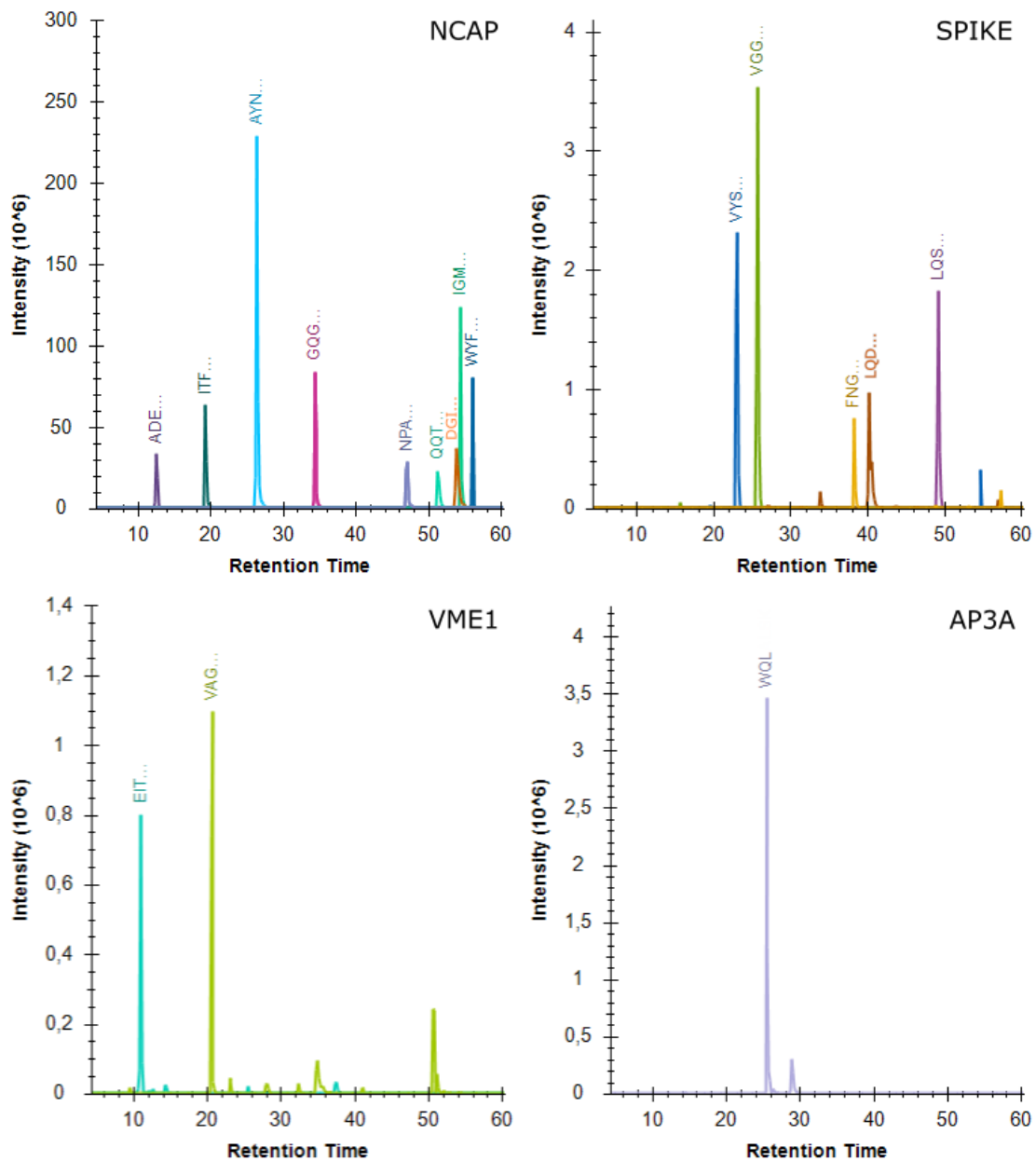
