégep de Shawinigan, 2263 Avenue du Collège, Shawinigan, Québec, Canada, G9N 6V8

[f] Department of Bioengineering, McGill University McConnell Engineering Building, 3480 University Street, Montreal, Québec, Canada, H3A 0E9

[g] Mammalian Cell Expression, Human Health Therapeutics Research Centre, National Research Council Canada, Montréal, Québec, Canada.

[h] National Laboratory for HIV Reference Services, National Microbiology Laboratory at the JC Wilt Infectious Diseases Research Centre, Public Health Agency of Canada, Winnipeg, Canada.

[i] Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Canada.

[j] Héma-Québec, Affaires médicales et innovation, 1070, avenue des Sciences-de-la-Vie, Québec City, Québec, Canada, G1V 5C3

[k] Centre de recherche du Centre hospitalier universitaire de Québec and Département de microbiologie-infectiologie et d'immunologie, Université Laval 2705, boulevard Laurier, Québec City, Québec, Canada G1V 4G2

* Corresponding authors: joelle.pelletier@umontreal.ca; tel: +1-514-343-2124

jf.masson@umontreal.ca; tel: +1-514-343-7342

⊥ Authors who contributed equally to the work.

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1) Additional experimental details for production of recombinant SARS-CoV-2 antigens

Unless otherwise specified, the nucleocapsid and spike ectodomain and RBD protein sequences correspond to the Wuhan-Hu-1 reference strain of SARS-CoV-2. Data for the purification of each protein is provided in Figure S1.

A) Nucleocapsid expressed in *E. coli*, with His-tag or cleaved:

The nucleocapsid construct was designed by Lemay and coworkers from the sequence YP_009724397.2 in GenBank and was *C*-terminally fused to the tobacco etch virus (TEV) ENLYFQG protease-specific cleavage site (underlined) and a DYDIPTT linker (italics) to facilitate the cleavage of the 6-His tag (bold) (Bio Basic). The sequence was codon-optimized for expression in *Escherichia coli*.

N (YP_009724397.2) **sequence:**
MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFT
ALTQHGKEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRW
YFYYLGTGPEAGLPYGANKDGIIWVATEGALNTPKDHIGTRNPANNAAIVLQLP
QGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSRGTSPARMAGNGGDA
ALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKAYNV
TQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEV
TPSGTWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADET
QALPQRQKKQQTVTLLPAADLDDFSKQLQQSMSSADSTQA<u>ENLYFQG</u>*DYDIPTT*
HHHHHH

The recombinant nucleocapsid (rN) protein was expressed in *E. coli* BD792 and purified using immobilized-metal-ion chromatography (IMAC). The rN protein was flanked by *Eco*RI and *Bam*HI restriction sites and was inserted into plasmid pQE-80-L-Kan (Qiagen).

The *E. coli* strain containing the plasmid was inoculated into 25 mL of 2×YT (16 g bacto-tryptone, 10 g yeast extract, 5 g NaCl, pH 7.0) culture medium and 25 μg/mL kanamycin and propagated at 37°C during 7 h at 180 rpm. Then, 10 mL of this pre-culture was used to inoculate 500 mL of 2×YT + 25 μg/mL kanamycin. This second pre-

culture was incubated during 10 h at 37°C at 180 rpm and 250 mL were used to inoculate 10 L of 2×YT + 25 μg/mL kanamycin in a bioreactor. The cells were grown at 37°C and 300-900 rpm. Air flow was at 1.00 vvm to maintain O_2 at >35% and pH was maintained at 7.0. When the OD_{600} reached ~1.0, 1 mM of IPTG (isopropyl-β-D-thiogalactopyranoside) was added to induce the protein expression at 20°C during 7 h.

Cells were harvested by centrifugating 15 min at 5000×g at 4 °C. Biomass (30 g) was resuspended in 200 mL of cold lysis buffer (10 mM Tris–HCl pH 8.0, 20 mM NaCl, 10 mM imidazole and 0.75% Tween). Lysozyme (0.3 mg/mL) and 1 mM phenylmethylsulfonyl fluoride (PMSF) were added and incubated on ice for 30 min. Bacteria were lysed by two passages through an Emulsiflex C5 homogenizer (Avestin) at 18,000–22,000 psi. Benzonase (2500 U; Sigma-Aldrich) and 2 mM of $MgCl_2$ were added to the cell lysate and incubated for 20 min with gentle agitation before clarification by centrifugation at 15,000×g for 15 min. The supernatant was adjusted to final concentrations of 10 mM Tris–HCl pH 8.0, 1000 mM NaCl, 10 mM imidazole and 0.75% Tween.

The clarified supernatant was loaded onto a 25 mL IMAC column packed with Ni-NTA agarose beads (Qiagen) and washed with 10 column volumes (CV) of wash 1 buffer (10 mM Tris–HCl pH 8.0, 1000 mM NaCl, 10 mM imidazole and 0.75% Tween), 10 CV of wash 2 buffer (10 mM Tris–HCl pH 7.5, 1000 mM NaCl, 25 mM imidazole and 0.75% Tween) and 10 CV of wash 3 buffer (10 mM Tris–HCl pH 7.5, 300 mM NaCl, 40 mM imidazole and 0.75% Tween). Protein was eluted with 5 mL of elution buffer (10 mM Tris–HCl pH 7.5, 150 mM, NaCl, 500 mM imidazole). The fractions with highest OD_{280} were pooled and dialyzed against PBS pH 7.4 and 10% glycerol in 10K MWCO cassettes (Thermo Scientific). The resulting solution was filtered through a 0.2 μM filter and stored at -80°C. Each step of the purification process was monitored by SDS-PAGE. This His-tagged antigen was directly used for immunoassays (Figure S1).

B) His-tagged Nucleocapsid expressed in CHO cells:

The SARS-CoV-2 nucleocapsid (YP_009724397) cDNA was synthesized (GenScript) with *Cricetulus griseus* codon bias. The protein construct designed by

Durocher and coworkers contains a FLAG tag (underlined), a Twin-Streptag (italics) and a 6-His-Glycine tag (bold) fused to its *C*-terminus.

N (YP_009724397) **sequence:**

MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTA
SWFTALTQHGKEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDL
SPRWYFYYLGTGPEAGLPYGANKDGIIWVATEGALNTPKDHIGTRNPANNAAIV
LQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSRGTSPARMAGN
GGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATK
AYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRI
GMEVTPSGTWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKK
KADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQSMSSADSTQATGGDYKDD
DDKG*WSHPQFEKGGGSGGGSWSHPQFEK*G**HHHHHHG**

Following cloning the cDNA into pTT5™ expression plasmid, expression was achieved by transient gene expression in CHO$^{BR1/55E1}$ cells following transfection with polyethylenimine [1]. As we found that a significant proportion of the nucleocapsid was released into the culture medium, the protein was purified from the clarified supernatant harvested at day 7 post-transfection. Following centrifugation and filtration, clarified supernatant was loaded on a Nickel Sepharose Excel column (Cytiva Life Sciences). The column was washed with 50 mM sodium phosphate buffer pH 7.0 containing 25 mM imidazole and 300 mM NaCl and protein was eluted with 50 mM sodium phosphate buffer pH 7.5 containing 300 mM imidazole and 300 mM NaCl. Nucleocapsid protein was further purified by affinity chromatography on a StrepTrap XT column (Cytiva Life Sciences) equilibrated in 100 mM Tris pH 8.0, 150 mM NaCl (Buffer W). Following washing with 5 CV of Buffer W, bound proteins were eluted with Buffer W containing 50 mM biotin and 1 mM EDTA. Purified nucleocapsid protein was buffer exchanged in Dulbecco's Phosphate-Buffered Saline (DPBS) using a Centripure P100 column, sterile-filtered through 0.2 µm membrane, aliquoted and stored at -80°C. All purification steps were performed at room temperature.

