

**Supplementary table 1.** Reagent information.

<b>Protein</b>	<b>Protein type</b>	<b>Expression mode</b>	<b>Typical purified yield (mg L<sup>-1</sup>)</b>	<b>% Main peak purity by SEC</b>
spike (SMT1)	570 kDa trimer	CHO stable pool	370	97
RBD (331–521)	37 kDa monomer	CHO transient	25	98
RBD (319–541)	37 kDa monomer	CHO transient	30	96
nucleocapsid (NCAP)	300 kDa hexamer <sup>†</sup>	CHO transient	60	99
VHH72-hFc1X7	100 kDa dimer	CHO transient	250	98
NRCov2-04-hFc1X7	90 kDa dimer	CHO transient	100	91
NRCov2-20-hFc1X7	100 kDa dimer	CHO transient	250	98
IgG#5-HRP	270 kDa mAb	CHO transient	15	60
ACE2-BAP	90 kDa monomer	CHO transient	150	99

<sup>†</sup>Hexamer predicted based on HPLC-SEC elution volume.

**Supplementary table 2.** Comparison of manual and automated ELISAs.

Reagent	Manual ELISA (96-well colorimetric)		Automated ELISA (384-well chemiluminescent)	
	Toronto	Ottawa	Toronto	Ottawa
<b>Antibody detection ELISA</b>				
spike	200 ng/well	100 ng/well	50 ng/well	50 ng/well
RBD (331–521)	75 ng/well	N/A	20 ng/well	N/A
RBD (319–541)	75 ng/well	100 ng/well	20 ng/well	50 ng/well
N	25 ng/well	100 ng/well	7 ng/well	50 ng/well
spike/RBD control antibody (Toronto: VHH72-hFc1X7 for IgG, hIGM2001 to IgM, CR3022 for IgA, Ottawa: CR3022)	8-point curve of 2-fold dilutions starting at 10 ng/well	10-point curve of 2-fold dilutions starting at 25 ng/well for IgM/IgA and 20 ng/well for IgG	8-point curve of 4-fold dilutions starting at 10 ng/well for IgG/IgA; 20 ng/well, 10 ng, then an 8-point curve of 4-fold dilutions for IgM	8-point curve of 2-fold dilutions starting at 5 ng/well, 4-fold dilutions starting at 0.039 ng/well
N control antibody (HC2003 for IgG, CR3018 for IgM and IgA)	8-point curve of 2-fold dilutions starting at 10 ng/well	N/A	20 ng/well, 10 ng/well, then an 8-point curve of 4-fold dilutions	8-point curve of 2-fold dilutions starting at 5 ng/well, 4-fold dilutions starting at 0.039 ng/well
IgG#5-HRP	3 ng/well (1:10K)	10 ng/well (1:3K)	0.9 ng/well (1:6.7K)	1.1 ng/well (1:5.4K)
Anti-IgA-HRP	2 ng/well (1:20K)	13 ng/well (1:3K)	0.8 ng/well (1:10K)	1 ng/well (1:8K)
Anti-IgM-HRP	0.67 ng/well (1:60K)	13 ng/well (1:3K)	0.66 ng/well (1:12K)	0.83 ng/well (1:9.6K)
Substrate	50 µL TMB/50 µL sulphuric acid	100 µL OPD/50 µL 3 M hydrochloric acid	1:4 dilution of ELISA Pico	1:2 dilution of ELISA Pico
<b>snELISA</b>				
spike	200 ng/well	N/A	50 ng/well	100 ng/well
RBD (319–541)	100 ng/well	N/A	34 ng/well	100 ng/well
ACE2-BAP	6.25 ng/well	N/A	2.08 ng/well (NRC), 17 ng/well (Rini)	6.5 ng/well
Streptavidin-HRP	25 (1:2K) ng/well	N/A	15 ng/well	25 ng/well
Substrate	50 µL TMB/50 µL sulphuric acid	N/A	1:4 dilution of ELISA Pico	1:2 dilution of ELISA Pico

**Supplementary table 3.** ELISA performance statistics for plasma or serum IgA and IgM (Toronto).

	spike		RBD		N		≥ 2 positive antigens	
	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM
<b>ROC analysis</b>								
<b>AUC</b>	0.982	0.949	0.978	0.955	0.954	0.759	N/A	N/A
<b>Cut point</b>	0.079	0.460	0.060	0.083	0.209	0.074	N/A	N/A
<b>FPR</b>	0.017	0.017	0.017	0.017	0.017	0.017	0.000	0.000
<b>TPR</b>	0.967	0.800	0.917	0.833	0.767	0.333	0.917	0.767
<b>3 SDs from the mean of the negative controls</b>								
<b>Cut point</b>	0.198	0.450	0.127	0.133	0.268	0.120	N/A	N/A
<b>FPR</b>	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
<b>TPR</b>	0.933	0.800	0.833	0.733	0.750	0.217	0.883	0.733
<b>Samples used to assess performance<sup>†</sup></b>								
Patients with COVID-19 20–40 d post-symptom onset						60		
Pre-COVID-19 (negative) controls						60		

<sup>†</sup>NML panel 4. Each sample was analyzed in duplicate (the unique numbers of samples are shown). Replicates were treated as separate samples in the ROC analysis. Negative samples were excluded from the analysis if the replicates were > 4 SDs from the mean.

