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 D2.5	**Report**
Title of Deliverable:	**Production of assay components in large quantities to meet requirements for screening of >10,000 samples**
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D2.5 Production of assay components in large quantities to meet requirements for screening of >10,000 samples

Following technical and clinical assay validation shown in previous deliverables D2.3 and D2.4, the established MULTICOV-AB assay was prepared to permit screening of large sample cohorts with consistent and reproducible results. To achieve this, production processes were scaled up where possible and all assay components were produced in mass.

For measurements with the assay, standard plate layouts were conceptualized using 96-well plates for manual processing or 384-well plates to enable high-content semi-automated screening. Plate layouts are shown in Figure 1. Furthermore, a new set of three quality control samples (QC) was created following the principles described in Planatscher et al. (*Scientific Reports (2013) 3, 3259*). They were titrated to match the cut-off values separating SARS-CoV-2 antibody-reactive from non-reactive samples as described in Deliverable D2.3. Such QC samples are crucial for large scale screenings, as they guarantee comparable results between assay runs by enabling normalization of sample MFI values to QC MFI values. A sufficient volume of QC samples and assay blanks (consisting of assay buffer only) was prepared to allow duplicate or octuple measurement on 96-well plates and 384-well plates, respectively.

Table 1 shows calculations for assay production to accommodate measurement of >10,000 samples. One MULTICOV-AB assay kits here consists of the following components: **Assay Buffer, 25x Bead Stock, PE-anti-human-IgG, MULTICOV-AB QC samples 1-3 and the MULTICOV-AB antigen panel.**

a

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S01	S09	S17	S25	S33	S41	S49	S57	S65	S73	S81
B	Blank	S02	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82
C	QC1	S03	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83
D	QC1	S04	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84
E	QC2	S05	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
F	QC2	S06	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
G	QC3	S07	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
H	QC3	S08	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88

b

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	Blank	Blank	S01	S17	S33	S49	S65	S81	S97	S113	S129	S145	S161	S177	S193	S209	S225	S241	S257	S273	S289	S305	S321	S337
B	Blank	Blank	S02	S18	S34	S50	S66	S82	S98	S114	S130	S146	S162	S178	S194	S210	S226	S242	S258	S274	S290	S306	S322	S338
C	Blank	Blank	S03	S19	S35	S51	S67	S83	S99	S115	S131	S147	S163	S179	S195	S211	S227	S243	S259	S275	S291	S307	S323	S339
D	Blank	Blank	S04	S20	S36	S52	S68	S84	S100	S116	S132	S148	S164	S180	S196	S212	S228	S244	S260	S276	S292	S308	S324	S340
E	QC1	QC1	S05	S21	S37	S53	S69	S85	S101	S117	S133	S149	S165	S181	S197	S213	S229	S245	S261	S277	S293	S309	S325	S341
F	QC1	QC1	S06	S22	S38	S54	S70	S86	S102	S118	S134	S150	S166	S182	S198	S214	S230	S246	S262	S278	S294	S310	S326	S342
G	QC1	QC1	S07	S23	S39	S55	S71	S87	S103	S119	S135	S151	S167	S183	S199	S215	S231	S247	S263	S279	S295	S311	S327	S343
H	QC1	QC1	S08	S24	S40	S56	S72	S88	S104	S120	S136	S152	S168	S184	S200	S216	S232	S248	S264	S280	S296	S312	S328	S344
I	QC2	QC2	S09	S25	S41	S57	S73	S89	S105	S121	S137	S153	S169	S185	S201	S217	S233	S249	S265	S281	S297	S313	S329	S345
J	QC2	QC2	S10	S26	S42	S58	S74	S90	S106	S122	S138	S154	S170	S186	S202	S218	S234	S250	S266	S282	S298	S314	S330	S346
K	QC2	QC2	S11	S27	S43	S59	S75	S91	S107	S123	S139	S155	S171	S187	S203	S219	S235	S251	S267	S283	S299	S315	S331	S347
L	QC2	QC2	S12	S28	S44	S60	S76	S92	S108	S124	S140	S156	S172	S188	S204	S220	S236	S252	S268	S284	S300	S316	S332	S348
M	QC3	QC3	S13	S29	S45	S61	S77	S93	S109	S125	S141	S157	S173	S189	S205	S221	S237	S253	S269	S285	S301	S317	S333	S349
N	QC3	QC3	S14	S30	S46	S62	S78	S94	S110	S126	S142	S158	S174	S190	S206	S222	S238	S254	S270	S286	S302	S318	S334	S350
O	QC3	QC3	S15	S31	S47	S63	S79	S95	S111	S127	S143	S159	S175	S191	S207	S223	S239	S255	S271	S287	S303	S319	S335	S351
P	QC3	QC3	S16	S32	S48	S64	S80	S96	S112	S128	S144	S160	S176	S192	S208	S224	S240	S256	S272	S288	S304	S320	S336	S352