Integrity and purity of the purified nucleocapsid was analyzed by SDS-PAGE and analytical size-exclusion high-performance liquid chromatography (SEC-HPLC). The SEC column (5 × 150 mm Superdex 200HR; Cytiva Life Sciences) mobile phase was

DPBS supplemented with 200 mM arginine. The nucleocapsid eluted as a major peak (>99% integrated area) of 300 kDa with no apparent aggregates (Figure S1).

C) RBD expressed in HEK or *Pichia* cells:

RBD was obtained by Kamen and coworkers in HEK293SF suspension cells cultured in serum-free medium, in 3L-controlled bioreactors, as recently described [2]. Briefly, RBD secreted expression to the culture medium of HEK293SF cells was mediated by human type 5 adenovirus infection, resulting in a glycosylated recombinant protein of approximately 38 kDa. The culture supernatant from the bioreactor was subjected to tangential flow filtration (TFF) using a hollow fiber filter to separate the adenovirus-containing retentate from the fraction containing the recombinant RBD. The permeate fraction was buffer-exchanged with equilibration buffer for purification by IMAC with the *N*-terminal 6-His tag, using HisTrap HP 5 mL affinity columns on an AKTA Avant 25 system (Cytiva Life Sciences). The protein, designated as pTPA_SP-RBD-His, was eluted in a single step with 150 mM imidazole and characterized by SDS-PAGE and Western blot, using SARS/SARS-CoV-2 Spike Protein (subunit 1) polyclonal antibody (Thermo Fisher Scientific, USA). The protein was aliquoted in storage buffer (PBS, 2 mM $MgCl_2$ and 2% sucrose, pH 7.2) at a concentration of 1.8 mg/mL and stored at -80°C.

Alternatively, RBD was produced in *Pichia pastoris* SuperMan5 (Biogrammatics) by Lemay and coworkers. The RBD (331-549) construct was designed from the GenBank sequence MN908947.3, codon-optimized for expression in its host. It was *C*-terminally fused to ENLYFQG as the TEV protease-specific cleavage site (underlined) and a DYDIPTT linker (italics) to facilitate the cleavage of the 6-His tag (bold). It was flanked by *Sap*I restriction sites (Bio Basic).

RBD (331-549; MN908947.3) **sequence:**
MITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPT
KLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSN
NLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQS
YGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLT
GTENLYFQG*DYDIPTT***HHHHHH**

It was inserted into plasmid pD912-AOX using the Electra kit (Atum). *P. pastoris* containing the plasmid was inoculated into 25 mL of BMGY (1% yeast extract, 2%

peptone, 100 mM potassium phosphate, pH 6.5, 1.34% yeast nitrogen base without amino acids, 0.4 mg/L biotin and 1% glycerol) culture medium containing 100 µg/mL zeocin and propagated at 28°C for 24 h at 240 rpm in a baffled flask. At that time, 25 mL of BMMY (1% yeast extract, 2% peptone, 100 mM potassium phosphate, pH 6.5, 1.34% yeast nitrogen base without amino acids, 0.4 mg/L biotin and 1% methanol) medium and 100 µg/mL zeocin was added. 1% methanol was added every 12 hours for the next 2 days.

The supernatant was harvested by centrifugating 15 min at 5000×g at 4℃. The supernatant was concentrated on 10K MWCO centrifugal filter units (Amicon). Equilibration buffer (5 mL of 10 mM Tris–HCl pH 8.0, 20 mM NaCl, 10 mM imidazole and 0.75% Tween) was added to the concentrated supernatant and gently agitated with 4 mL of Ni-NTA agarose beads (Qiagen) during 1 h at 4°C. The solution was packed into an empty column (Bio-Rad). The column was washed with 2 CV of wash 1 buffer (10 mM Tris–HCl pH 8.0, 1000 mM NaCl, 10 mM imidazole and 0.75% Tween) and 2 CV of wash 2 buffer (10 mM Tris–HCl pH 7.5, 300 mM NaCl, 50 mM imidazole and 0.75% Tween). Protein was eluted with 5 mL of elution buffer (10 mM Tris–HCl pH 7.5, 150 mM NaCl, 500 mM imidazole). Fractions with high OD_{280} were pooled and diafiltrated on 10K MWCO centrifugal filter units with PBS pH 7.4 and 10% glycerol. The proteins were stored at -80°C. Each step of the purification was verified by SDS-PAGE.

D) His-tagged Spike ectodomain expressed in CHO cells:

SARS-CoV-2 spike ectodomain (MN908947) construct with the furin site (aa 682-685: RRAR) mutated to GGAS, the stabilizing prefusion mutations K986P/V987P, and with the human resistin as trimerization partner [1], (named SmT1) was expressed and purified by Durocher and coworkers, as described [3] with some modifications. The trimeric and stabilized spike (SmT1) sequence contains the human resistin (underlined), a FLAG tag (italics), and a hexa-His-Glycine tag (bold).

Spike ectodomain (MN908947) **construct (SmT1):**

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLF
LPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLD
SKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANN
CTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGF

SALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFL
LKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNIT
NLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKL
NDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNL
DSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYG
FQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG
VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQ
VAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYE
CDIPIGAGICASYQTQTNSPGGASSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNF
TISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQ
DKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLAD
AGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSG
WTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLS
STASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQID
RLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYH
LMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHW
FVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKN
HTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQGTGGS
MEEAINERIQEVAGSLIFRAISSIGLECQSVTSRGDLATCPRGFAVTGCTCGSACGS
WDVRAETTCHCQCAGMDWTGARCCRVQP*DYKDDDDK*G**HHHHHHG**

CHO cells were transfected with pTT5-SmT1 plasmid using polyethylenimine and the culture was harvested at day 7 post-transfection. Clarified harvested medium was purified by a single affinity chromatography step (Ni-Sepharose Excel) using a 500 mL IMAC column. The column was washed with 50 mM sodium phosphate, pH 7.0, containing 300 mM NaCl and 25 mM imidazole and eluted with the same buffer containing 300 mM imidazole. In order to achieve near homogeneity, additional purification steps were required. IMAC eluate was buffer exchanged by diafiltration for 20 mM Tris-Cl pH 7.5, 25 mM NaCl using a Minimate tangential flow capsule (30 kDa cut-off; Pall). Purification by anion exchange chromatography (HiTrap Q HP; Cytiva) was conducted through NaCl step gradient of 100, 200, 300 and 1000 mM NaCl. Cleaner fraction eluting at 200 mM NaCl was further purified by gel filtration chromatography (Sephacryl S300 16/600, Cytiva) in DPBS. Fractions corresponding to trimeric spike were pooled and concentrated using centrifugal filter units of NMWL 30 kDa (Amicon Ultra-15, Millipore-Sigma), 0.2 μm filtered (Millipore) and stored at -80°C at protein concentration not exceeding 2 mg/mL. The purified spike protein integrity and purity were analyzed by SDS-PAGE and analytical size-exclusion ultra-high-performance liquid

chromatography using an Acquity BEH200 column (2.5 µm bead size; Waters Limited) equilibrated in DPBS + 0.02% Tween-20 and coupled to a MALS detector.

