

Table 1. Automated Western immunoblotting results of 27 sera from COVID-19-positive patients and 37 sera from negative controls used to fix automated Western immunoblotting parameters. (Results expressed as median with 25 and 75 percentile).

Negative controls (n=37)					
	Early sera from COVID-19- positive patients (n=10)	sera from negative HCoV's patients collected before the pandemic (n=13)	sera from healthcare workers highly exposed to SARS-CoV-2 (n=14)	All	sera from COVID-19-positive patients (n=27)
Nucleocapside (56 kDa)					
S/N ratio	44 (29.98-64.4)	62.1 (33-78.7)	49.85 (34.95-70.95)	49.85 (34.95-70.95)	421 (214.1-666.8)
Height	6511 (5674-7729)	8854 (6428-12995)	4934 (4397-5605)	6100 (4586-9813)	64065 (32338-121517)
Area	82169 (65588-94895)	124541 (84495-147319)	67780 (61427-76234)	79634 (66110-128111)	839470 (524393-1597656)
Spike (217 kDa)					
S/N	0	0	0	0	27.9 (0-43.1)
Height	0	0	0	0	3174 (0-8621)
Area	0	0	0	0	40699 (0-182108)

Table 2. Sensitivity and specificity of automated Western immunoblotting determined with 27 sera from COVID-19-positive patients and 37 sera from non COVID-19 patients.

n=64	AUC	Youden index	Optimal Cut-off value	Sensitivity (%)	Specificity (%)	True positive (nb)	True negative (nb)	False positive (nb)	False negative (nb)
Nucleocapside									
S/N	0.975	0.907	110.4	96.3	94.6	26	35	2	1
Height	0.982	0.885	24922	88.9	100	24	37	0	3
Area	0.970	0.885	287005	88.9	100	24	37	0	3
Spike									
S/N	0.833	0.667	0	66.7	100	18	37	0	9
Height	0.833	0.667	0	66.7	100	18	37	0	9
Area	0.833	0.667	0	66.7	100	18	37	0	9

AUC= area under the curve.

Table 3. Results of automated Western immunoblotting including the 602 sera tested.

		Negative controls							
	Early sera from COVID-19 positive patients (n=10)	Sera from negative HCoVs patients collected before the pandemic (n=13)	Sera from healthcare workers highly exposed to SARS-CoV-2 (n=14)	All (n=37)	Sera from patients diagnosed with others HCoVs (n=76)	Sera collected before the pandemic from patients with unknown status for HCoVs (n=266)	Sera from COVID-19 positive patients (n=223)	Total (n=602)	
Positive sera									
Nucleocapside reactivity	1 (10%)	0	2 (14%)	3 (8%)	9 (12%)	12 (4.5%)	180 (81%)	204 (34%)	
Spike reactivity	0	0	0	0	2 (3%)	0	67 (30%)	69 (11%)	
Total	1 (10%)	0	2 (14%)	3 (8%)	11 (14.5%)	12 (4.5%)	181 (81%)	207 (34%)	

Table 4. Comparison between automated Western immunoblotting and commercial SARS-CoV-2 IgG ELISA on 197 sera.

	AWB IgT Nb (%)	ELISA IgG Nb (%)
Sera from COVID-19 positive patients (n=27)	27 (100)	22 (81)
Sera from negative control group (n=37)	3 (8)	0
Early sera from COVID-19 positive patients (n=10)	1 (10)	0
Sera from healthcare workers highly exposed to SARS-CoV-2 (n=14)	2 (14)	0
Sera from negative HCoV patients collected before the pandemic (n=13)	0	0
Sera from patients diagnosed with others HCoV (n=45)	6 (13)	0
Sera collected before the pandemic from patients with unknown status for HCoV (n=88)	5 (6)	6 (7)