Supplementary Materials:

Materials and Methods:

Samples and RT-PCR

Positivity of the assays was assessed on a set of prospectively collected serum samples of 75 COVID-19 patients (positive RT-PCR (1), see **Table S1** for clinical characteristics). The first blood sample available after hospitalization was analyzed with the aim i) to provide primary data on performance of various automated SARS-COV antibody tests at the time our patients enter the health care setting for inpatient service and ii) to analyze differences in sensitivity of the various assay formats, which will be especially prominent in the early phase of disease. In our own diagnostic facilities 66/75 patients were tested. For these patients, the initial test consisted of a modified E-gene assay as described by Corman et al. (2), adapted as 'cobas Omni Utility Channel'-protocol and performed on the cobas6800 system (1). All 66 patients received a PCR result of cycle threshold value (Ct) < 34 in at least two independent samples, using the above-mentioned method or the Roche SARS-CoV-2 IVD-Test for the cobas6800 system. The remaining nine patients received positive PCR results from external (certified) diagnostic laboratories, these patients are marked by an arrow in figure 1F. The mean time between onset of symptoms and blood sampling was 11.4 days (±6.6), ranging from 1 to 38 days. To analyse specificity, a set of anonymized retained samples of a pre-pandemic blood donor cohort (n=320, equally distributed between the age of 18-70; m/f ratio 1:1, collected 01.03.17 – 09.04.17) was used.

Test setup and statistics

All assays were performed according to the manufacturer´s recommendations (Table 1). GraphPad Prism version 8.4.2 (GraphPad Software, La Jolla, California, USA) was used for statistical analysis. For correlation assessments, Pearson correlation coefficient was utilized. The Mann-Whitney test was computed for analysis of comparison between test results of patients categorized as critical and as severe (categorization based on the WHO case definitions). P-values <0.05 were considered significant. The 95% confidence intervals were calculated according to the method of Wilson-Brown (3).

References:

1. Pfefferle S, Reucher S, Nörz D, Lütgehetmann M. Evaluation of a quantitative RT-PCR assay for the detection of the emerging coronavirus SARS-CoV-2 using a high throughput system. Euro Surveill. 2020;25(9).
2. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3).
3. Brown, L. D., Cai, T., & Dasgupta, A. (2001). Interval Estimation for a Binomial Proportion. Statistical Science, 16 (2), 101-133. http://dx.doi.org/10.1214/ss/1009213286

Table S1 Clinical characteristics of SARS-CoV-2 PCR positive patients (naso-/oropharyngeal swab, n=75)

age - years		
	mean	60.2 ± 15.4
	range	16 - 93
sex - n (%)		
	female	25 (33.3)
	male	50 (66.7)
time since onset of symptoms - days		
	mean	11.4 ± 6.6
	range	1 - 38
severity of SARS-CoV-2 infection - n (%)		
	critical	31 (41.4)
	severe	36 (48)
	mild	7 (9.3)
	asymptomatic	1 (1.3)

Table S1 I Clinical characteristics of 75 patients who tested positive for SARS-CoV-2 RNA by PCR from naso-/oropharyngeal swabs in March and April of 2020. Patients were grouped in critical, severe, mild and asymptomatic SARS-CoV-2 infection based on the WHO case definitions.

Table S2 I Test results of SARS-CoV-2 PCR positive patients (n=75) and pre-pandemic blood donors (n=320)

	SARS-CoV-2 neg.		SARS-CoV-2 pos.									
	total				1-5 d		6-10 d		11-15 d		> 15 d	
result	n	%	n	%	n	%	n	%	n	%	n	%
Euroimmun												
positive	3/320	0.9	36/75*	48.0	4/12*	33.3	6/25	24.0	15/22	68.2	11/16	68.8
negative	317/320	99.1	39/75	52.0	8/12	66.7	19/25	76.0	7/22	31.8	5/16	31.2
Diasorin												
positive	3/320*	0.9	37/75***	49.3	4/12	33.3	7/25*	28.0	15/22	68.2	11/16**	68.8
negative	317/320	99.1	38/75	50.7	8/12	66.7	18/25	72.0	7/22	31.8	5/16	31.2
Roche												
positive	1/320	0.3	47/75	62.7	6/12	50.0	11/25	44.0	18/22	81.8	12/16	75.0
negative	319/320	99.7	28/75	37.3	6/12	50.0	14/25	56.0	4/22	18.2	4/16	25.0
Wantai												
positive	2/320	0.6	58/75*	77.3	8/12	66.7	19/25	76.0	19/22*	86.4	12/16	75.0
negative	318/320	99.4	17/75	22.7	4/12	33.3	6/25	24.0	3/22	13.6	4/16	25.0
Siemens												
positive	0/320	0.0	41/75	54.7	6/12	50.0	7/25	28.0	16/22	72.7	12/16	75.0
negative	320/320	100.0	34/75	45.3	6/12	50.0	18/25	72.0	6/22	27.3	4/16	25.0

Table S2 I Test results of samples from SARS-CoV-2 PCR positive patients (naso-/oropharyngeal swab, n=75) and samples from pre-pandemic blood donors (2017, n=320) for all five tests. Test results of the SARS-CoV-2 positive cohort are sorted by time since onset of symptom in days. Cut-off values for positivity are 1.1 for Euroimmun (0.8-1.1 borderline), 15 for Diasorin (12-15 borderline) and 1 for Roche, Wantai and Siemens. Borderline test results were considered positive.

