Supporting Information

LIPS method for the detection of SARS-CoV-2 antibodies to spike and nucleocapsid proteins

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Methods and materials

SARS-CoV-2 (NCBI Acc # NC_045512.2) two S (S1 aa 1-680 and S2 aa 820-1211) and N (aa 2-419) gene fragments were cloned into in-house modified pNanoLuc vector, the full sequences of the vector and cloned inserts are given below. After the transfection into HEK293 cells, the cell lysates containing NanoLuc-fusion proteins were collected at 72 h and stored at -20°C.

For Western blot, the cell lysates containing NanoLuc-fusion proteins were loaded to a 10% SDS-polyacrylamide gel and subjected to gel electrophoresis (1 h at 50 V and 2 h at 100 V). Proteins were then electrotransferred to a PVDF membrane (Millipore, 0,45 μm pore diameter) for 15 min at 10 V and 1 hour at 15 V at room temperature. Non-specific binding was blocked by incubation with 5% non-fat milk in TBST (Tris-buffered saline containing 0,1% Tween-20) for 1 h at room temperature, followed by an overnight incubation at 4 °C with primary antibodies diluted in 5% non-fat milk in TBST. The following primary antibodies were used: anti-Spike1 (diluted 1:2000, GeneTex), anti-NanoLuc (diluted 1:500, Promega). The membranes were washed three times with TBST, followed by an 1 hour incubation at room temperature with HRP-labeled secondary antibodies (Jackson ImmunoResearch) diluted in 5% non-fat milk in TBST. The chemiluminescent signal was produced using the Amersham ECL Reagents (GE Healthcare) and later detected using ImageQuant™ RT ECL™ Imager (GE Healthcare).

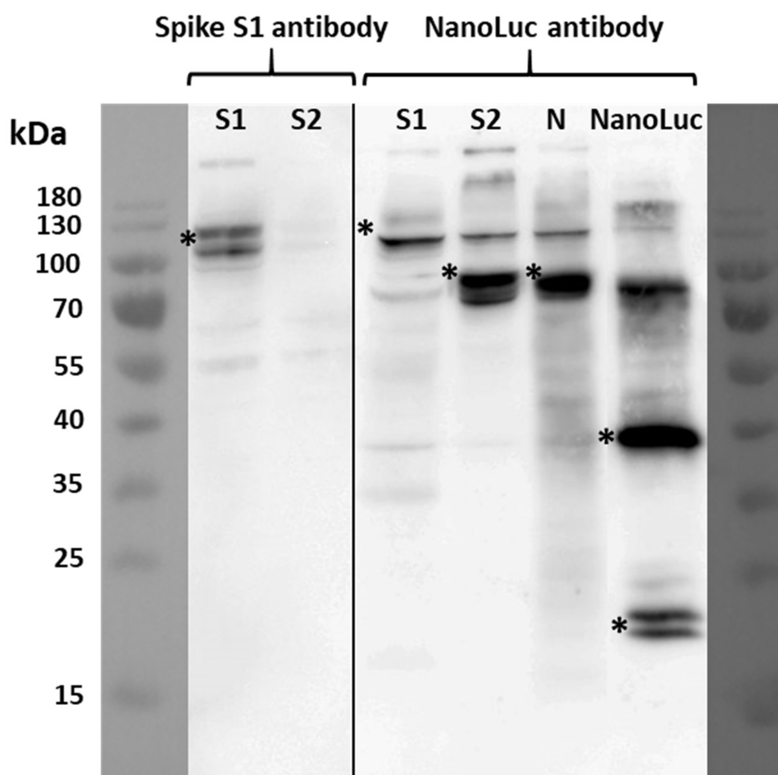
Plasma samples were obtained from 26 COVID-19 patients (age range 33-91 years) hospitalized at the Tartu University Hospital, Estonia. The diagnosis were confirmed by PCR analysis for SARS-CoV-2 virus. In addition, we studied 26 healthy controls (age range 23-54 years) without recent infection or COVID-19 symptoms (fever or cough) for last month. The study was conducted in accordance with the Helsinki Declaration, with the approval from the Ethics Committee of the University of Tartu.

The plasma samples were incubated with lysates containing S1, S2 and N fusion protein solutions (0.5 - 1 × 10^6 luminescence units; LU) for 1 h at RT. The Protein G Sepharose beads (25 μl of 4% suspension, Creative BioMart) were added and incubated at room temperature for 1 h in 96-well microfilter plates (Merck Millipore) to capture antibodies (in 1:40 dilution) and immune complexes to the beads. After the washing to remove unbound fusion proteins, luciferase substrate was added (Nano-Glo™ Luciferase Assay Substrate, Promega), and luminescence was measured in VICTOR X Multilabel Plate Reader (PerkinElmer Life Sciences). Results are expressed as fold changes (FC) of luminescence units (LU) (FC=LU sample/average LU of 5 healthy control samples). The positive/negative discrimination level was set to the mean plus 2 standard deviations of the healthy control samples. The experiments were performed three times in three experimental replicates. Statistics was performed using unpaired Student's t-test and Pearson correlation analysis in Graphpad Prism.

