coresma

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 D2.4	**Report**
Title of Deliverable:	**Final clinical validation of the multiplex serological immunoassay finished**
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]

D2.4 Final clinical validation of the multiplex serological immunoassay finished

Following the initial establishment and technical validation of MULTICOV-AB, a multiplex-bead based serological assay (described already in deliverables D2.1, D2.2 and D2.3), we proceeded to the next phase of assay development and determined diagnostic accuracy of MULTICOV-AB. Diagnostic accuracy is defined as the correct separation of blood samples from SARS-CoV-2 infected or COVID-19 convalescents from those of SARS-CoV-2 naïve individuals. For this purpose, we investigated a large sample set of 866 serum or plasma samples from SARS-CoV-2 naïve or pre-pandemic donors and 310 serum or plasma samples from infected or convalescent donors and screened it with MULTICOV-AB. A breakdown of individual sample characteristics is listed in Table 1

More in detail, we assessed the performance of each individual SARS-CoV-2-specific antigen within the MULTICOV-AB multiplex panel using Receiver operating characteristic (ROC) analysis as shown in Figure 1. The analysis revealed that the best predictors of infection were the presence of SARS-CoV-2 Spike Trimer- and RBD-specific IgG and IgA. Cut-off values, which are key for defining a sample status were assigned to create maximum levels of specificity to avoid false-positive test results, which is crucial in serology, as the presence of antibodies implies a level of immune protection and leads to inaccurate epidemiological seroprevalence estimates. We found that sample classification based on combination of RBD and Spike antigen-specific antibodies (both cut-off-values >1) lead to a 100% specificity while retaining high levels of sensitivity. By using a combination of IgG and IgA positivity, we were able to further increase assay sensitivity to 90% as IgA antibody isotypes develop earlier than the IgG humoral response (see Table 2).

Table 1: Characteristics of samples used for clinical validation (Table is adapted from Supplementary Table 2 of *Becker, Strengert et al., Nature Communications (2021)12:1152*).

