SUPPORTING INFORMATION

Aptamer sandwich assay for the detection of SARS-CoV-2 spike protein antigen

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Table S1. Recombinant proteins with C-terminal His-tag used in this work.

Human coronavirus	Protein	Protein information	Catalog number
SARS-CoV-2	S1	Val16-Arg685, 76.5 kDa	40591-V08B1
	RBD	Arg319-Phe541, 26.5 kDa	40592-V08H
SARS-CoV	S1	Met1-Arg667, 74.4 kDa	40150-V08B1
MERS-CoV	S1	Met1-Glu725, 79.9 kDa	40069-V08B1
HCoV-NL63	S1	Cys19-Val717, 78.8 kDa	40600-V08H
HCoV-HKU1	S1	Ala13-Arg756, 85.2 kDa	40602-V08H
HCoV-OC43	S1	Met1-Leu794, 89.0 kDa	40607-V08H1
HCoV-229E	S1	Cys16-Asn536, 58.3 kDa	40601-V08H

Table S2. Aptamers used in this work with 5'-biotin or 5'-thiol-C6 modifications.

Aptamer	**Sequence (5' – 3')**	**Length (nt)**	**% GC**
Apt1	ATCCAGAGTGACGCAGCACCGACCTTGTGCTTTGGGAGTGCTGG TCCAAGGGCGTTAATGGACACGGTGGCTTAGT	76	56.6
Apt2	ATCCAGAGTGACGCAGCATCGAGTGGTGGGCTGGTCGGGTTTGG ATTCCCTTAGATGCTGGACACGGTGGCTTAGT	76	56.6
Apt3	ATCCAGAGTGACGCAGCACTGCGTAGGCGCGGCCAATGTGTAGG ATTGCTCAGGTCTGCTGGACACGGTGGCTTAGT	77	58.4
Apt4	ATCCAGAGTGACGCAGCATTTCATCGGGTCCAAAAGGGGCTGCT CGGGATTGCGGATATGGACACGGTGGCTTAGT	76	55.3
Apt5	ATCCAGAGTGACGCAGCAGGACTGCTTAGGATTGCGAAGCTGAG GAGCTCCCCCGCCTTGGACACGGTGGCTTAGT	76	59.2
Apt6	ATCCAGAGTGACGCAGCAGTAGGGGGATTGGCTCCAGGGCCTGG CTGACGGTTGCACGTGGACACGGTGGCTTAGT	76	61.8
Apt1C	CAGCACCGACCTTGTGCTTTGGGAGTGCTGGTCCAAGGGCGTTA ATGGACA	51	56.9
Apt4C	ATCCAGAGTGACGCAGCATTTCATCGGGTCCAAAAGGGGCTGCT CGGGATTGCGGATATGGACACGT	67	55.2

Table S3. Biosensors and assays developed for the detection of the SARS-CoV-2 S antigen.

Platform	**Biorecognition molecule**	**Target**	**Sensitivity (LOD)**	**Reference**
Lateral flow assay	Sialic acids – sialic acids/gold nanoparticles sandwich	S1	5 µg/mL in buffer	1. Baker et al., ACS Centr. Sci. 2020, 6, 2046-2052
Field-effect transistor biosensor	Antibody immobilized on graphene	S1	1 fg/mL in buffer 100 fg/mL in VTM	2. Seo et al., ACS Nano 2020, 14, 5135-5142
Colorimetric assay	Antibody immobilized on gold nanoparticles	S	1 ng/mL in buffer (visual)	3. Pramanik et al., Nanoscale Adv. 2021, 3, 1588-1596
SERS assay			4 pg/mL in buffer	
Plasmonic biosensor	Antibody immobilized on gold nanoparticles	S1	4.2 fM in buffer	4. Ahmadivand et al., Biosens. Bioelectron. 2021, 117, 112971
Magnetic particle spectroscopy assay	Antibody immobilized on magnetic nanoparticles	RBD	84 pM in buffer	5. Zhong et al., ACS Sens. 2021, 6, 976-984.
Lateral flow assay	ACE2 – antibody/gold nanoparticles conjugate sandwich	S1	50 ng/mL in buffer	6. Lee et al., Biosens. Bioelectron. 2021, 171, 112715
Near-Infrared biosensor	ACE2 immobilized on single-walled carbon nanotubes	RBD	12.6 nM in buffer	7. Pinals et al., Nano Lett. 2021, 21, 2272-2280
Direct colorimetric assay	Biotinylated aptamer	S	2 nM in buffer	8. Gupta et al., Mol. Ther. Nucleic Acids 2021, 26, 321-332
Electrochemical aptasensor	Aptamer with methylene blue label	S1	35.4 pM in buffer	9. Idili et al., ACS Sens. 2021, 6, 3093-3101
Plasmonic aptasensor	Biotinylated aptamer	S1+S2	37 nM in buffer	10. Cennamo et al., Talanta 2021, 122532
Electrochemical aptasensor	Aptamer with methylene blue label	S1	1 ag/mL in buffer	11. Zakashansky et al., Anal. Methods 2021, 13, 874
Gold nanoparticles colorimetric assay	Thiolated aptamer	S1	16 nM in buffer	12. Aithal et al., Talanta 2022, 122841
Microplate assay	Aptamers sandwich	S1	21 ng/mL (270 pM) in buffer 15 ng/mL (190 pM) in VTM	This work