The SmT1-1 spike Reference Material can be purchased at: https://nrc.canada.ca/en/certifications-evaluations-standards/certified-reference-materials/list/131/html

Variant Spike ectodomain construct:

A point-mutated variant of the B.1.1.7 variant of concern (with the native D614 residue) was similarly produced in CHO cells. The DNA sequence (PRO6430-1(SmT1-UK)) includes the following variations in the spike ectodomain relative to the SmT1 construct: del69–70 HV, del144 Y, N501Y, A570D, P681H, T716I, S982A, D1118H. All other aspects of the construct are as described above for the SmT1 construct. [3]

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLF
LPFFSNVTWFHAISGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSK
TQSLLIVNNATNVVIKVCEFQFCNDPFLGVYHKNNKSWMESEFRVYSSANNCTF
EYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSAL
EPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKY
NENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNINLCP
FGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLC
FTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKV
GGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT
YGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLT
ESNKKFLPFQQFGRDIDDTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVA
VLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECD
IPIGAGICASYQTQTNSHGGASSVASQSIIAYTMSLGAENSVAYSNNSIAIPINFTIS
VTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDK
NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGF
IKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTF
GAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTAS
ALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILARLDPPEAEVQIDRLIT
GRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSF
PQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQ
RNFYEPQIITTHNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSP
DVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQGTGGSMEEAI
NERIQEVAGSLIFRAISSIGLECQSVTSRGDLATCPRGFAVTGCTCGSACGSWDVR
AETTCHCQCAGMDWTGARCCRVQP*DYKDDDDKG***HHHHHHG**

Following expression in CHO cells, single-step IMAC purification was performed as described above. Following elution, buffer exchange into DPBS adjusted to pH 7.8 was performed by tangential flow diafiltration (30 kDa cut-off) and 0.2 µm filtered for

storage at -80°C at protein concentration not exceeding 2 mg/mL. The purified variant spike protein integrity and purity were analyzed by SDS-PAGE and analytical size-exclusion ultra-high-performance liquid chromatography using an Acquity BEH450 column (2.5 μm bead size; Waters Limited) equilibrated in DPBS + 0.02% Tween-20 and coupled to a MALS detector.

2) ELISA Protocol

The ELISA protocol is based on that of Krammer and colleagues with modifications inspired by reports led by Finzi and by Bazin [4-6]. Unless otherwise mentioned, the relevant SARS-CoV-2 antigen was diluted in PBS at a concentration of 2.5 μg/mL. Immulon 2 HB 96-well plates (Thermo Fischer Scientific, cat. no. 14-245-78) were coated with 100 μL of diluted antigen and incubated at 4°C overnight. The following day, plates were washed 4 times with PBS-T using a 50 TS Microplate Washer (Biotek) automated plate washer followed by addition of 300 μl of blocking solution (PBS-T + 3% (w/v) milk powder) to each well. After 1 h of incubation at RT, plates were washed 4 times with PBS-T and 100 μL of diluted commercial primary antibody was added to each well. Negative controls were treated identically, without inclusion of primary antibody. Plates were incubated for 1 h at RT and washed 4 times with PBS-T. The appropriate secondary antibody (100 μL of 1:10000 dilution) was added. Plates were incubated for 1 h at RT then washed 4× with PBS-T. Addition of 100 μL of TMB (3,3',5,5'-tetramethylbenzidine, Sigma-Aldrich) to each well was followed by a 20 min incubation at RT. Color development was initiated by addition of 100 μL of 2 M HCl. Absorbance was immediately recorded at 450 nm in a FLUOstar Optima microplate reader (BMG Labtech).

For ELISA assays using commercial primary antibodies from animals, commercial human serum (H4522, Sigma-Aldrich) was diluted 10-fold in PBS-T. Primary antibody was added at a concentration of 10 μg/mL; antibodies were those used for SPR (specified in the main text). The diluted antibody was further diluted 10-fold in PBS-T 1% (w/v) milk powder in a 96-well polystyrene dilution plate for use. The HRP-conjugated secondary antibodies used were Goat Anti-Mouse IgG Fc (Abcam, cat. no. ab97265) or Goat Anti-Rabbit IgG Fc (Abcam, cat. no. ab97200; GeneTex, GTX213110-01).

For ELISA assays of clinical serum samples, the same protocol was used with the following modifications. While the plates were being coated with the antigen, clinical samples were inactivated at 56 °C in a water bath for 1 h, then kept at 4 °C overnight. The following day, serum samples were diluted 1:50 or as specified in PBS with 0.1% Tween20 and 1% milk powder, in a 96-well polystyrene dilution plate and 100 µl was added to each well. The secondary antibody (Anti-Human IgG (gamma-chain specific)-Peroxidase antibody produced in goat, Sigma-Aldrich, cat. no. A6029-1ML) was diluted 1:10000.

The results were analyzed using R. The cutoff value was determined for each experiment according to Frey *et al*. [7], where $\bar{X}$ is the mean of control readings, SD is the standard deviation, n is the number of controls, t is the 95th percentile of the one-tailed t-distribution with $v = n - 1$ degrees of freedom.

$$Cutoff = \bar{X} + SD \times f, \quad where\ f = t\sqrt{1 + \frac{1}{n}}$$

3) SPR Protocol optimization – Nucleocapsid

Establishing the optimal experimental parameters for the immobilization of SARS-CoV-2 proteins is part of the SPR assay development process for the detection of antibodies. We rapidly observed that EDC-NHS coupling of hexa-histidine tagged rN in standard conditions led to satisfactory immobilization of the nucleocapsid protein for sensitive detection of matching animal antibodies from commercial sources (Figure S2). These early tests were performed with a commercial source of hexa-histidine tagged rN (MyBiosource, cat. No. MBS569934) and antibodies from a murine source (Table S3 and S4), acting as a surrogate assay. Composition of the immobilization buffer was investigated to optimize the SPR response for antibody detection at 10 µg/mL. Acetate buffers (pH 4.4 and 5.5) and PBS (pH 6.5 and 7.4) are commonly used SPR immobilization buffers in the context of EDC-NHS chemistry. Immobilization of the rN protein led to shifts between 534 and 1240 RU (Table S3, 1 RU is approximately equal to 1 pg/mm^2). More importantly, antibody detection led to shifts of approximately 200 RU in all immobilization buffers, with the exception of PBS pH 6.5, which was significantly lower. As the acetate buffer led to the largest concentration of rN protein bound to the

surface among the better immobilization buffers for anti-rN detection, it was selected for all further studies.

A linear relationship was observed between the concentration of rN protein applied and that immobilized on the surface (Table S3). The highest response for murine anti-rN antibody detection was 226 RU, obtained upon immobilization of 10 μg/mL rN protein. Increasing the concentration of the rN protein on the surface led to a significant decrease in the detection of the antibody, suggesting that steric hindrance reduced access to the binding site on the rN protein at higher concentrations. Thus, 10 μg/mL rN protein was used for the remaining experiments.

Table S1 - Authorized medical devices for uses related to COVID-19: List of authorized testing devices by serological technology

Device Name	Manufacturer	Date Authorized	Positive percent agreement (%)
Liaison IgG S1/S2 SARS-Cov-2	Diasorin Inc. (United States)	2020-05-12	100
SARS-CoV-2 IgG Reagent kit (06R8620)	Abbott Laboratories, Division des diagnostics (United States)	2020-05-14	96.8
Elecsys Anti-SARS-CoV-2	Roche Diagnostics Gmbh (Germany)	2020-06-05	99
Vitros Immunodiagnostic Products Anti-Sars-Cov-2 Total Reagent Pack	Ortho-Clinical Diagnostics Inc. (United States)	2020-06-08	93.0
SARS-CoV-2 IgG Reagent kit (06R9030)	Abbott Laboratories Diagnostics Division (United States)	2020-06-11	100
Advia Centaur Sars-Cov-2 Total (Cov2t)	Siemens Healthcare Diagnostics Inc. (United Sates)	2020-08-14	100
Atellica Im Sars-CoV-2 Total (Cov2t)	Siemens Healthcare Diagnostics Inc. (United States)	2020-08-14	100
Dimension Exl Sars-CoV-2 Total Antibody (cv2t) assay	Siemens Healthcare Diagnostics Inc. (United States)	2020-09-08	100
Dimension Vista Sars-CoV-2 Total Antibody (cov2t)	Siemens Healthcare Diagnostics Inc. (United States)	2020-09-08	100
Anti-SARS-CoV-2 Elisa (IgG)	Euroimmun Medizinische Labordiagnostika Ag (Germany)	2020-09-25	90.7
Platelia SARS-CoV-2 Total AB	Bio-Rad (France)	2020-10-07	97.5
Anti-SARS-Cov-2 IgG Elisa Kit	Diagnostics Biochem Canada Inc. (Canada)	2020-10-26	98.6
Anti-SARS-CoV-2 Total Antibody (ab) Elisa Kit	Diagnostics Biochem Canada Inc. (Canada)	2020-12-23	98.2
Nadal COVID-19 IgG/IgM Test	Nal Von Minden Gmbh (Germany)	2021-02-05	92
Rapid Response COVID-19 IgG/IgM Rapid Test Device	Btnx Inc. (Canada)	2021-02-12	100 (IgG); 89.3 (IgM)
COVID-19 IgG/IgM Rapid Test Device	Assure Tech. (Hangzhou) Co. Ltd. (China)	2021-02-12	100 (IgG); 89.3 (IgM)

