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CORESMA - COVID-19-Outbreak Response combining E-health, Serolomics, Modelling, Artificial Intelligence and Implementation Research

WP 2	**Differential serolomics to assess sero-prevalence, cross and pre-existing immunity against coronaviruses**
Deliverable D2.3	**Report**
Title of Deliverable:	**Pre-final technical validation of the multiplex serological immunoassay finished**
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D2.3 Pre-final technical validation of the multiplex serological immunoassay finished

To establish MULTICOV-AB, a multiplex based–serological assay, a final antigen panel was selected and assay development was started as described in deliverables D2.1 and D2.2. In order to assure reproducible analyses, final technical validation was performed. The validation protocol included assessment of intra- and inter-assay variance, dilution linearity, reagent and sample stability, and comparison of serum and plasma sample pairs. The entire process was performed according to FDA bioanalytical guidelines.

In more detail, we assigned three Quality Control (QC) samples which were processed on every assay plate. Those QC samples were chosen to cover a range of high and low mean fluorescence (MFI) signals for the respective antigens. Figure 1a) shows stable assay performance across 17 runs. The linear assay range also showed good parallelism for selected antigens (Fig 1b). Intra- and inter-assay variance were generally around 5% indicating good data reproducibility. Details on inter- and intra-assay variance for all antigens are listed in Table 1. By measuring paired serum and plasma samples from individuals, we could show that serum and plasma can be used interchangeably for MULTICOV-AB analyses. Later on the assay was adapted for the analysis of saliva (details are described in *Becker et al. 2021b, Nature Communications 12:3109*).

All technical assay validation steps were successfully completed and published as part of "Exploring beyond clinical routine SARS-CoV-2 serology using MULTICOV-AB to evaluate endemic coronavirus cross-reactivity" in the open access journal Nature Communications (*Becker et al., Nature Communications (2021)12:1152*). Further assay validation steps such as diagnostic value is part of the clinical validation work package (deliverable D2.4).