ACE2: angiotensin-converting enzyme II; S1: S1 subunit of SARS-CoV-2 spike protein; S2: S2 subunit of SARS-CoV-2; RBD: receptor-binding domain of SARS-CoV-2 spike protein; S: SARS-CoV-2 spike protein; SERS: surface enhanced Raman spectroscopy; VTM: viral transport medium.

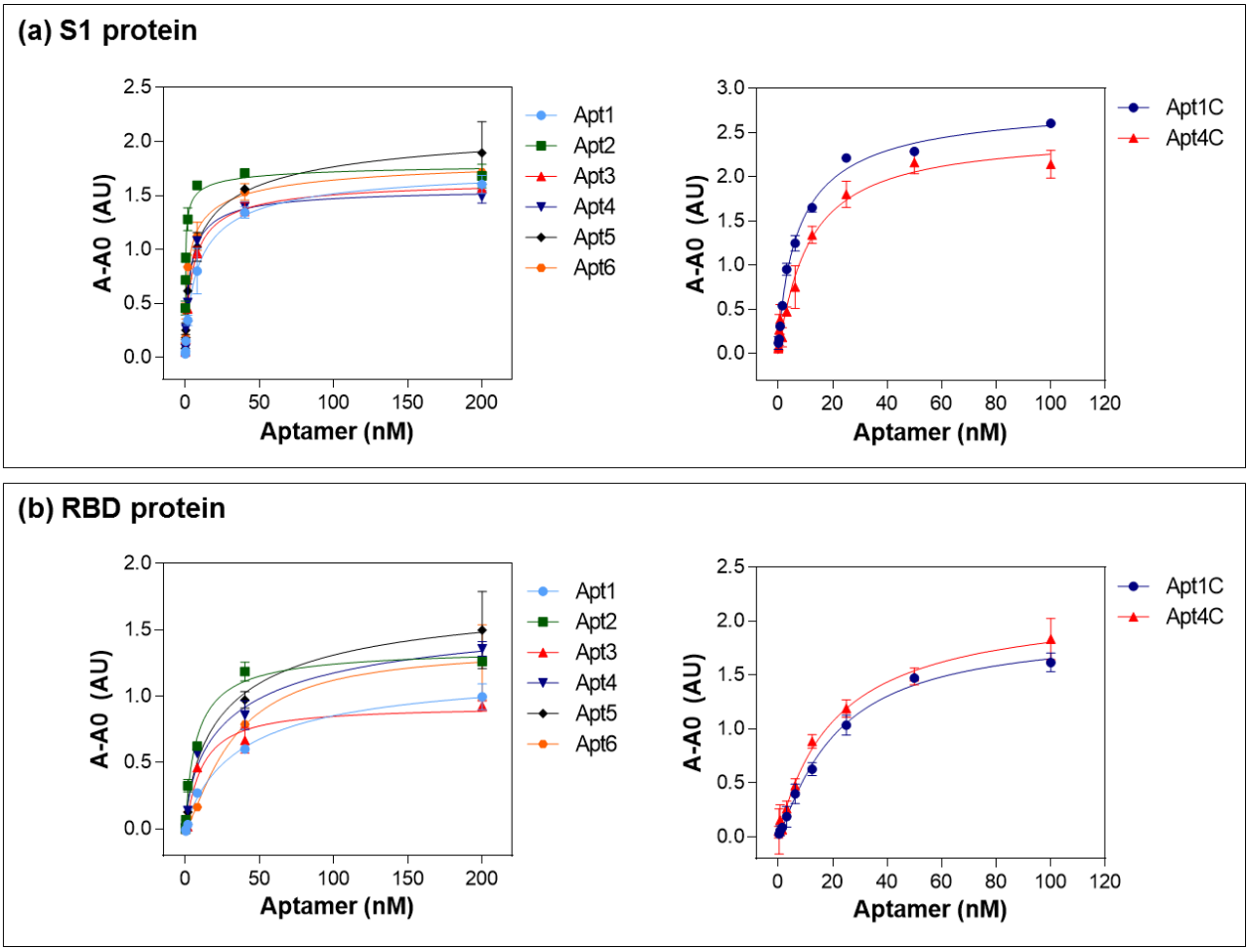


Figure S1. Binding studies of aptamers using (a) S1 and (b) RBD proteins of SARS-CoV-2 by direct Enzyme Linked Aptamer Assay (ELAA). Biotinylated aptamers (12.8 pM - 200 nM) were used in combination with constant concentrations (2 µg/mL) of the proteins.