Source: https://www.canada.ca/fr/sante-canada/services/medicaments-produits-sante/covid19-industrie/instruments-medicaux/autorises/liste.html#wb-auto-4 (last accessed 25/02/2021)

Table S2 – Comparison of the common SARS-CoV-2 serological assays with expected performance[1]

Types of immunoassays	Manufacturer	Test	SARS-CoV-2 biomarkers	Targeted SARS-CoV-2 antibodies	Time to results (min)	Performance measure (%)				FDA/HC* authorizations
						Sensitivity		Specificity		
						IgM	IgG	IgM	IgG	
Chemiluminescence-immunoassay (CLIA or CMIA)	Diazyme	Diazyme DZ-Lite SARS-CoV-2 IgM or IgG CLIA kit	Spike and Nucleocapsid	IgM or IgG detection separately	n.a.	94.4	100	98.3	97.4	FDA/HC
	DiaSorin, Inc.	DiaSorin LIAISON SARS-CoV-2 IgM or S1/S2 IgG	Spike	IgM or IgG detection separately	~35	91.8	97.6	99.3	99.3	FDA/HC
	Abbott Laboratories, Inc.	SARS-CoV-2 IgM or IgG assays	Nucleocapsid (IgG) or Spike (IgM)	IgM or IgG detection separately	~30	95	100	99.6	99	FDA/HC
	Siemens Healthcare Diagnostics	Atellica IM SARS-CoV-2 Total (COV2T/COV2G)	Spike	Total antibody/IgG detection	~10	100 (Pan-IgG)/ 100 (IgG)		99.8 (Pan-Ig)/ 99.9 (IgG)		FDA/HC
	Roche	Elecsys Anti-SARS-CoV-2/S	Nucleocapsid (or Spike)	Total antibody	~18	100/96.6 (Pan-Ig)		99.8/100 (Pan-Ig)		FDA/HC
Lateral flow immunoassay (LFA)	BioMedomics	COVID-19 IgM/IgG Rapid Test	n.a.	IgM or IgG detection separately	15	100 (combined)[¥]		99 (combined)[¥]		none
	Pharmact AG	BELTEST-IT COV-2	n.a.	IgM or IgG detection separately	20	98.1[2]	98.2[2]	99.5[2]	99.7[2]	none
	Biohit Healthcare	Biohit SARS-CoV-2 IgM/IgG antibody test kit	Nucleocapsid	IgM or IgG detection separately	~20	96.7	96.7	95.0	95.0	FDA
	Cellex, Inc.	qSARS-CoV-2 IgG/IgM Rapid Test	Spike and Nucleocapsid	Both IgG and IgM	~20	93.8 (combined)		96.0 (combined)		FDA

Enzyme-linked immunosorbent assay (ELISA or ELFA)	Epitope Diagnostics	EDI™ Novel Coronavirus COVID-19 IgM or IgG ELISA /COVID-19 Nucleocapsid IgG Quantitative ELISA	n.a./ Nucleocapsid	IgM or IgG detection separately/ IgG quantification	~ 90	92.2[§] (PPA)	98.4/ 90.3[§] (PPA)	100[§]	98.3/ 98.3[§]	none
	Euroimmun	SARS-COV-2 ELISA (IgG)	Spike	IgG detection	~ 300	n.a.	90.0	n.a.	100	FDA/HC
	Bio-Rad Laboratories, Inc.	Platelia SARS-CoV-2 Total Ab	Nucleocapsid	Total antibody	~100	98.0 (Pan-Ig)		99.3 (Pan-Ig)		FDA/HC
	bioMérieux SA	VIDAS SARS-COV-2 IgM and IgG	Spike	IgM or IgG detection separately	27	100	100	99.4	99.9	FDA

n.a: not available.

[1]https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance

* FDA: U.S Food and Drug Administration; HC: Health Canada (COVID-19 Testing Device Applications Authorized by Health Canada, lab-based test). Last accessed 02/12/2020.

[¥]https://www.biomedomics.com/products/infectious-disease/covid-19-rt/

[2] https://pharmact.de/en/beltest-it-cov-2/

[§] http://www.epitopediagnostics.com/covid-19-elisa

Table S3. Optimization of the SPR conditions for the immobilization of rN protein

Buffer optimization			**Concentration optimization**		
Buffer	10 µg/mL rN shift (RU)	10 µg/mL Murine anti-rN shift (RU)	rN conc. (µg/mL)	rN shift (RU)	10 µg/mL Murine anti-rN shift (RU)
Acetate pH 4.5	689 ± 64	230 ± 23	5	263 ± 120	192 ± 22
Acetate pH 5.5	866 ± 86	226 ± 23	10	734 ± 236	226 ± 23
PBS pH 6.5	1240 ± 162	162 ± 41	20	1871 ± 128	183 ± 22
PBS pH 7.4	534 ± 144	225 ± 31	40	3111 ± 70	80 ± 30

Table S4. Optimization of the murine anti-rN surrogate source with the immobilization of rN protein in 10 mM acetate pH 4.5

Source	*Cat. no.*	*Lot*	*rN conc. (μg/mL)*	*Murine anti-rN shift (RU)*
MyBioSource	MBS569904	A0562	10	60 ± 14
SinoBiological	HD14MA2713	40588	1:500 *	241 ± 3
MyBiosource	MBS569905	A1335	10	395 ± 79
MyBioSource	MBS569903	A1336	10	983 ± 45

* No concentration provided, a dilution ratio of 1:500 was tested

Table S5. SPR data for the immobilization of various spike protein and domains in 10 mM acetate pH 4.5

	Immobilization (RU)	**Anti-rS Detection (RU)**	**IgG secondary detection (RU)**
S1	533 ± 123	139 ± 33 *	N/A
RBD	1592 ± 222	1036 ± 96 **	1859 ± 96 ***
Spike	1785 ± 190	161 ± 15 **	976 ± 76 ***

* For 25 μg/mL rabbit anti-Spike pAb (Beta Lifescience, cat no. BLSN-005P)

** For 1:125 dilution rabbit Anti-Spike MAb (IgG) (Sino Biologicals, cat no. 40150-R007)

*** 10 μg/mL goat Anti-Rabbit IgG (H+L) (Jackson Immunoresearch, cat no. 111-005-003)

N/A: data not available

Table S6. Optimization of immobilization concentration of the spike protein ectodomain from CHO cells on the SPR surface in 10 mM acetate buffer pH 4.5

Concentration (µg/mL)	***Immobilization of rS (RU)***	***Detection of rabbit anti-rS at 1:500 dilution (RU)***
5	260 ± 23	4 ± 18
10	1146 ± 279	168 ± 35
20	2427 ± 208	402 ± 14
30	3542 ± 53	429 ± 10

Table S7. ELISA measurement of human anti-rN and human anti-rS in clinical sera of COVID-19 positive and negative individuals (PCR tested)

	Sample ID	*Anti-rN (OD)*	*Anti-rS (OD)*
Positives	4907	1.470	2.499
	4911	0.253	1.264
	5905	1.029	0.874
	6902	0.984	2.211
	7001	1.261	2.440
Negatives	C002	0.143	0.201
	C005	0.250	0.185
	C007	0.130	0.152
	C008	0.236	0.318

	C009	0.280	0.369

Table S8. Compilation of the DBS and plasma panel of positive and negative blood donors. Assays are for antibodies against spike (SPR and ELISA) or S1 (EuroImmun). Secondary detection with anti-human IgG is reported for SPR.

