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 D2.2	**Report**
Title of Deliverable:	**Development of the multiplex serological immunoassay finalized**
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D2.2 Development of the multiplex serological immunoassay finalized

After selection, in-house production or purchase of an initial antigen panel, serological assay development was started. As platform technology, we used multiplex MAP technology by Luminex which allows semi-quantitative simultaneous detection of antibody levels towards multiple antigens using serum and plasma samples with volumes as low as 5µL.

First, in-house produced and purchased proteins or fragments were immobilized on Luminex MAGPLEX microspheres in 2-3 different concentrations to identify the optimal coupling concentration. The initial set of antigens was then assessed for performance with samples from COVID-19 convalescent individuals and healthy controls. Further, serial dilutions to identify the sample concentration to best separate SARS-CoV-2 convalescent from healthy donors were performed. Different assay buffers and incubations times were assessed to identify best performing blocking buffers, diluents and optimal assay processing conditions. Detectable immunoglobulin isotypes from the serum samples were identified by using Phycoetythrin-labelled antibodies against human IgG and IgA. IgM was initially tested, but did not show an acceptable performance. The antigen panel was then narrowed down to a set of core antigens, which showed either promising performance in distinguishing healthy from convalescent donors or allowed for equivalent comparison between SARS-CoV-2 and human endemic coronaviruses. For example, SARS-CoV-2 S1 showed good performance and therefore hCoV S1 was included to be able to directly compare antibody reactions using the homologous protein. As additional controls, the assay contains human IgG, human IgA, goat-anti-human IgG and goat-anti-human IgA coupled MAGPLEX microspheres to confirm sample and signal system addition.

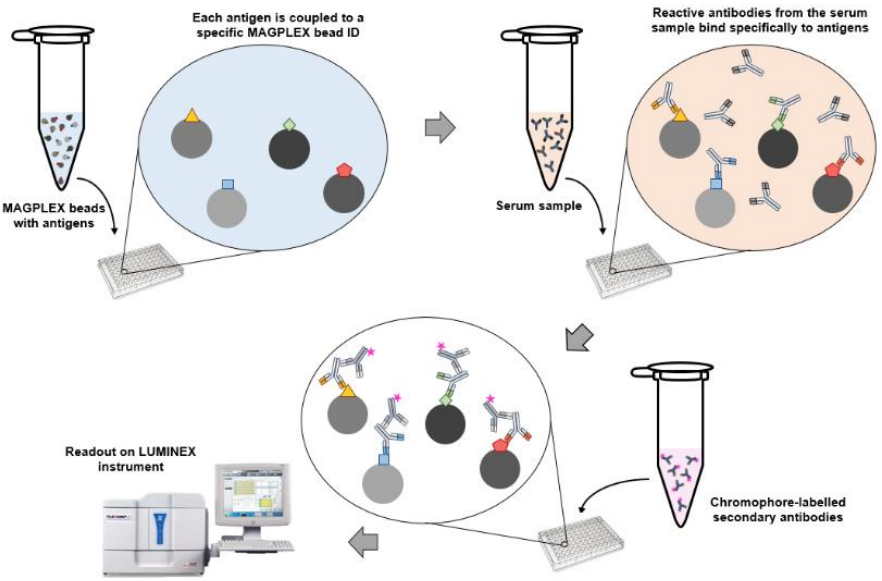

Figure 1: Schematic presentation of a Luminex-based multiplex serologic assay. Different antigens are first immobilized on MAGPLEX beads which are then incubated with individual samples. During the incubation period, reactive antibodies bind to the immobilized antigens. Unreactive antibodies are

afterwards removed by washing steps. Readout on a Luminex platform is performed via chromophore-labelled secondary antibodies, which are specific to human immunoglobulins.

The validated assay was named MULTICOV-AB and contains an antigen panel of 18 recombinant proteins to assess antibody responses for both SARS-CoV-2 and endemic coronaviruses. For SARS-CoV-2 full-length trimeric Spike, receptor binding domain (RBD), S1 domain, S2 domain, Nucleocapsid and N-terminal domain of the Nucleocapsid are included, hCoV antigens are limited to S1 domain, Nucleocapsid and N-terminal domain of the Nucleocapsid. The complete list and description of antigens used in the assay are listed in Table 1.

Additional details about the assay setup can be found in our publication “Exploring beyond clinical routine SARS-CoV-2 serology using MULTICOV-AB to evaluate endemic coronavirus cross-reactivity” which we published in the open access journal Nature Communications (*Becker et al., Nature Communications (2021)12:1152*).

Table 1: Final MULTICOV-AB antigen panel. *For commercial antigens, catalogue number is given and information is provided as available from the data sheets. NA: Information was not available. The table is according to supplementary table 5 from Becker et al., Nature Communications (2021)12:1152*

Construct	Manufacturer	Sequence Identifier	Fragment	Mutations	Expression system	Tag	Tag position
SARS-CoV-2 Spike Trimer	In-house expressed	QHD43416.1	1-1213	^{682}RRAR to A, K986P and V987P	Expi293	Thrombin cleavage-site/ T4 foldon/ His_6	C-terminus
SARS-CoV-2 RBD	In-house expressed	QHD43416.1	1-14 + 319-541		Expi293	His_6	C-terminus
SARS-CoV-2 S2	Sino Biological #40590-V08B	YP_009724390.1	686-1213		Baculovirus-Insect cells	His_6	C-terminus
SARS-CoV-2 S1	In-house expressed	QHD43416.1	1-681		Expi293	His_6	C-terminus
hCoV-OC43 S1	In-house expressed	AVR40344.1	1-760		Expi293	His_6	C-terminus
hCoV-NL63 S1	In-house expressed	APF29071.1	1-744		Expi293	His_6	C-terminus
hCoV-229E S1	In-house expressed	APT69883.1	1-561		Expi293	His_6	C-terminus
hCoV-HKU1 S1	In-house expressed	AGW27881.1	1-755		Expi293	His_6	C-terminus
SARS-CoV-2 N	Aalto Bioreagents #CK 6406-b	NA	full length		*E. coli*	His_6	C-terminus
SARS-CoV-2 N-NTD	In-house expressed	QHD43423.2	1-174		*E.coli* BL21	His_6	N-terminus
hCoV-OC43 N	In-house expressed	YP_009555245.1	1-448		*E.coli* BL21	His_6	N-terminus
hCoV-OC43 N-NTD	In-house expressed	YP_009555245.1	1-189		*E.coli* BL21	His_6	N-terminus
hCoV-NL63 N	In-house expressed	YP_003771.1	1-377		*E.coli* BL21	His_6	N-terminus
hCoV-NL63 N-NTD	In-house expressed	YP_003771.1	1-139		*E.coli* BL21	His_6	N-terminus
hCoV-229E N	In-house expressed	NP_073556.1	1-389		*E.coli* BL21	His_6	N-terminus
hCoV-229E N-NTD	In-house expressed	NP_073556.1	1-141		*E.coli* BL21	His_6	N-terminus
hCoV-HKU1 N	In-house expressed	YP_173242.1	1-441		*E.coli* BL21	His_6	N-terminus
hCoV-HKU1 N-NTD	In-house expressed	YP_173242.1	1-188		*E.coli* BL21	His_6	N-terminus