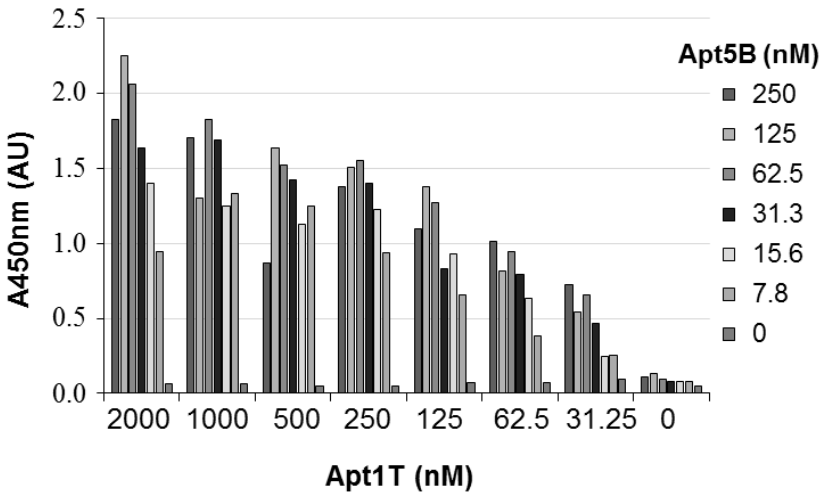
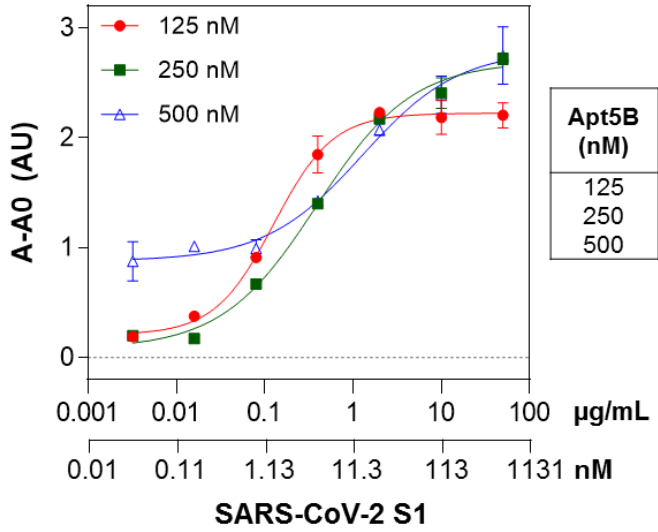


Figure S2. Checkerboard titration to optimize the concentration of Apt1T capture and Apt5B reporter aptamers for sandwich SARS-CoV-2 S1 protein detection.



Apt5B (nM)	LOD		EC50		R^2
	µg/mL	nM	µg/mL	nM	
125	0.022	0.28	0.127	1.66	0.9916
250	0.021	0.27	0.393	5.14	0.9937
500	0.128	1.68	1.269	16.59	0.9722

Figure S3. Sensitivity of the Apt1T/Apt5B aptamer sandwich assay for SARS-CoV-2 S1 protein detection at different concentrations of the reporter aptamer Apt5B.

SARS-CoV-2 RNA detection in nasopharyngeal swab samples by droplet digital PCR analysis

SARS-CoV-2 RNA quantification was performed at Galicia Sur Health Research Institute Laboratories. Viral RNA was extracted from nasopharyngeal swab samples with the QIAmp viral RNA mini kit (QIAGEN, Hilden, Germany) by the automatized QIAcube system (QIAGEN, Hilden, Germany), and their quantification was performed by QX200 droplet digital PCR System (Bio-Rad Laboratories) using a one-step reverse transcription (One-Step RT-ddPCR Advanced Kit for Probes, Bio-Rad Laboratories) and a triplex probe assay for the PCR amplification (2019-nCoV CDC ddPCR Triplex Probe Assay, Bio-Rad Laboratories). This assay contains in a single tube the primers and probes reported by Chinese CDC that targets two regions of the SARS-CoV-2 nucleocapsid gene (N1 and N2) as well as primers and probe for the human RNase P (*RPP30*). The results obtained were analyzed using the QuantaSoft Analysis Pro Software (Bio-Rad Laboratories). A sample was considered positive for SARS-CoV-2 if it had a minimal concentration of N (N1 and/or N2) of 0.1 copies/µL of 1X ddPCR reaction, and two or more positive droplets for N1 and/or N2 per well.

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