Age	**≤39**				**40-59**				**≥60**				**NA**				**∑**
n	**299**	**(25.4 %)**			**241**	**(20.5 %)**			**475**	**(40.4 %)**			**161**	**(13.7 %)**			**1,176**
Gender	**M**		**F**		**M**		**F**		**M**		**F**		**M**		**F**	**NA***	
n	**139**	**(11.8 %)**	**160**	**(13.6 %)**	**144**	**(12.2 %)**	**97**	**(8.2 %)**	**271**	**(23 %)**	**204**	**(17.3 %)**	**5**	**(0.4 %)**	**3**	**(0.3 %)**	**153**	**(13 %)**	**1,176**
SARS-CoV-2-infected (total)	**60**	**(19.4 %)**	**51**	**(16.5 %)**	**71**	**(22.9 %)**	**63**	**(20.3 %)**	**42**	**(13.5 %)**	**17**	**(5.5 %)**	**3**	**(1 %)**	**3**	**(1 %)**	**0**	**(0 %)**	**310**
Hospitalized (for COVID19)	**6**	**(10.9 %)**	**2**	**(3.6 %)**	**14**	**(25.5 %)**	**6**	**(10.9 %)**	**23**	**(41.8 %)**	**4**	**(7.3 %)**	**0**	**(0 %)**	**0**	**(0 %)**	**0**	**(0 %)**	55
Non-Hospitalized	**52**	**(25 %)**	**43**	**(20.7 %)**	**49**	**(23.6 %)**	**43**	**(20.7 %)**	**13**	**(6.3 %)**	**8**	**(3.8 %)**	**0**	**(0 %)**	**0**	**(0 %)**	**0**	**(0 %)**	208
Hospitalisation NA	**2**	**(4.3 %)**	**6**	**(12.8 %)**	**8**	**(17 %)**	**14**	**(29.8 %)**	**6**	**(12.8 %)**	**5**	**(10.6 %)**	**3**	**(6.4 %)**	**3**	**(6.4 %)**	**0**	**(0 %)**	47
Patients with time series	**2**	**(40 %)**	**0**	**(0 %)**	**0**	**(0 %)**	**0**	**(0 %)**	**2**	**(40 %)**	**1**	**(20 %)**	**0**	**(0 %)**	**0**	**(0 %)**	**0**	**(0 %)**	5
SARS-CoV-2-uninfected (total)	**79**	**(9.1 %)**	**109**	**(12.6 %)**	**73**	**(8.4 %)**	**34**	**(3.9 %)**	**229**	**(26.4 %)**	**187**	**(21.6 %)**	**2**	**(0.2 %)**	**0**	**(0 %)**	**153**	**(17.7 %)**	**866**
Sample during pandemic	**10**	**(15.4 %)**	**10**	**(15.4 %)**	**12**	**(18.5 %)**	**14**	**(21.5 %)**	**7**	**(10.8 %)**	**5**	**(7.7 %)**	**1**	**(1.5 %)**	**0**	**(0 %)**	**6**	**(9.2 %)**	**65**
Sample pre-pandemic	**69**	**(8.6 %)**	**99**	**(12.4 %)**	**61**	**(7.6 %)**	**20**	**(2.5 %)**	**222**	**(27.7 %)**	**182**	**(22.7 %)**	**1**	**(0.1 %)**	**0**	**(0 %)**	**147**	**(18.4 %)**	**801**
Previous PCR confirmed hCoV Infection	19	(12.9 %)	18	(12.2 %)	45	(30.6 %)	20	(13.6 %)	29	(19.7 %)	16	(10.9 %)	0	(0 %)	0	(0 %)	0	(0 %)	147
NL63	*2*	*(20 %)*	*0*	*(0 %)*	*3*	*(30 %)*	*1*	*(10 %)*	*2*	*(20 %)*	*2*	*(20 %)*	*0*	*(0 %)*	*0*	*(0 %)*	*0*	*(0 %)*	*10*
229	*5*	*(25 %)*	*1*	*(5 %)*	*4*	*(20 %)*	*1*	*(5 %)*	*5*	*(25 %)*	*4*	*(20 %)*	*0*	*(0 %)*	*0*	*(0 %)*	*0*	*(0 %)*	*20*
OC43	*0*	*(0 %)*	*1*	*(3.7 %)*	*14*	*(51.9 %)*	*1*	*(3.7 %)*	*6*	*(22.2 %)*	*5*	*(18.5 %)*	*0*	*(0 %)*	*0*	*(0 %)*	*0*	*(0 %)*	*27*
HKU1	*3*	*(20 %)*	*1*	*(6.7 %)*	*4*	*(26.7 %)*	*2*	*(13.3 %)*	*5*	*(33.3 %)*	*0*	*(0 %)*	*0*	*(0 %)*	*0*	*(0 %)*	*0*	*(0 %)*	*15*
unknown hCoV	*9*	*(12 %)*	*15*	*(20 %)*	*20*	*(26.7 %)*	*15*	*(20 %)*	*11*	*(14.7 %)*	*5*	*(6.7 %)*	*0*	*(0 %)*	*0*	*(0 %)*	*0*	*(0 %)*	*75*
Pregnant	0	(0 %)	9	(90 %)	0	(0 %)	1	(10 %)	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	10
RF/HAMA samples	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	6	(100 %)	6
PCT > 3 ng/mL	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	0	(0 %)	21	(100 %)	21
Neuroinflammatory disease	6	(40 %)	6	(40 %)	1	(6.7 %)	0	(0 %)	1	(6.7 %)	1	(6.7 %)	0	(0 %)	0	(0 %)	0	(0 %)	15