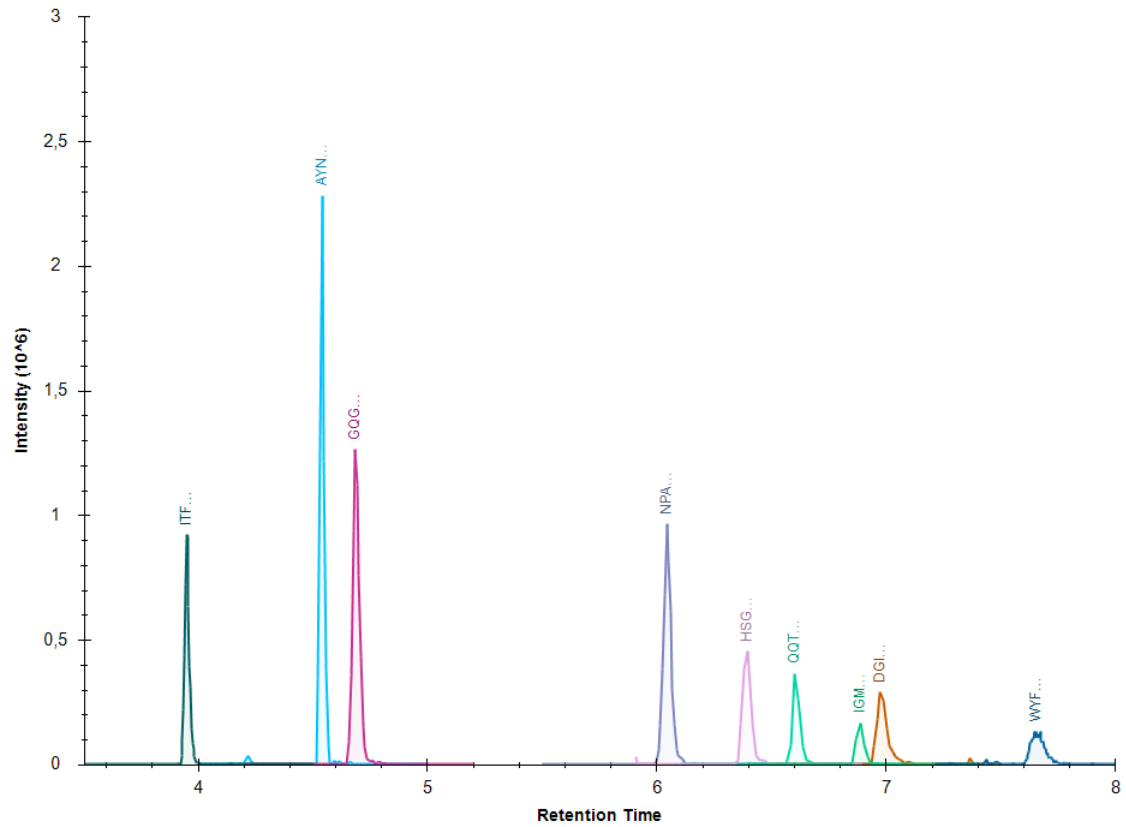