Figure 1: MULTICOV-AB plate layouts for 96-well (a) and 384-well (b) plates

Table 1: Material calculation overview – Upscaling of MULTICOV-AB from a single kit unit (88 samples) to 115 kit units (10,120 samples)

Material Calculation overview		Kit Unit	Samples	Kit Unit	Samples
		1	88	115	10120
Reagent	Description	Required amount*	Unit	Required amount*	Unit
Assay Buffer	Ready-to use assay buffer	28	mL	3220	mL
25x Bead Stock	Bead stock 25x concentrated (800 Beads per well per Bead ID)	110	µL	12650	µL
PE-anti-human-IgG	PE-labelled anti-human-IgG antibody	20	µL	2300	µL
MULTICOV-AB QC sample 1	Pre-diluted sample aliquots to be processed with every assay run	1	Aliquots	115	Aliquots
MULTICOV-AB QC sample 2	Pre-diluted sample aliquots to be processed with every assay run	1	Aliquots	115	Aliquots
MULTICOV-AB QC sample 3	Pre-diluted sample aliquots to be processed with every assay run	1	Aliquots	115	Aliquots

*including at least 10% excess volume for any stock solutions

For optimal technical processing of assay runs, all MULTICOV-AB kit reagents are prepared as follows:

Assay Buffer

MULTICOV-AB assay buffer was prepared in one large batch using high volume containers. The produced batch was aliquoted at 30mL and aliquots were stored at -20°C so that only one aliquot has to be thawed per assay run.

25x Bead Stock

MULTICOV-AB antigens (listed in in the previous deliverable D2.2) were produced or purchased in sufficient quantities to accommodate production of antigen-coupled beads for screening of >10,000 samples. Coupling of antigens to MAGPLEX microspheres (beads) was performed to generate 1mL (equal to $12.5*10^6$ beads) per MULTICOV-AB bead ID. The process of antigen production and coupling is described in more detail in Becker, Strengert et al. *(Nature Communications (2021)12:1152)*. For ease of use, the single MULTICOV-AB bead IDs were pre-mixed at a 25x concentration per bead ID matching a final assay concentration of 800 beads per well per ID. The resulting 25x bead stock was aliquoted at 1mL and stored at 4°C.

PE-anti-human-IgG

Phycoetythrin-labelled antibodies against human IgG were purchased from a commercial vendor in a lyophilized state. To ensure uniform performance of the secondary antibody, all vials came from the same production lot. Reconstitution of multiple vials was performed simultaneously and reconstituted PE-anti-human-IgG solutions were mixed to ensure uniform reactivity throughout the assay batch. The PE-anti-human-IgG stock solution was aliquoted at 500µL and stored at 4°C protected from light.

MULTICOV-AB QC samples 1-3

QC samples consisting of a mixture of different serum samples were prepared and pre-diluted to a 1:200 dilution in assay buffer, aliquoted at 60µL and stored at -80°C. The resulting aliquots can be thawed and readily used to add directly onto the assay plate (resulting in a final dilution of 1:400, as is used for all serum samples).