		DBS			**PLASMA**		
SAMPLE ID	Test result	EuroImmun S/CO	SPR (RU)	ELISA OD	EuroImmun S/CO	SPR (RU)	ELISA OD
5	Negative	0.46	-149	0.174	0.40	165	1.341
6	Negative	0.07	-137	0.100	0.04	-28	0.152
7	Negative	0.10	-163	0.136	0.08	-60	0.634
9	Negative	0.38	-37	0.121	0.50	38	0.278
13	Negative	0.08	17	0.115	0.04	88	0.344
14	Negative	0.16	44	0.113	0.14	-82	0.829
16	Negative	0.13	26	0.125	0.11	158	0.758
17	Negative	0.17	-39	0.102	0.13	193	0.412
8	Positive	3.45	238	1.002	3.20	645	3.160
10	Positive	2.90	151	0.974	3.20	2204	3.273
11	Positive	3.60	158	1.224	4.00	2573	3.570
12	Positive	6.97	819	2.260	6.49	3873	3.430
15	Positive	2.05	208	0.345	3.59	1172	2.794
18	Positive	2.29	144	0.499	2.24	1500	3.559
19	Positive	4.90	395	0.956	4.80	2378	3.573
20	Positive	4.35	268	0.839	5.18	2625	3.253

Table S9. Compilation of the DBS and plasma panel of positive and negative blood donors. All assays are for antibodies against nucleocapsid. Secondary detection with anti-human IgG is reported for SPR.

		DBS		**PLASMA**	
SAMPLE ID	Test result	SPR (RU)	ELISA OD	SPR (RU)	ELISA OD
5	Negative	56	0.208	57	0.156
6	Negative	35	0.116	-28	0.139
7	Negative	62	0.179	-16	0.202
9	Negative	-7	0.130	-38	0.128
13	Negative	-23	0.141	-28	0.196
14	Negative	-10	0.189	-7	0.346
16	Negative	42	0.151	1	0.174
17	Negative	-151	0.180	-5	0.209
8	Positive	144	0.758	183	1.467
10	Positive	80	0.387	169	1.245
11	Positive	98	0.812	200	1.756
12	Positive	257	1.466	718	2.069
15	Positive	80	0.498	273	1.596
18	Positive	153	0.294	272	1.379
19	Positive	313	1.070	523	1.722
20	Positive	274	1.921	360	1.732

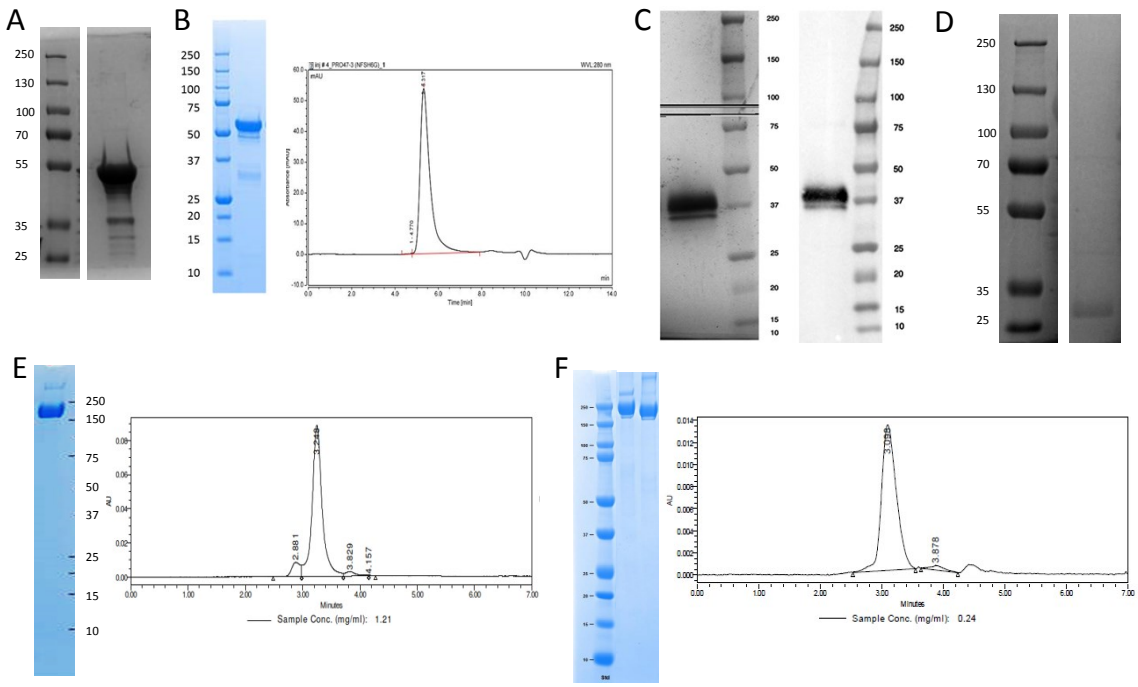

Figure S1. (**A**) Hexa-his-tagged nucleocapsid expressed in *E. coli* characterized on Coomassie blue-stained SDS-PAGE (Lemay and coworkers). (B) Hexa-His-tagged nucleocapsid expressed in CHO cells characterized on non-reducing Coomassie blue-stained SDS-PAGE (left panel) and analytical size-exclusion high-performance liquid chromatography (SEC-HPLC; right panel) (Durocher and coworkers). (**C**) RBD expressed in HEK293SF suspension cells, characterized on silver-stained SDS-PAGE (left panel) and by Western blotting (right panel) (Kamen and coworkers). (**D**) RBD expressed in Pichia cells characterized on Coomassie blue-stained SDS-PAGE (Lemay and coworkers). (**E**) His-tagged Spike ectodomain expressed in CHO cells characterized on non-reducing Coomassie blue SDS-PAGE (left panel) and analytical size-exclusion high-performance liquid chromatography (SEC-UPLC; right panel) (Durocher and coworkers). (**F**) His-tagged point-mutated variant of the B.1.1.7 variant of concern Spike ectodomain expressed in CHO cells characterized on reducing (r.) and non-reducing (n.-r.) Coomassie blue SDS-PAGE (left panel) and analytical size-exclusion high performance liquid chromatography (SEC-UPLC; right panel) (Durocher and coworkers).

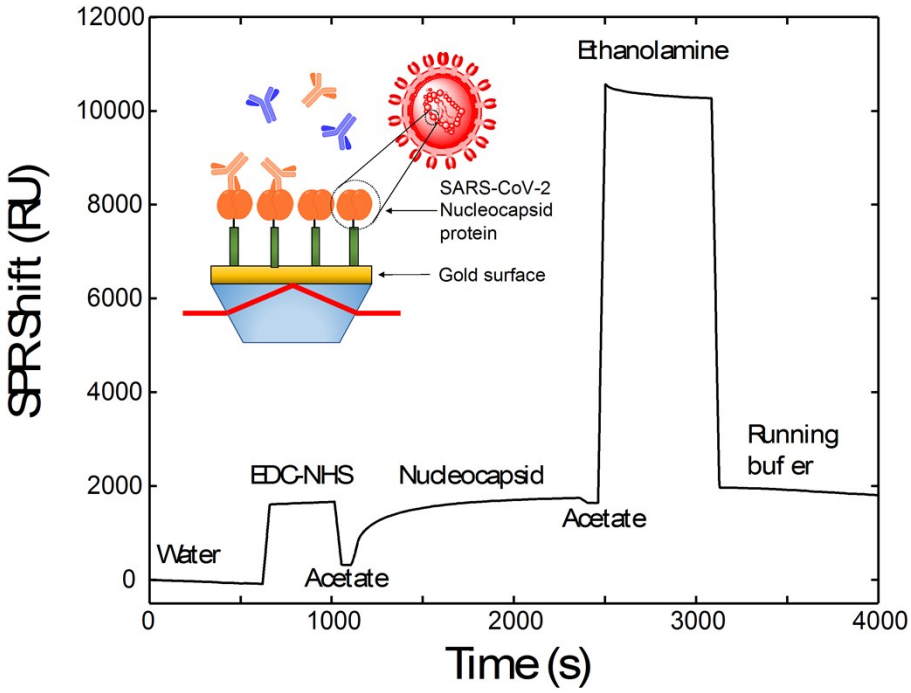

Figure S2. SPR sensorgram of the surface functionalization with rN. After EDC-NHS activation of the AffiCoat surface, the rN protein of SARC-CoV-2 was bound to the surface of the SPR chip and remaining activated sites were passivated with ethanolamine.