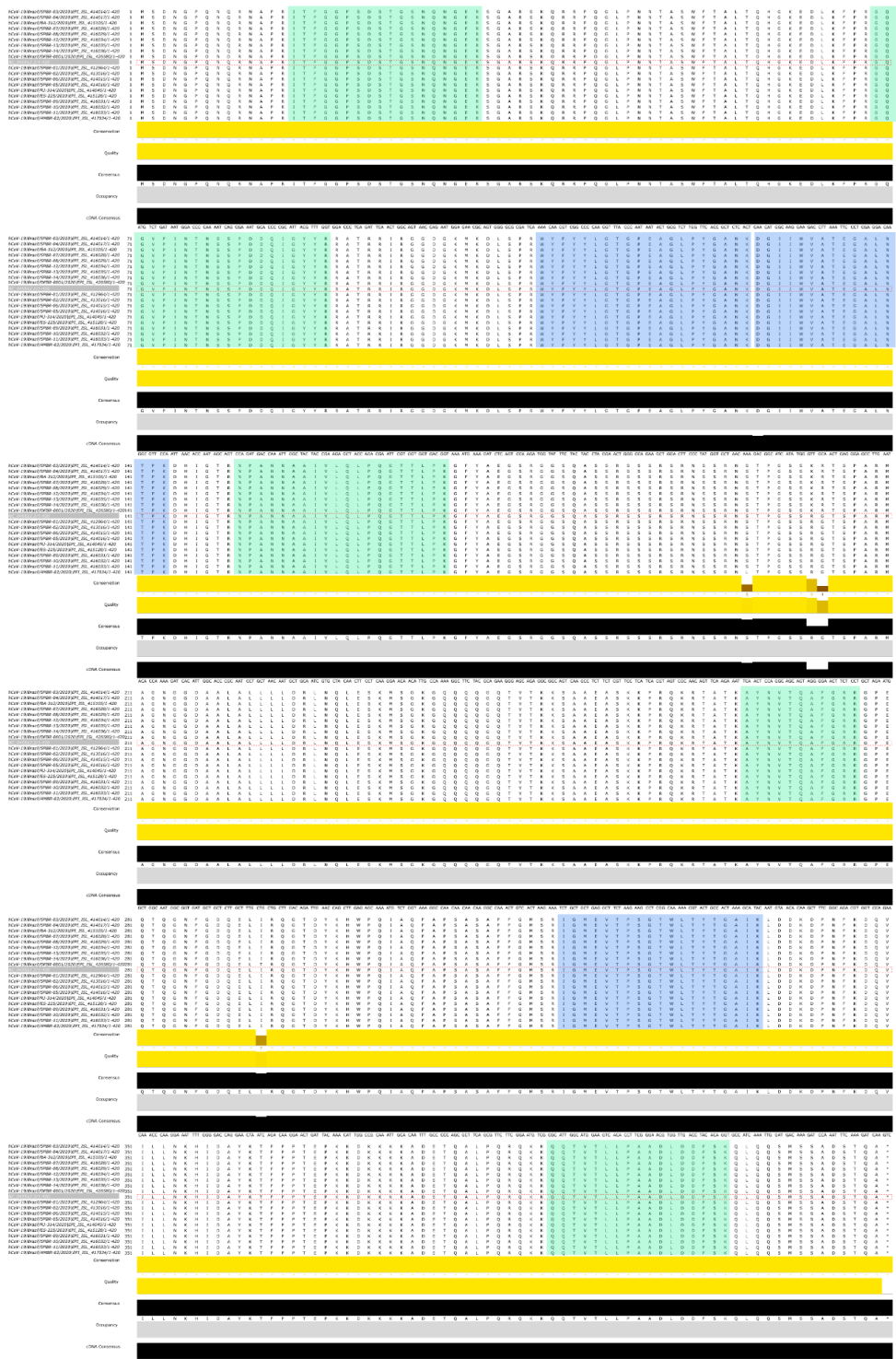
Supplemental Figures



Supplemental figure 1. Parallel reaction monitoring (PRM) chromatograms of SARS-CoV-2-positive respiratory tract samples showing the most promising peptides for nucleoprotein (NCAP), spike glycoprotein (SPIKE), membrane protein (VME1), and protein 3a (AP3A). The peptide peaks were labeled with the first three residues. NCAP (nucleoprotein): ADE (ADETQALPQR), ITF (ITFGGSPDSTGSNQNGER), AYN (AYNVTQAFGR), GQG (GQGVPIINTNSSPDDQIGYYR), NPA (NPANNAIIVLQLPQGTTLPK), QQT (QQTVTLLPAADLDDFSK), DGI (DGIWVATEGALNTPK), IGM (IGMEVTPSGTWLTYTGAIK), WYF (WYFYLTGPEAGLPYGANK). SPIKE (spike glycoprotein): VYS (VYSTGSNVFQTR), VGG (VGGNYNYLYR.), FNG (FNGIGVTQNVLYENQK), LQD (LQDVVNQNAQALNTLVK), LQS (LQSLQTYVTQQLIR). VME1 (membrane protein): VAG (VAGDSGFAAYSR), EIT (EITVATSR). AP3A (protein 3a): WQS (WQLALSK).