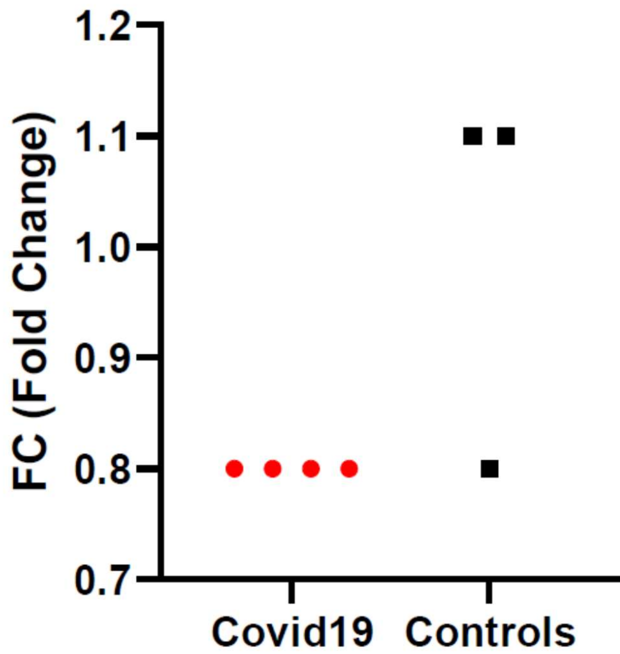
The Anti-SARS-CoV-2 IgG ELISA (Euroimmun Medizinische Labordiagnostika; Cat # EI 2668-9601 G) was performed according to the manufacturer's instructions. In semi-quantitative ELISA, IgG antibodies against SARS-CoV-2 S1 protein subunit S1 were detected. Briefly, 1:101 diluted plasma samples were added to wells coated with recombinant SARS-CoV-2 antigen and incubated for 60 minutes at 37 °C. Wells were washed three times followed by the addition of HRP-conjugated anti-human IgG and subsequent incubation for 30 minutes at 37 °C. Wells were washed three times again and a chromogen solution was added. Following 30 minutes of incubation at room temperature, the reaction was stopped and the resultant absorbance was read on a microplate reader at 450 nm with reference at 620 nm. A ratio between the extinction of the sample and calibrator on plate was calculated. According to the manufacturer's recommendations, a ratio <0.8 is considered negative, ≥0.8 and <1.1 borderline, and ≥1.1 positive.

Supporting Table 1. Main characteristics, LIPS and ELISA results of each studied patient. Patient sex, age and plasma sampling day, SARS-CoV-2 antibody values in LIPS with three antigens and EUROIMMUNE ELISA assay optical density (OD) values and its ratios to controls are given.

				LIPS			ELISA	
Patient nr	**Sex (M/F)**	**Age (years)**	**Sample day**	**SP1**	**SP2**	**N**	**OD**	**Ratio**
Pat1	M	33	11	0.8	1	3.7	0.4	1.7
Pat2	F	50	14	2.4	6.7	9.1	2.4	11.6
Pat3	M	55	12	5.1	3.9	8.4	3.4	16.2
Pat4	M	56	21	6.5	4.5	7.9	3.0	14.5
Pat5	F	58	26	11	9.3	7.7	3.4	16.6
Pat6	M	60	17	4.7	8	9	3.0	14.7
Pat7	M	61	14	2	3.3	5.2	1.0	4.8
Pat8	M	63	29	1.4	15.3	7.8	2.0	9.5
Pat9	M	70	16	3.9	7.4	10.2	2.8	13.4
Pat10	M	73	18	1.3	2.7	5.2	1.1	5.4
Pat11	M	83	20	4	2.6	7.2	3.0	14.5
Pat12	F	63	15	2.3	2.7	3.8	1.9	9.4
Pat13	F	83	17	1.5	1.2	2.7	2.4	11.5
Pat14	M	73	22	3.2	1.9	4.6	3.2	15.3
Pat15	M	57	22	2.4	1	3.4	2.9	14.2
Pat16	M	58	21	2.9	2.1	4.2	2.9	13.8
Pat17	M	63	37	2	5.1	4.9	2.1	10.2
Pat18	F	62	14	1.6	2.1	3.3	2.6	12.6
Pat19	F	62	14	2.4	2.2	5.7	2.9	13.8
Pat20	M	72	13	1.3	2.2	2.7	1.0	4.8
Pat21	F	91	14	1.1	5.6	4.4	0.8	4.1
Pat22	M	65	16	4.1	5.7	5.7	3.4	16.6
Pat23	F	55	25	2.3	1.9	5.1	2.8	13.7
Pat24	M	90	8	1.9	4.6	3.4	2.6	12.8
Pat25	M	60	15	1.5	1.6	3.5	2.1	10.2
Pat26	M	42	15	2.4	2.5	3.2	3.1	14.7