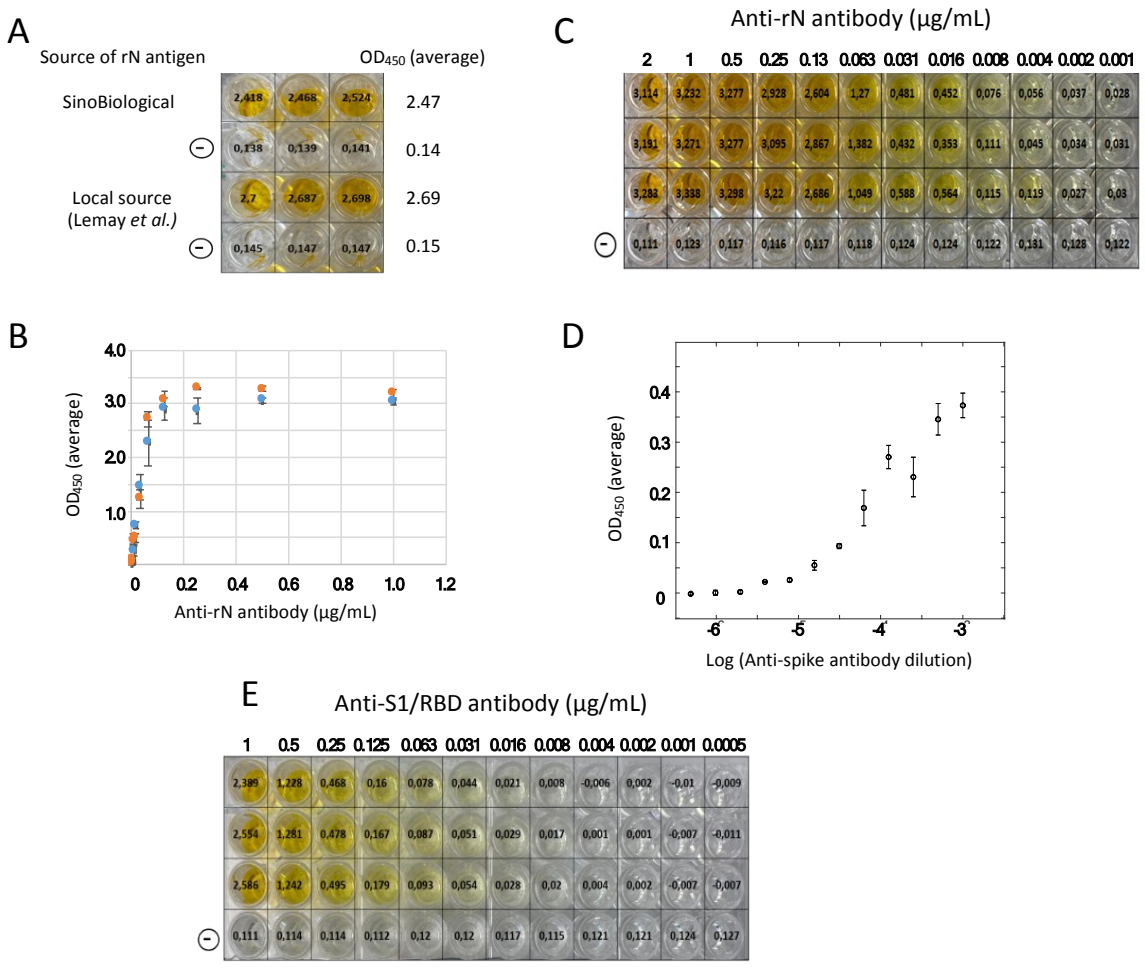

Figure S3. Development of the ELISA assays. In all panels, assays were performed in triplicate. (**A)** ELISA assay of hexa-His-tagged rN antigen expressed in *E. coli*. Top: rN from SinoBiological, cat. no. 40588-V08B. Bottom: locally-sourced rN (Lemay and coworkers). The ELISA assay used 2 µg/mL of either rN antigen, 1 µg/mL murine anti-rN (MyBioSource MBS569905) and a 1:50,000 dilution of Abcam goat anti-mouse IgG Fc. The OD_{450} read-out value is shown on each well, where the average of the negative controls (no anti-rN, raw values shown) has been subtracted. (**B**) Comparison of two lot numbers of a murine anti-rN primary antibody in ELISA. The assay used 2.5 µg/mL of locally-sourced rN antigen (Lemay and coworkers), a serial dilution of murine anti-rN primary antibody from MyBioSource (blue dots: lot MBS569903; orange dots: lot MBS569905), as indicated, and a 1:50,000 dilution of Abcam goat anti-mouse IgG Fc. The average value is plotted, where the average of the negative controls (no anti-rN, n=12) has been subtracted. (**C**) Limit of detection (LOD) for anti-rN antibody by ELISA

assay. The assay used 2.5 µg/mL of locally-sourced rN) antigen (Lemay and coworkers), a serial dilution of murine anti-rN primary antibody from MyBioSource (MBS569905) as indicated and a 1:50,000 dilution of Abcam goat anti-mouse IgG Fc. The OD_{450} read-out value is shown on each well, where the average of the negative controls (no anti-rN, raw values shown) has been subtracted. The LOD under these conditions was 0.016 µg/mL. (**D**) ELISA assay of hexa-His-tagged RBD antigen expressed in HEK cells. The assay used 2.5 µg/mL of the locally-sourced RBD (Kamen and coworkers), a serial dilution of rabbit anti-spike primary antibody from SinoBiological (40150-R007), as indicated, and a 1:50,000 dilution of GeneTex anti-rabbit IgG Fc. The average value is plotted, where the average of the negative controls (no anti-rN, n=12) has been subtracted. (**E**) Limit of detection (LOD) for anti-S1/RBD antibody by ELISA assay. The assay used 2.5 µg/mL of locally-sourced spike ectodomain antigen (spike SmT1, Durocher and coworkers), a serial dilution of rabbit anti-S1/RBD primary antibody from Raybiotech (130-10759), as indicated, and a 1:50,000 dilution of Abcam goat anti-rabbit IgG Fc. The OD_{450} read-out value is shown on each well, where the average of the negative controls (no anti-S1/RBD, raw values shown) has been subtracted. The LOD under these conditions was 0.125 µg/mL.

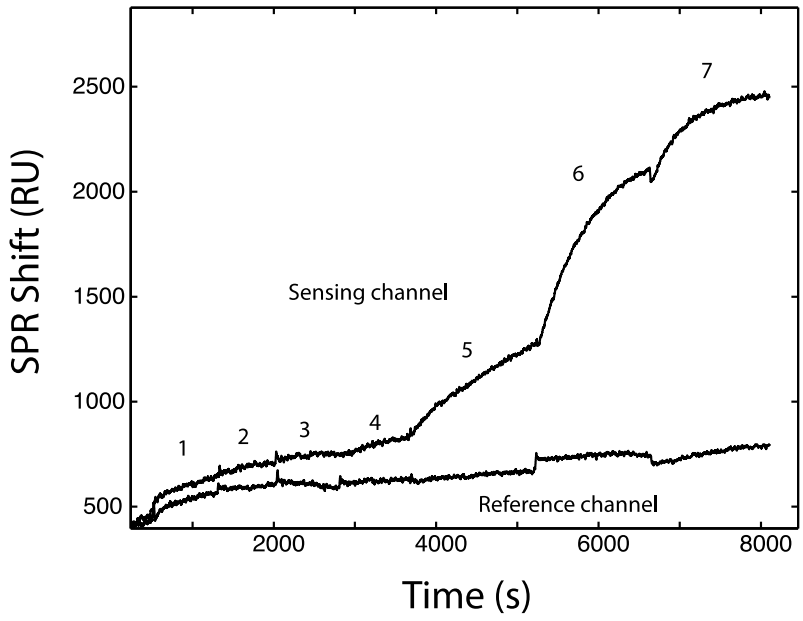

Figure S4. SPR sensorgram for the detection of SARS-CoV-2 anti-rN murine surrogate antibody in human serum. Concentrations of murine anti-rN: (1) 100 ng/mL, (2) 500 ng/mL, (3) 1 µg/mL, (4) 5 µg/mL, (5) 10 µg/mL, (6) 25 µg/mL, and (7) 75 µg/mL. The reference channel confirms that nonspecific adsorption is minimal.