**Supplementary table 4.** Conversion of relative ratios to international units (BAU mL<sup>-1</sup>) for plasma or serum.

Nucleocapsid <sup>†</sup>						
Dilution fold (d)		160		2560		Note
Volume equivalent (μL)		0.0625		0.00391		
Relative Ratio (RR)	log <sub>2</sub> (RR)	log <sub>2</sub> (BAU mL <sup>-1</sup> )	BAU mL <sup>-1</sup>	log <sub>2</sub> (BAU mL <sup>-1</sup> )	BAU mL <sup>-1</sup>	
2	1.0	8.38	333.98	12.38	5,343.76	Upper limit of linear range
1	0.0	6.98	126.34	10.98	2,021.37	
0.5	-1.0	5.58	47.79	9.58	764.62	
0.396	-1.3	5.11	34.46	9.16	571.19	Positivity Threshold
0.25	-2.0	4.18	18.08	8.17	289.23	
0.125	-3.0	2.77	6.84	6.77	109.41	
0.0625	-4.0	1.37	2.59	5.37	41.38	Lower Limit of linear range
<sup>†</sup> Formula: $\log_2(\text{BAU mL}^{-1} \text{ @ sample dilution fold } d) = (\log_2(\text{RR}) - 0.243) / 0.713 + \log_2(d)$ . See Figure 3D for a graph plotting the international standard curve used to calculate the conversion formula and its relationship to the reference curve. With standard 1:160 and 1:2560 dilutions, the range is 2.59 to 5343.8 BAU mL <sup>-1</sup> .						
RBD <sup>‡</sup>						
Dilution fold (d)		160		2560		Note
Volume equivalent (μL)		0.0625		0.00391		
Relative Ratio (RR)	log <sub>2</sub> (RR)	log <sub>2</sub> (BAU mL <sup>-1</sup> )	BAU mL <sup>-1</sup>	log <sub>2</sub> (BAU mL <sup>-1</sup> )	BAU mL <sup>-1</sup>	
1	0.0	8.12	278.37	12.12	4,453.99	Upper limit of linear range
0.5	-1.0	6.82	112.63	10.81	1,802.02	
0.25	-2.0	5.51	45.57	9.51	729.07	
0.186	-2.4	4.95	30.97	8.95	495.58	Positivity Threshold
0.125	-3.0	4.20	18.44	8.20	294.97	
0.0625	-4.0	2.90	7.46	6.90	119.34	
0.03125	-5.0	1.59	3.02	5.59	48.28	Lower Limit of linear range
<sup>‡</sup> Formula: $\log_2(\text{BAU mL}^{-1} \text{ @ sample dilution fold } d) = (\log_2(\text{RR}) + 0.612) / 0.766 + \log_2(d)$ . With standard 1:160 and 1:2560 dilutions, the range is 3.02 to 4,449.72 BAU mL <sup>-1</sup> .						

Spike <sup>§</sup>						
Dilution fold (d)		160		2560		Note
Volume equivalent (μL)		0.0625		0.00391		
Relative Ratio (RR)	log <sub>2</sub> (RR)	log <sub>2</sub> (BAU mL <sup>-1</sup> )	BAU mL <sup>-1</sup>	log <sub>2</sub> (BAU mL <sup>-1</sup> )	BAU mL <sup>-1</sup>	
1	0.0	6.55	93.80	10.55	1,500.80	Upper limit of linear range
0.5	-1.0	5.28	38.75	9.27	619.95	
0.25	-2.0	4.00	16.01	8.00	256.09	
0.19	-2.4	3.50	11.28	7.49	180.45	Positivity Threshold
0.125	-3.0	2.72	6.61	6.72	105.78	
0.0625	-4.0	1.45	2.73	5.45	43.70	
0.03125	-5.0	0.17	1.13	4.17	18.05	Lower Limit of linear range

<sup>§</sup>Formula:  $\log_2(\text{BAU mL}^{-1} \text{ @ sample dilution fold } d) = (\log_2(\text{RR}) - 0.604)/0.784 + \log_2(d)$ . With standard 1:160 and 1:2560 dilutions, the range is 1.13 to 1,500.80 BAU mL<sup>-1</sup>.