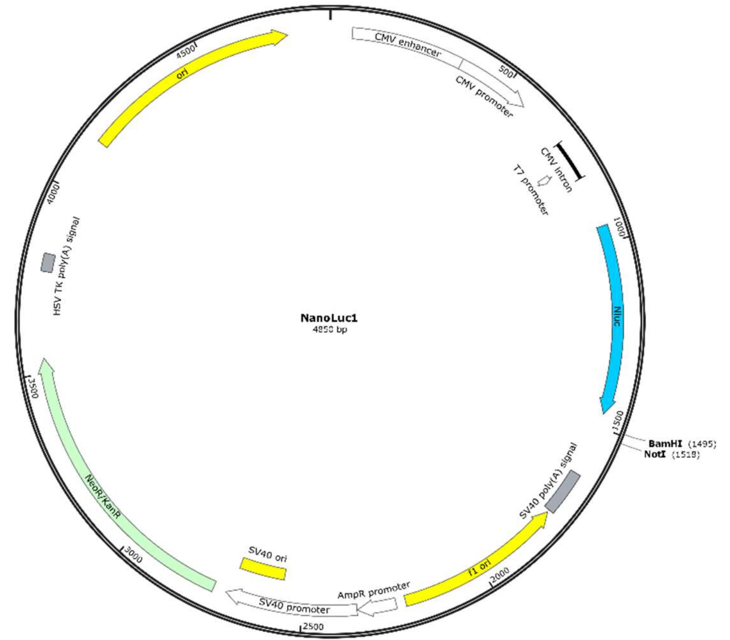


Supporting Figure 1. Western blot for the detection of NanoLuc-S1, NanoLuc-S2 and NanoLuc-N fusion proteins. The cell lysates were run a single 10% polyacrylamide gel, transferred to PVDF membrane, which was cut into two after the transfer. Left side: transfected HEK293 cell lysates of NanoLuc-S1 (S1) and NanoLuc-S2 (S2) were probed with anti-Spike1 antibody (diluted 1:2000, GeneTex). Right side: transfected HEK293 cell lysates of NanoLuc-S1, NanoLuc-S2, NanoLuc-N (N) and NanoLuc were probed with anti-NanoLuc antibody (diluted 1:500, Promega). The membranes were further incubated with HRP-labeled anti-rabbit (for S1) and anti-mouse (for NanoLuc) (both from Jackson ImmunoResearch). The detected proteins are shown by asterisks. The molecular marker lanes are shown on both sides of the membrane. The anti-S1 antibody (on left side) detects NanoLuc-S1 protein as a double band at approximately 110-120 kDa. The anti-NanoLuc antibody detects NanoLuc-S1 protein at the same location albeit weaker than seen with anti-S1 antibody. NanoLuc-S2 and NanoLuc-N are seen as strong bands at 75-80kDa. NanoLuc is seen at 20kDa but also at 38-40kDa. The predicted molecular mass of the proteins without post-translational modifications are 100kDa (NanoLuc-S1), 62kDa (NanoLuc-S2) and 59kDa (NanoLuc-N).



Supporting Figure 2. Empty NanoLuc vector without SARS-CoV-2 antigens was assayed with 4 Covid-19 and 3 control plasma samples. The NanoLuc (Promega) gene was expressed in HEK293 cells. The cell lysates were incubated with plasma samples (in 1:40 dilution) and bound to Protein G Sepharose to capture antibody complexes with viral proteins. After the washing, luciferase substrate Nano-Glo™ (Promega) was added and luminescence was measured in VICTOR X multilabel reader (PerkinElmer Life Sciences). Results are expressed as fold changes (FC) of luminescence units (LU) (FC=LU sample/average LU of 3 control samples). The luminescence values were in low range in LIPS assay and similar in Covid-19 and control individuals.

pNanoLuc1 vector

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Spike 1 gene fragment with BamHI and NotI restriction enzyme sites

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Nucleocapsid gene fragment with BamHI and NotI restriction enzyme sites

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//
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