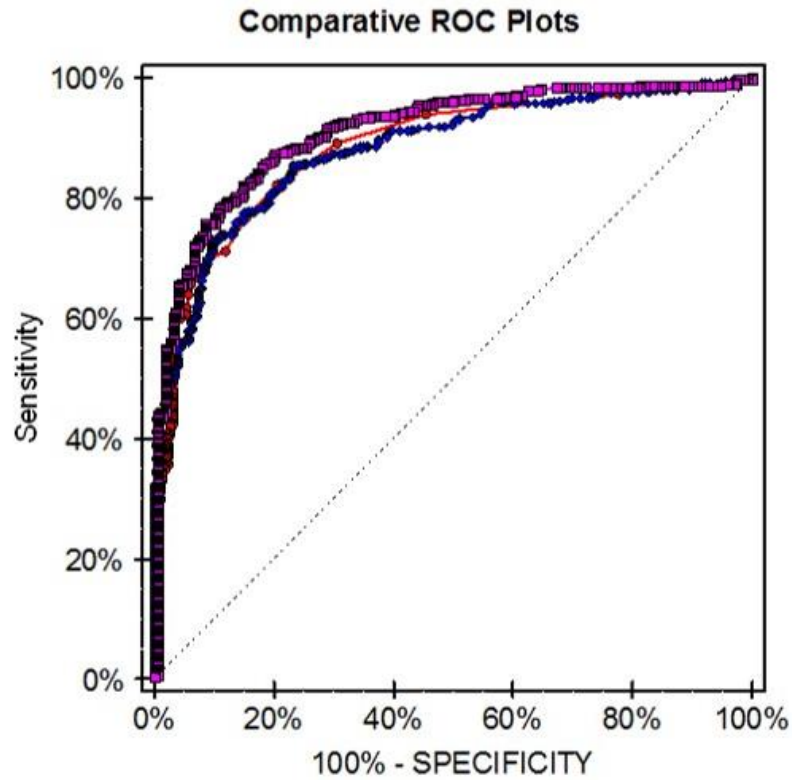


Supplemental figure 2. Nine-min parallel reaction monitoring (PRM) chromatogram of SARS-CoV-2-positive respiratory tract specimen showing nucleoprotein target peptides. The peptide peaks are labeled with the first three residues. NCAP (nucleoprotein): ITF (ITFGGSPDSTGSNQNGER), AYN (AYNVTQAFGR), GQG (GQGVPIINTSSPDDQIGYYR), NPA (NPANNAIIVLQLPQGTTLPK), QQT (QQTVTLLPAADLDDFSK), DGI (DGIWVATEGALNTPK), IGM (IGMEVTPSGTWLTYTGAIK), WYF (WYFYLLGTGPEAGLPYGANK). ¹⁵N-labeled global standard: HSG (HSGFEDELSEVLENQSSQAEK).