*/**/***=one/two/three borderline test results considered positive

Abbreviations: d, days; neg., negative; pos., positive

Table S3 I Test results of patients with a negative test results in at least one of the five assays

Euroimmun - Ratio	Diasorin - AU/ml	Roche - COI	Wantai - A/C.O.	Siemens - Index	RT-PCR - Ct-value
11-15 days since onset of symptoms					
0.4	4.9	0.1	0	0.1	n/a
0.2	7	0.1	0.1	0.1	20.35
0.4	6.7	13	18	1.7	28.73
1.1	11.6	5.9	9	5	34.71
0.3	7.4	0.5	1.4	0.1	29.66
0.1	6.2	0.1	0.9	0.1	21.5
0.3	38.5	1.4	15.9	1.2	27.06
0.3	5.7	2.1	0.5	0.1	32.75
1.3	75.2	17.5	2.9	0.8	27.0
> 15 days since onset of symptoms					
0.5	4.9	0.1	0.1	0.2	27.58
0.2	3.8	0.1	0	0.1	36.77
0.2	4.3	0.1	0	0.1	31.5
1.6	11	24.6	9.6	1.3	31.21
1.7	17.2	3.9	0	6.8	35.74*
0.3	3.8	0.1	14.1	0.4	36.77
0.5	24.6	5.1	18	10	27.03

Table S3 I Test results for patients that tested negative in at least one assay and experienced first symptoms > 10 days before blood sampling are displayed. Each row represents one patient. RT-PCR was performed at our center from naso-/oropharyngeal swabs by a modified version of the E-gene assay. Cut-off value for positivity was a Ct-value of < 34. For one patient RT-PCR analysis was performed by an external certified laboratory. Therefore the Ct-value can not be provided. Cut-off values for positivity for the serology assays were 1.1 for Euroimmun, 15 for Diasorin and 1 for Roche, Wantai and Siemens. Borderline test results were considered positive (0.9-1.1. for Euroimmun and 12-15 for Diasorin).

* RT-PCR from EDTA blood

Abbreviations: A/C.0., absorbance/cut-off; AU/ml, arbitrary units/ml; COI, Cut-off index (sample signal/cut-off); Ct-value, cycle threshold value; Ratio, ratio (extinction sample/extinction calibrators); n/a, data not available

Figure S1 – Comparisons of disease severity and test results of examined SARS-CoV-2 serology assays

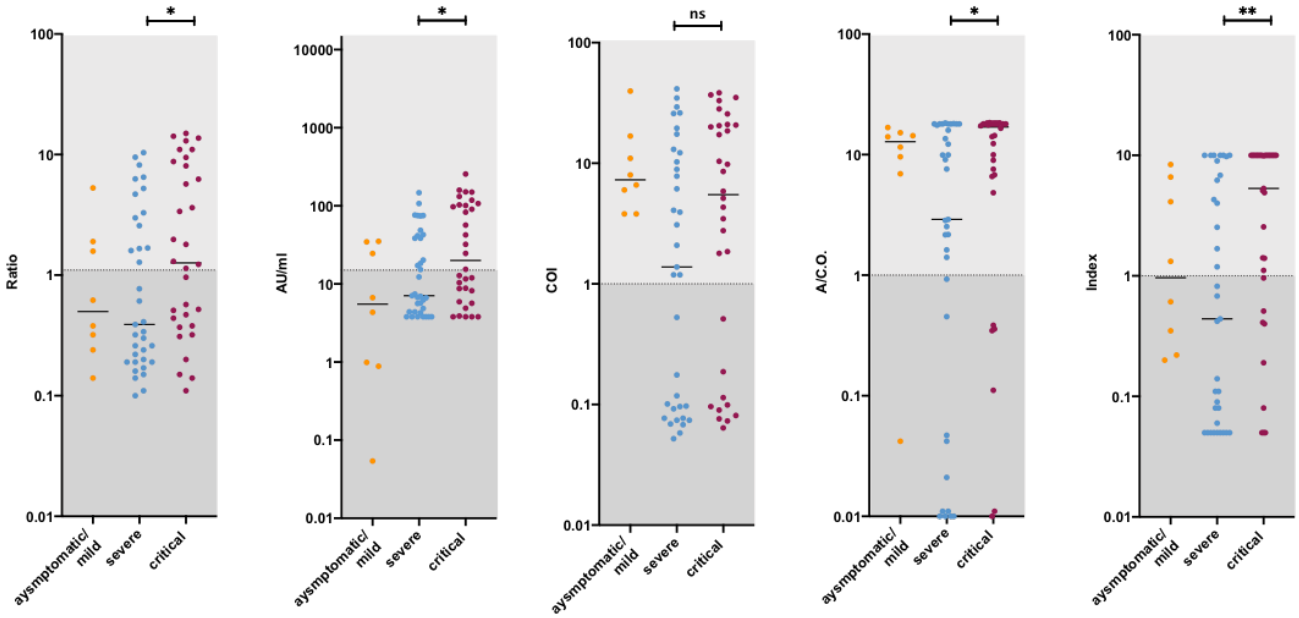

Figure S1 I SARS-CoV-2 RT-PCR positive patients (n=75) were categorized in asymptomatic/mild (orange dots), severe (blue dots) and critical (magenta dots) according to the WHO case definitions. Each dot represents one sample. For statistical analysis the Mann-Whitney test was computed. P-values > 0.05 were considered non-significant. P-values ≤ 0.05 are marked by * and those ≤ 0.01 by **. Cut-off values for positivity are 1.1 for Euroimmun, 15 for Diasorin and 1 for Roche, Wantai and Siemens (dotted line). Median is indicated for each group and assay (black line).

Abbreviations: A/C.O., Absorbance/cut-off value; AU/ml, Arbitrary units/ml; COI, cut-off index (signal sample/cut-off); Ratio, ratio (extinction sample/extinction calibrator)