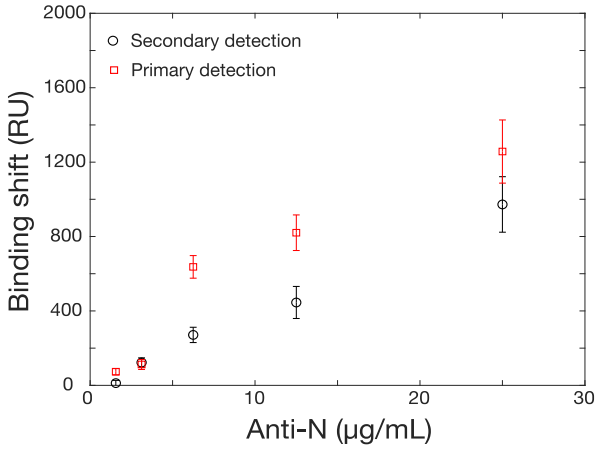

Figure S5. Comparison of the primary and secondary detection step for a secondary anti-mouse IgG coupled to HRP. The magnitude of the SPR response and the gain is lower than for an anti-IgG without HRP when compared to the response in Figure 2(right).

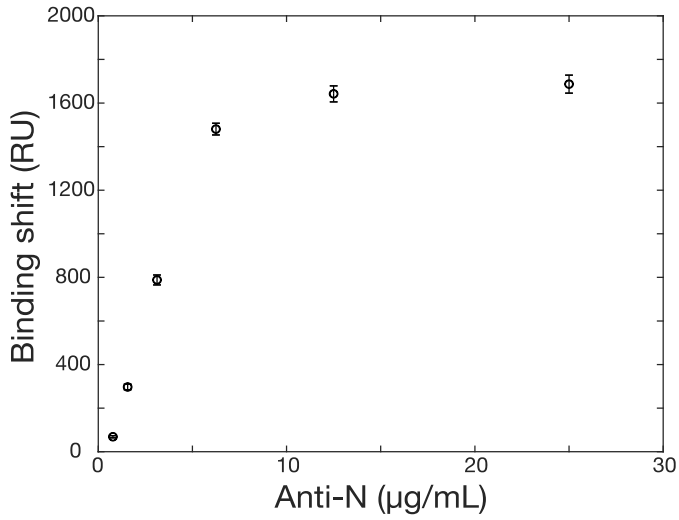

Figure S6. Calibration curve for the humanized anti-rN with rN (with the hexa-His tag). The strong response in SPR is indicative of interaction between the humanized anti-rN and rN.

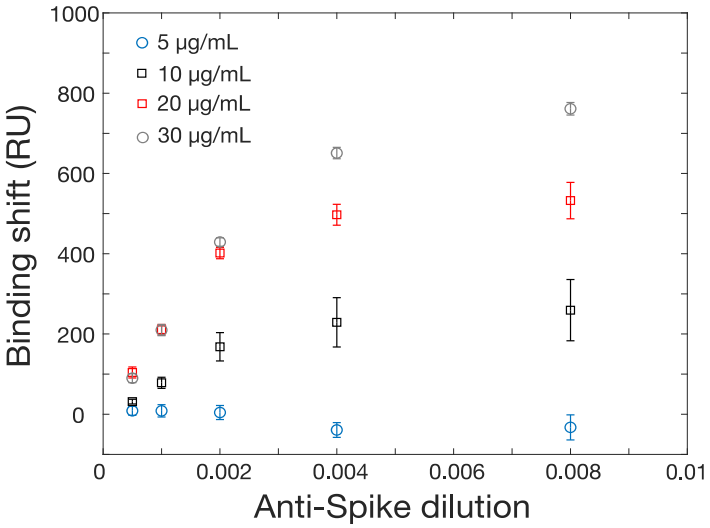

Figure S7. Calibration curves for different rS concentrations for the immobilization on the SPR chip. SPR detection for rabbit anti-rS is reported in the x-axis.

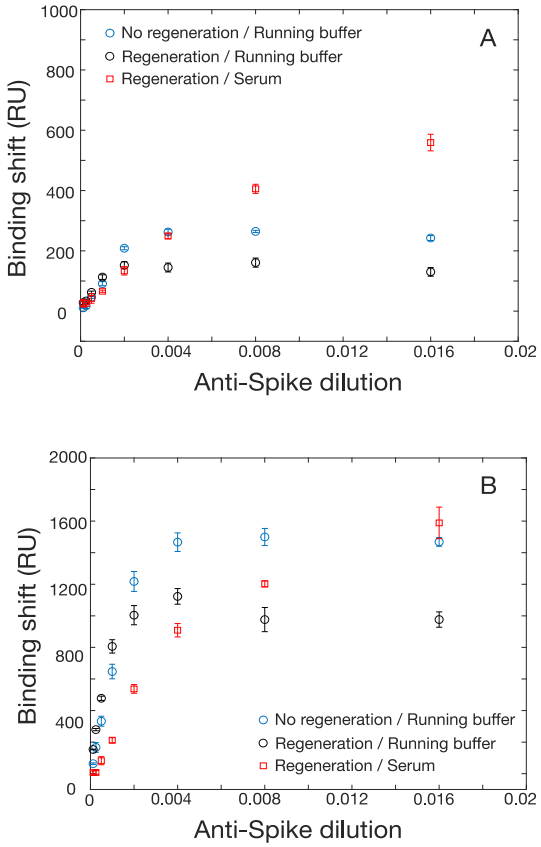

Figure S8. Comparison of the calibration curve for rabbit anti-rS without (blue) and with (black) regeneration of the surface with glycine between concentrations for the direct (panel A) and secondary (panel B) detection. Detection is also shown in serum for both panels.

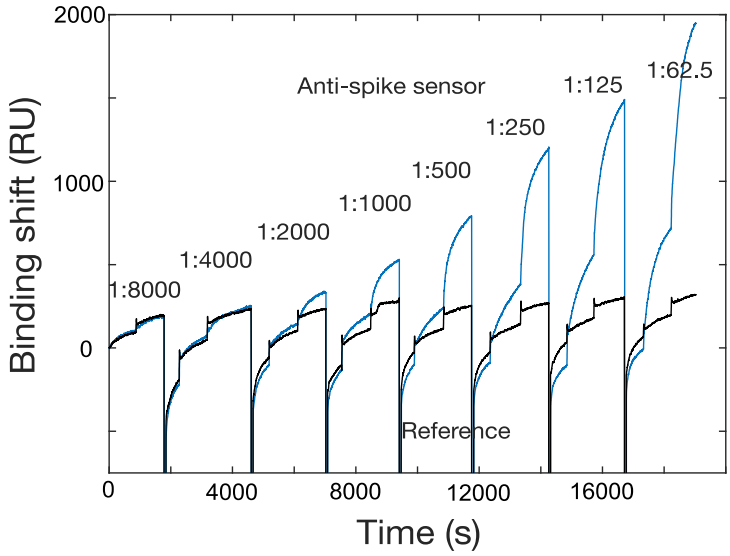

Figure S9. Regeneration of the SPR sensor for rabbit anti-spike between calibration points in undiluted serum. Anti-spike dilution factors are provided in the figure. All detection steps consisted of baseline stabilization in serum, injection of the serum sample and secondary detection with 10 µg/mL anti-rabbit IgG. The large refractive index different during the glycine regeneration steps caused the massive spike in response at negative RUs. A blank serum was used in the reference channel and measured in otherwise identical conditions.