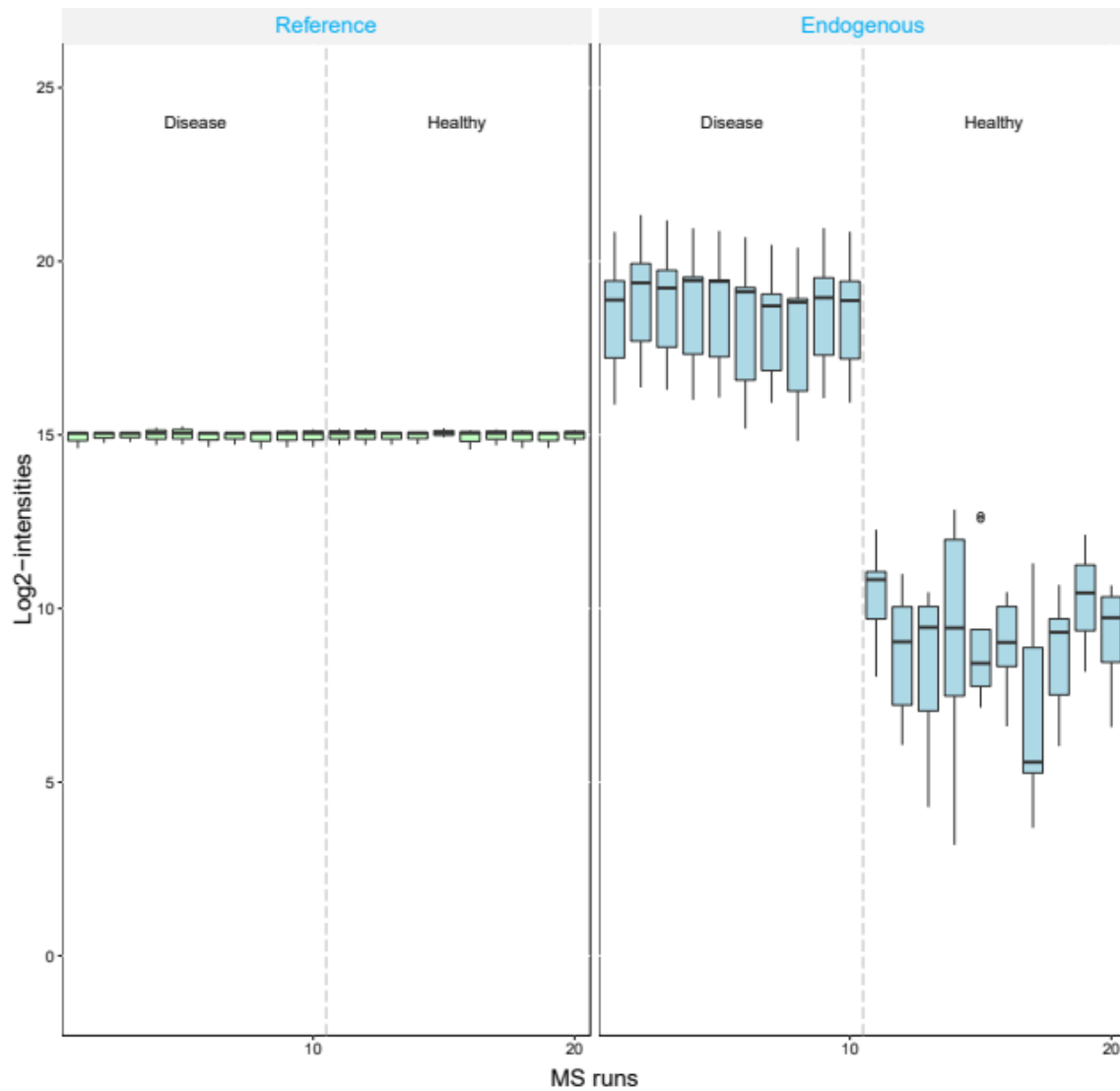
Supplemental figure 3. Multiple sequence alignment (EMBL-EBI Clustal Omega) corresponding to a set of SARS-CoV-2 nucleocapsid phosphoprotein CDSs deposited in GISAID. NCBI reference sequence NC_04512.2 (red dashed box) was included for comparison. Searches were filtered from location and only those sequences with full coverage on this genomic region were included. Segments corresponding to targeted peptides used in the selected reaction monitoring (SRM) method are shaded in blue and the remaining targeted peptides in the PRM analysis are shaded in green.

Multiple Test Comparison



	AUC	Std Err	95%CI	Cutoff
—■— SN IGM	0.912	0.012	0.88 to 0.94	≥ 1.65
—◆— SN DGI	0.883	0.014	0.85 to 0.91	≥ 0.83
—●— IGM/IS	0.886	0.014	0.86 to 0.91	≥ 0.04

Supplemental figure 4. Receiver operating curve (ROC) for the parameters used for virus detection in respiratory tract samples (n = 562). SN IGM: signal-to-noise for peptide IGMEVTPSGTWLTYTGAIK; SN DGI: signal-to-noise for peptide DGIIWVATEGALNTPK; and IGM/IS: ratio of peptide IGMEVTPSGTWLTYTGAIK to the ¹⁵N-labeled global internal standard.