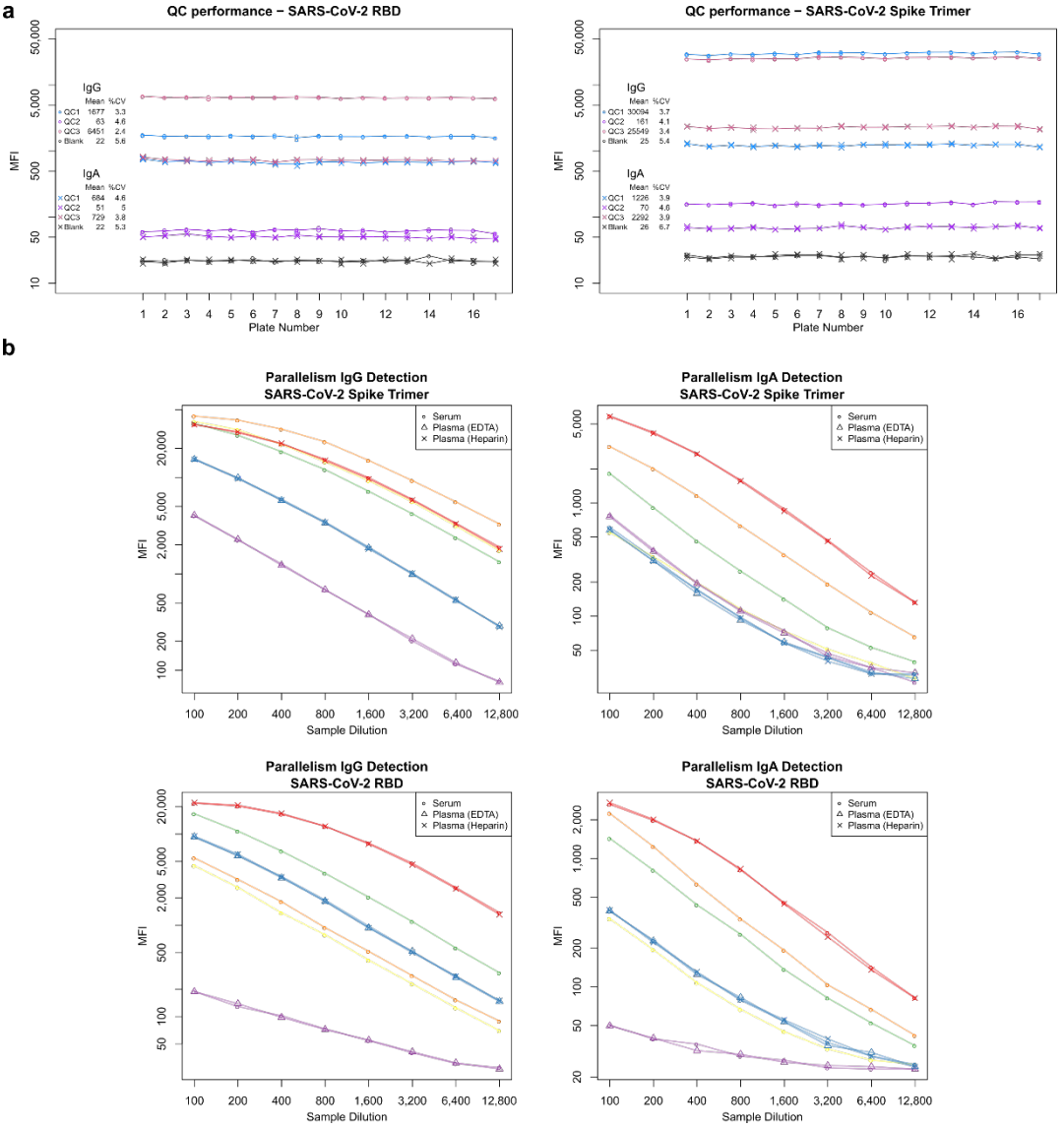

***Figure 1: Technical assay validation for SARS-CoV-2 RBD and Spike Trimer antigens*. a)** Three quality control (QC) samples and a sample of assay buffer (blank) were processed in duplicates on every plate. Performance across 17 assay runs is depicted and mean and %CVs are shown on the left side (n=34). For plate 14, a processing error lead to exclusion of one blank sample from this evaluation. **b)** To assess parallelism of signals from different samples, 6 unique serum samples were processed over a 8-step dilution range from 1:100 to 1:12,800. For 3 samples, paired plasma (EDTA or Heparin) and serum samples were available and processed together. For IgG and IgA detection of Spike Trimer and RBD, mean fluorescence intensity (MFI) is plotted against sample dilution. Color indicates unique sample and shapes indicate sample type. The data represent a single measurement per sample and dilution (n=1). This figure is Supplementary Figure 2 from Becker et al., Nature Communications (2021)12:115.

Table 1: Assay variance and LOD. *Intra- and inter-assay variance were determined by repeated measurement of QC and blank samples as replicates on one plate and in duplicates over 17 plates, respectively. Standard deviation relative to mean (%CV) is given for each antigen. A limit of detection (LOD) was calculated from 24 blank sample replicates on the same plate as the mean MFI + 3 times standard deviation. This is table is Supplementary Table 1 from Becker et al., Nature Communications (2021)12:11*

			SARS-CoV-2						hCoV NL63			hCoV 229E			hCoV OC43			hCoV HKU1		
			Spike Trimer	RBD	S1	S2	N	N-NTD	S1	N	N-NTD	S1	N	N-NTD	S1	N	N-NTD	S1	N	N-NTD
Inter-assay variance (%CV) n = 34, duplicates, 17 plates	IgG	QC1	3.7	3.3	3.4	3.7	2.8	7.4	3.5	3.2	4.4	3.3	2.8	5.2	3.1	6.0	4.4	3.4	4.7	5.4
		QC2	4.1	4.6	6.9	3.4	5.3	4.8	3.0	2.2	6.3	2.4	2.1	6.7	2.7	4.5	2.3	2.7	5.1	2.8
		QC3	3.4	2.4	2.3	3.6	2.1	4.6	3.1	2.5	3.5	2.9	2.0	4.7	2.9	6.4	4.6	3.2	3.2	3.5
		Blank	5.4	5.6	6.7	6.4	5.6	6.1	6.3	7.1	5.7	9.1	6.1	6.1	5.6	7.3	4.1	4.9	6.1	8.3
	IgA	QC1	3.9	4.6	4.9	4.0	5.1	5.0	4.2	3.6	3.9	4.0	5.3	7.4	4.3	7.4	5.0	4.2	6.0	5.0
		QC2	4.6	5.0	5.1	3.9	3.9	4.2	3.7	2.4	7.6	2.9	2.2	6.0	4.1	16.4	4.2	4.3	5.5	3.9
		QC3	3.9	3.8	4.5	3.4	3.4	4.8	3.9	2.8	4.0	3.0	5.1	4.5	3.6	6.1	4.1	3.7	4.5	5.1
		Blank	6.7	5.3	8.2	6.3	5.3	5.3	3.3	5.0	5.0	6.7	7.0	6.1	5.3	7.1	4.7	6.0	6.8	6.3
Intra-assay variance (%CV) n = 24	IgG	QC1	2.5	1.9	2.0	2.1	1.8	2.1	2.4	1.7	2.8	2.0	2.7	3.2	1.9	2.0	2.2	2.7	2.4	2.2
		QC2	5.9	4.3	4.1	2.8	2.7	3.2	1.9	1.9	2.6	2.0	2.2	2.7	2.2	1.6	2.1	2.5	3.1	2.5
		QC3	1.6	4.3	5.1	1.9	1.9	4.5	4.2	1.7	3.2	3.3	4.1	5.7	3.2	3.1	5.5	5.6	6.0	8.4
		Blank	6.0	5.6	5.2	5.8	5.2	4.2	5.0	4.8	4.8	7.3	6.2	6.3	6.5	6.2	4.4	6.1	6.0	6.2
	IgA	QC1	2.5	3.3	5.2	3.8	3.7	4.2	3.2	2.3	2.2	2.0	4.8	4.7	2.9	4.7	3.4	3.3	4.5	4.3
		QC2	4.8	5.7	5.7	3.2	4.1	4.3	3.4	2.0	5.7	2.1	2.1	6.1	3.0	3.1	1.9	3.9	6.4	3.8
		QC3	3.1	4.7	5.5	3.0	4.1	4.4	3.7	2.7	3.7	2.7	5.4	5.8	2.6	4.1	3.1	2.4	4.5	4.3
		Blank	5.8	5.3	6.3	5.0	5.4	5.5	4.5	5.6	5.3	7.2	6.3	7.0	6.7	9.5	7.3	5.2	8.8	7.0
LOD* (MFI) n = 24	IgG		32	26	23	29	38	33	29	28	26	65	35	24	33	30	33	37	33	25
	IgA		31	26	26	27	37	32	57	28	40	28	36	22	35	28	32	33	39	28