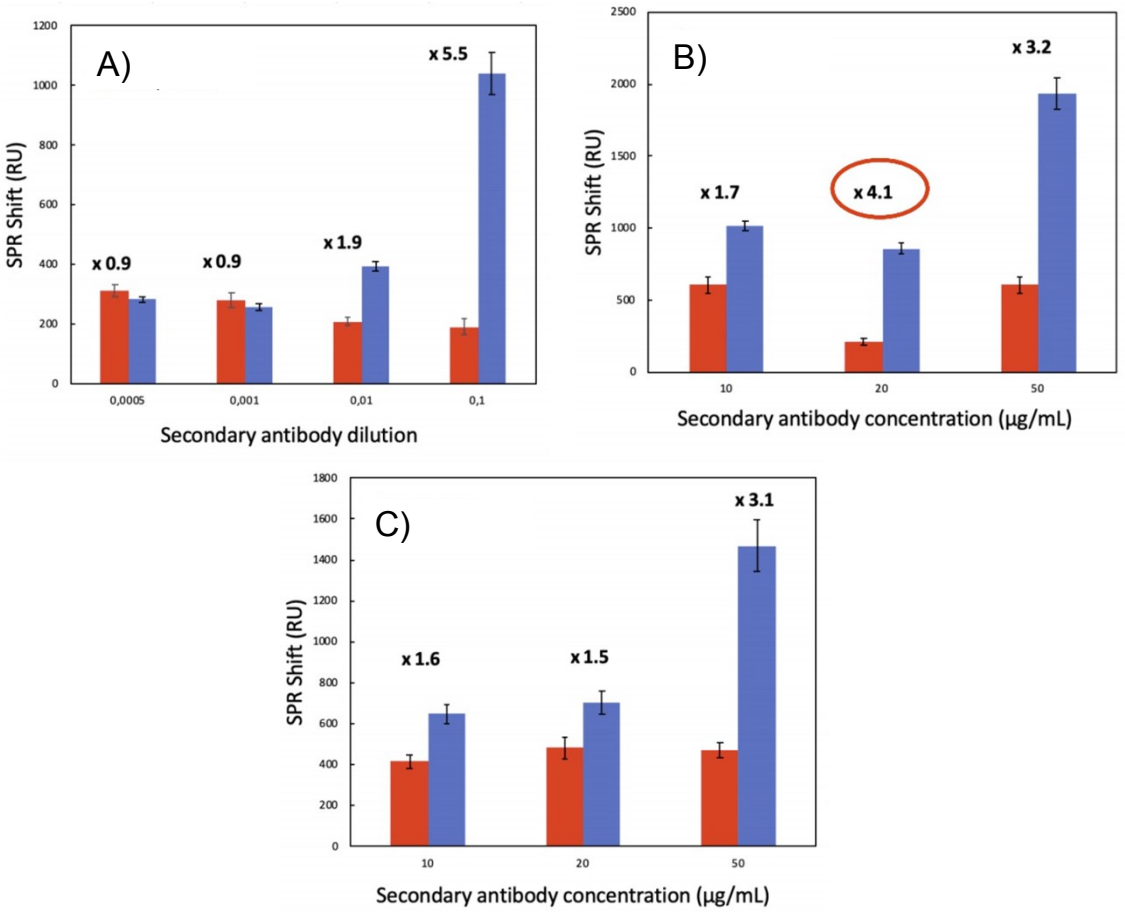

Figure S10. Test for three different sources of secondary antibodies at different dilutions for the detection of human anti-spike in 10% human serum, panel A) shows data for anti-human IgG (gamma-chain specific) coupled to peroxidase (source is Sigma-Aldrich cat no. A6029), panel B) shows the results for a goat polyclonal antibody to human IgG coupled with HRP (Abcam cat no. ab209702) and panel C) has data for an AffiniPure goat anti-human IgG (H+L) (Jackson Immunoresearch cat no. 109-005-003). The ratio of secondary to primary detection is used to determine the best amplification signal, which was observed for the Abcam secondary antibody at a concentration of 20 µg/mL.

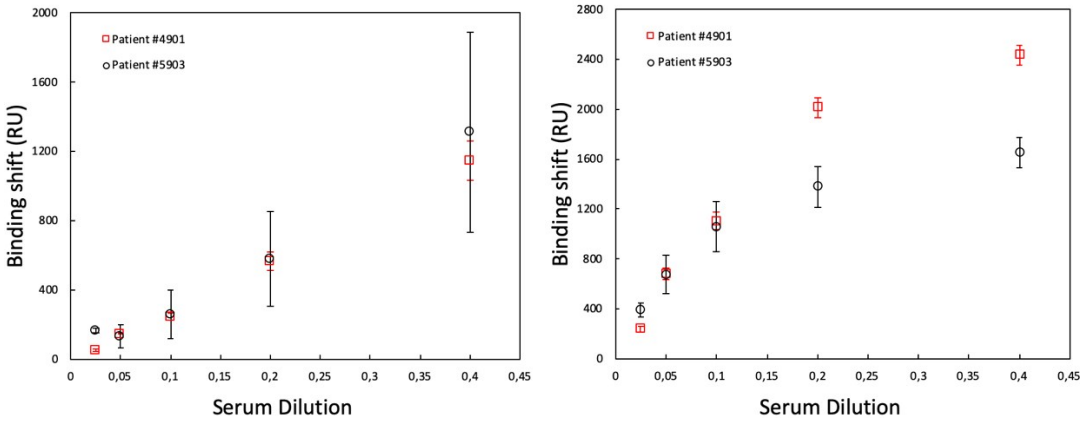

Figure S11. SPR response for different serum dilutions with direct detection (left) and secondary detection (right). The response was optimal at a dilution of 1:5 (0.2) in secondary detection.

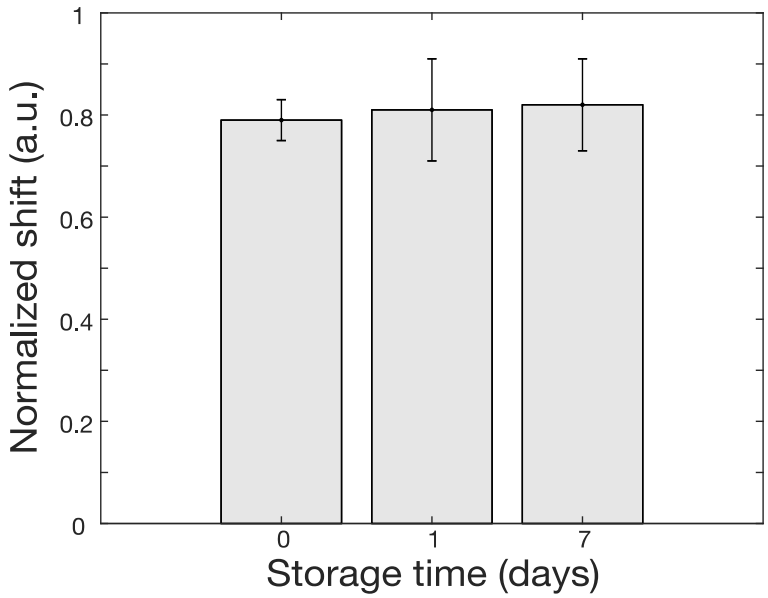

Figure S12. Normalized SPR response for anti-rN sensors stored at -20°C for periods of time up to 7 days. Data represent a triplicate measurement on independently prepared sensors. Normalization was performed to account for slight changes in immobilization of rN from chip to chip.

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