Supplemental figure 5. MSstats QC plot of sample repeatability. Y-axis: log ratio of IGMEVTPSGTWLTYTGAIK, DGIWVATEGALNTPK, and WYFYLLGTGPEAGLPYGANK intensities normalized by ^{15}N -labeled HSGFEDELSEVLENQSSQAEK (global internal standard). X-axis: runs for disease (SARS-CoV-2 detected) and healthy (SARS-CoV-2 not detected) conditions.

Supplemental tables

Supplemental table 1. Parallel reaction monitoring (PRM) parameters for determination of SARS-CoV-2 targeted peptides on a Q-Exactive HF-X high-resolution mass spectrometer.

Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	NCE	Comment
423.2476			2	Positive			27	WQLALSK (light)
912.4114			2	Positive			27	ITFGGSDSTGSNQNGER (light)
775.4013			3	Positive			27	RPQGLPNNTASWFTALTQHGK (light)
1091.014			2	Positive			27	GQGVPIINTNSSPDDQIGYYR (light)
727.6784			3	Positive			27	GQGVPIINTNSSPDDQIGYYR (light)
1134.044			2	Positive			27	WYFYLLGTGPEAGLPYGANK (light)
756.3651			3	Positive			27	WYFYLLGTGPEAGLPYGANK (light)
842.9489			2	Positive			27	DGIWVATEGALNTPK (light)
1030.579			2	Positive			27	NPANNAIIVLQLPQGTTLPK (light)
687.3881			3	Positive			27	NPANNAIIVLQLPQGTTLPK (light)
835.9483			2	Positive			27	MAGNGGDAALALLLDR (light)
563.7856			2	Positive			27	AYNVQTAFGR (light)
894.9292			2	Positive			27	GPEQTQGNFGDQELIR (light)
1013.022			2	Positive			27	IGMEVTPSGTWLTYTGAIK (light)
471.7846			2	Positive			27	DQVILLNK (light)
458.7424			2	Positive			27	TFPTEPK (light)
564.7858			2	Positive			27	ADETQALPQR (light)
931.4807			2	Positive			27	QQTVTLLPAADLDDFSK (light)
741.3305			2	Positive			27	QLQQSMSSADSTQA (light)
748.3806			2	Positive			27	TQLPPAYTNSFTR (light)
1080.518			2	Positive			27	FDNPVLPFNDGVYFASTEK (light)
612.8166			2	Positive			27	GWIFGTTLDSK (light)
609.7987			2	Positive			27	VGGNYNYLYR (light)
570.3035			2	Positive			27	FLPFQQFGR (light)
679.8386			2	Positive			27	VYSTGSNVFQTR (light)
613.3268			2	Positive			27	SFIEDLLFNK (light)
912.468			2	Positive			27	FNGIGVTQNVLYENQK (light)
689.3646			2	Positive			27	IQDLSSTASALGK (light)
934.5154			2	Positive			27	LQDVVNQNAQALNTLVK (light)
623.3461			3	Positive			27	LQDVVNQNAQALNTLVK (light)
1011.037			2	Positive			27	QLSSNFGAISSVLNDILSR (light)
845.978			2	Positive			27	LQSLQTYVTQQLIR (light)
430.7461			2	Positive			27	IAGHHLGR (light)
438.7429			2	Positive			27	EITVATSR (light)
600.7858			2	Positive			27	VAGDSGFAAYS (light)
907.45			2	Positive			27	LNTDHSSSDNIALLVQ (light)

Supplemental table 2. Limits of blank (LoB) for peptides IGMEVTPSGTWLTYTGAIK and DGIIWVATEGALNTPK calculated according to the equation $LoB = \text{mean}_{\text{blank}} + 1.645(\text{SD}_{\text{blank}})$.

