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 D2.1	**Report**
Title of Deliverable:	**Identification of an appropriate antigen set to fit the assay goals described**
Author:	**Natural and Medical Sciences Institute, University Tübingen, Germany**

Authors: Nicole Schneiderhan-Marra [1], Matthias Becker [1], Alex Dulovic [1], Monika Strengert [2]

Affiliations: Natural and Medical Sciences Institute, University Tübingen [1], Helmholtz Centre for Infection Research [2]

D2.1 Identification of an appropriate antigen set to fit the assay goals

Aim of work package 2 was to develop a novel serological assay, capable of not only an in-depth assessment of the SARS-CoV-2 antibody response but also to distinguish the SARS-CoV-2 antibody response from those towards human endemic coronaviruses (hCoVs). Such an assay will enable seroprevalence studies, which correlate previous hCoV infections with potential protection from SARS-CoV-2.

The correct antigen selection and production to identify diagnostic targets of interest is a crucial step for the development of each serological screening project. We performed in-silico analyses and literature research to determine useful antigens for SARS-CoV-2 serology. Initially, we targeted all four structural proteins, present in the SARS-CoV-2 virion, namely the Spike (S), the Membrane (M), Envelope (E) and Nucleocapsid (N) protein. Sequence analysis identified the S protein as most promising target to distinguish coronaviruses species, as the SARS-CoV-2 S1 subdomain was most divergent compared to hCoVs whereas higher sequence conservation was observed in the S2 domain of the Spike protein and the N protein.

Table 1 - Percentage Identity of sequence alignments of used hCoV and corresponding SARS-CoV-2. *Alignments were calculated using version 1.2.4. of Clustal Omega[3]. Sequences of constructs used in alignment are provided as a Source Data file. The table is according to supplementary table 3 from Becker et al., Nature Communications (2021)12:1152*

		% of sequence identity of corresponding antigen				
Protein	Identifier	hCoV-NL63	hCoV-229E	SARS-CoV-2	hCoV-OC43	hCoV-HKU1
hCoV-NL63 S1	APF29071.1	100.0	50.2	17.8	19.7	18.1
hCoV-229E S1	APT69883.1	50.2	100.0	18.5	20.2	18.7
SARS-CoV-2 S1	QHD43416.1	17.8	18.5	100.0	24.2	24.2
hCoV-OC43 S1	AVR40344.1	19.7	20.2	24.2	100.0	58.0
hCoV-HKU1 S1	AGW27881.1	18.1	18.7	24.2	58.0	100.0
hCoV-NL63 N	YP_003771.1	100.0	47.5	30.9	29.0	29.7
hCoV-229E N	NP_073556.1	47.5	100.0	30.4	30.1	32.2
SARS-CoV-2 N	QHD43423.2	30.9	30.4	100.0	37.1	36.7
hCoV-OC43 N	YP_009555245.1	29.0	30.1	37.1	100.0	65.5
hCoV-HKU1 N	YP_173242.1	29.7	32.2	36.7	65.5	100.0
hCoV-NL63 N-NTD	YP_003771.1	100.0	63.4	35.3	34.4	35.8
hCoV-229E N-NTD	NP_073556.1	63.4	100.0	38.2	37.9	39.2
SARS-CoV-2 N-NTD	QHD43423.2	35.3	38.2	100.0	42.0	42.8
hCoV-OC43 N-NTD	YP_009555245.1	34.4	37.9	42.0	100.0	68.3
hCoV-HKU1 N-NTD	YP_173242.1	35.8	39.2	42.8	68.3	100.0

An initial antigen panel therefore utilized different constructs of SARS-CoV-2 Spike protein, as well as SARS-CoV-2 M, E and N protein. For the four seasonal hCoVs NL63, 229E, OC43 and HKU1, we included the S1 domain and constructs from N proteins. While we bought some antigens from commercial vendors, a substantial amount was produced by our in-house protein production facility. Some antigens were both purchased and produced in-house to assure rapid assay development. For in-house production, antigen sequences were selected

by searching genomic databases (e.g. GISAID). From selected sequences, expression constructs were generated by molecular biology techniques such as PCR and ligation. Final plasmids were verified by DNA sequence analysis and then expressed in Expi293 cells by transient transfection. Next, supernatants containing the expressed proteins of interest were filtered and purified by AKTA purification. Last, expressed proteins were subject to quality control by mass spectrometry and SDS PAGE. The plasmids encoding the stabilized trimeric Spike protein and the receptor binding domain (RBD) of SARS-CoV-2 were kindly provided by F. Krammer (Amanat, F. et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. 2020 Nat. Med. 26, 1033–1036). Table 2 provides a comprehensive overview of initially chosen antigens and their source. More detailed information about protein production and antigen selection can be found in "Exploring beyond clinical routine SARS-CoV-2 serology using MultiCoV-Ab to evaluate endemic coronavirus cross-reactivity" which was published in the open access journal Nature Communications (*Becker et al., Nature Communications (2021)12:1152*).

Table 2: Overview of antigens tested throughout assay development

No.	CoV strain and antigen	Manufacturer / Vendor	Catalogue number
1	SARS2 Spike Trimer	NMI	in-house produced
2	SARS2 S2	Sino Biological	40590-V08B
3	SARS2 S1	MyBiosource	MBS8574739
4	SARS2 S1	NMI	in-house produced
5	SARS2 S1	Sino Biological	40591-V08H
6	SARS2 RBD	BeiResources	NR-52306
7	SARS2 RBD	MyBiosource	MBS8574741
8	SARS2 RBD	NMI	in-house produced
9	SARS2 RBD	Sino Biological	40592-V08H
10	SARS2 N NTD	NMI	in-house produced
11	SARS2 N	Aalto Bioreagents	CK 6404-b
12	SARS2 N	MyBiosource	MBS8309646
13	SARS2 N	NMI	in-house produced
14	SARS2 N	ProSpecBio	SARS-015-b
15	SARS2 E	MyBiosource	MBS8309649
16	SARS S1	Sino Biological	40150-V08B1
17	SARS S dTM	BeiResources	NR-722
18	SARS RBD	Sino Biological	40150-V08B2
19	SARS N	BeiResources	NR-48761
20	SARS M	BeiResources	NR-878
21	SARS E	BeiResources	NR-4284
22	SARS E	ProSpecBio	SARS-252-a
23	OC43 S1	NMI	in-house produced
24	OC43 S	Sino Biological	40607-V08B
25	OC43 N NTD	NMI	in-house produced
26	OC43 N	NMI	in-house produced
27	NL63 S1	NMI	in-house produced
28	NL63 S1	Sino Biological	40600-V08H

No.	CoV strain and antigen	Manufacturer / Vendor	Catalogue number
29	NL63 N NTD	NMI	in-house produced
30	NL63 N	NMI	in-house produced
31	NL63 N	ProSpecBio	SARS-003-a
32	MERS S2	Sino Biological	40070-V08B
33	MERS S1	Sino Biological	40069-V08H
34	MERS RBD	MyBiosource	MBS430256
35	HKU1 S1	NMI	in-house produced
36	HKU1 S1	Sino Biological	40021-V08H
37	HKU1 N NTD	NMI	in-house produced
38	HKU1 N	NMI	in-house produced
39	229E S1	NMI	in-house produced
40	229E S1	Sino Biological	40601-V08H
41	229E N NTD	NMI	in-house produced
42	229E N	NMI	in-house produced
43	229E N	ProSpecBio	SARS-001-a