Sample	Signal-to-noise	
	IGMEVTPSGTWLTYTGAIK	DGIIWVATEGALNTPK
1	0.83	0.24
2	0.70	0.57
3	0.36	0.34
4	0.50	0.34
5	0.27	0.37
6	0.34	0.66
7	0.40	0.57
8	0.17	0.46
9	0.79	0.30
10	0.39	0.55
11	0.40	0.54
12	1.11	0.14
13	0.57	0.43
14	1.08	0.24
15	0.87	0.90
16	0.55	0.68
17	0.47	0.27
18	3.91	0.50
19	0.45	0.43
20	0.29	0.75
21	0.89	0.34
22	0.83	0.88
23	0.40	0.48
24	0.62	0.24
25	0.32	0.58
26	0.25	0.95
27	0.46	0.88
28	0.66	0.24
29	0.94	0.43
30	1.07	0.22
31	0.79	0.33
32	0.72	0.23
33	0.31	0.89
34	0.38	0.35
35	0.57	0.41
36	0.80	0.22
37	0.14	0.15
38	0.92	0.35
LoB	1.65	0.83

Supplemental table 3. Limits of detection for peptides IGMEVTPSGTWLTYTGAIK and DGIIWVATEGALNTPK calculated according to the equation $LoD = LoB + 1.645(SD_{low\ concentration\ sample})$.

Sample	Signal-to-noise	
	IGMEVTPSGTWLTYTGAIK	DGIIWVATEGALNTPK
1	0.15	0.30
2	1.59	0.57
3	1.64	0.29
4	1.03	0.18
5	2.47	0.08
6	3.40	0.14
7	0.08	0.62
8	1.17	0.67
9	0.24	0.18
10	0.98	0.52
11	0.85	0.26
12	0.95	0.40
13	0.98	0.39
14	1.19	0.17
15	1.71	0.42
16	0.91	0.34
17	0.72	0.30
18	1.23	0.22
19	2.80	0.14
20	0.54	0.29
LOD	3.03	1.10

Supplemental table 4: Stability of SARS-CoV-2 targeted protein in sterile saline and virus transport media conserved at 21 °C, 4 °C, and -20 °C. P-values were calculated from triplicate analyses compared to samples stored at -80 °C. Low: pool of samples with low viral loads; High: pool of samples with high viral loads.

Storage temperature	Sterile saline		Virus transport media	
	Low	High	Low	High
21 °C	0.8141	0.1834	0.0359*	0.8875
4 °C	0.0869	0.6353	0.0758	0.1811
-20 °C	0.4452	0.5284	0.9293	0.3838

Supplemental table 5: Thermal inactivation of SARS-CoV-2 targeted protein in sterile saline and virus transport media after inactivation of virus by heating process at 90 °C for 5 min. P-values calculated from triplicate analyses compared to samples not submitted to high heat. Low: pool of samples with low viral loads; High: pool of samples with high viral loads.

Viral load	Sterile saline	Virus transport media
Low	0.4962	0.5713
High	0.4680	0.7897

Supplemental table 6. Lack of cross-reactivity of respiratory samples in non **SARS-Cov-2** infections.

Sample	Interferent	SARS-Cov-2 presence
1	Rhinovirus/Enterovirus	Undetected
2	Rhinovirus/Enterovirus/Human metapneumovirus	Undetected
3	Rhinovirus/Enterovirus	Undetected
4	Coronavirus 229E	Undetected
5	Rhinovirus/Enterovirus	Undetected
6	Respiratory syncycial virus	Undetected
7	Rhinovirus/Enterovirus	Undetected
8	Coronavirus HKU1/Rhinovirus/Enterovirus	Undetected
9	Parainfluenza 4	Undetected
10	Influenza A/H1-2009	Undetected
11	Influenza A/H1-2009	Undetected
12	Parainfluenza 1	Undetected
13	Rhinovirus/Enterovirus/Human metapneumovirus	Undetected
14	Coronavirus NL63	Undetected
15	Human metapneumovirus	Undetected
16	Rhinovirus/Enterovirus	Undetected
17	Rhinovirus/Enterovirus	Undetected
18	Rhinovirus/Enterovirus	Undetected
19	Rhinovirus/Enterovirus	Undetected
20	Rhinovirus/Enterovirus	Undetected
21	Rhinovirus/Enterovirus	Undetected
22	Rhinovirus/Enterovirus	Undetected
23	Rhinovirus/Enterovirus	Undetected
24	Rhinovirus/Enterovirus	Undetected
25	Rhinovirus/Enterovirus	Undetected
26	Rhinovirus/Enterovirus	Undetected