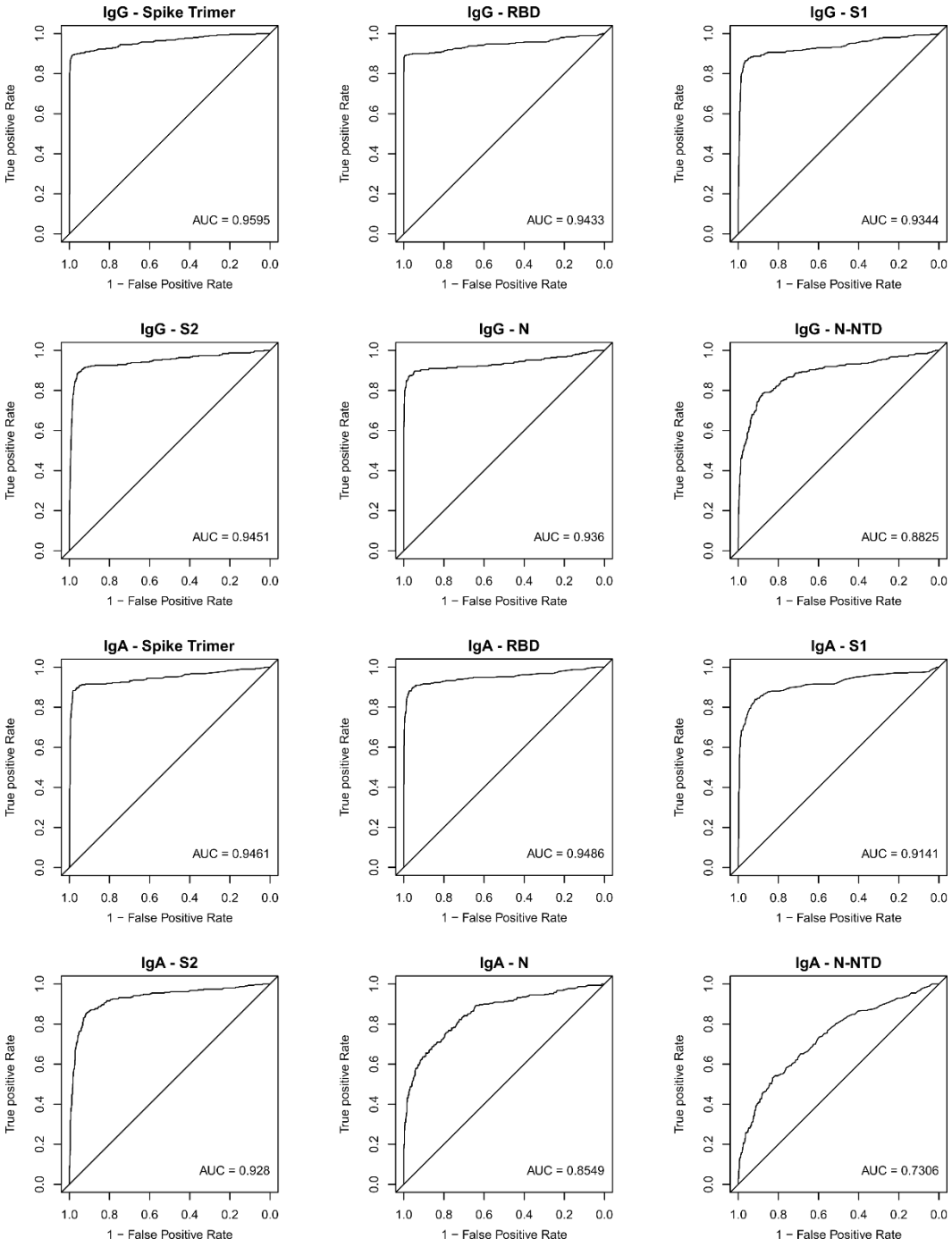

Figure 1: ROC analysis for IgG and IgA detection of SARS-CoV-2 antigens based on a sample set from 866 uninfected and 310 infected individuals used for MULTICOV-AB clinical validation. True positive rate is displayed against 1 - false positive rate, corresponding to sensitivity and specificity at a given cut-off. Area under the curve (AUC)-values indicating individual antigen performance are shown. (This figure is published as Supplementary Figure 4 of *Becker, Strengert et al., Nature Communications (2021)12:1152*).

Table 2: MULTICOV-AB assay specification based on a sample set from 866 uninfected and 310 infected individuals. Sensitivity and specificity sample set for IgA and IgG based on a single antigen or a combined cut-off of Spike Trimer and RBD (IgG or IgA overall) or combined isotype cut-off (IgG and IgA) are shown. (This table is published as Table 3 of *Becker, Strengert al., Nature Communications (2021)12:1152*).

	correctly classified		Sensitivity	Specificity	PPV at 3%	NPV at 3%
	infected	uninfected	(95 % CI)	(95 % CI)	prevalence	prevalence
IgG Spike Trimer	277	849	89.4 % (85.4 - 92.6 %)	98.0 % (96.9 - 98.9 %)	58.5 %	99.7 %
IgG RBD	276	862	89.0 % (85 - 92.3 %)	99.5 % (98.8 - 99.9 %)	85.7 %	99.7 %
IgG overall	275	866	88.7 % (84.6 - 92 %)	100.0 % (99.6 - 100 %)	100 %	99.7 %
IgA Spike Trimer	272	850	87.7 % (83.6 - 91.2 %)	98.2 % (97 - 98.9 %)	59.5 %	99.6 %
IgA RBD	255	855	82.3 % (77.5 - 86.3 %)	98.7 % (97.7 - 99.4 %)	66.7 %	99.4 %
IgA overall	254	864	81.9 % (77.2 - 86.1 %)	99.8 % (99.2 - 100 %)	91.7 %	99.4 %
Combined IgA & IgG	279	866	90.0 % (86.1 - 93.1 %)	100.0 % (99.6 - 100 %)	100 %	99.7 %

To additionally validate assay performance, we compared MULTICOV-AB sensitivity and specificity to three commercial CE-certified in vitro-diagnostic SARS-CoV-2 serology assays, which were in the 2nd quarter of 2020 most commonly used in clinical laboratories. This comparison was carried out in a subset of samples from our extended MULTICOV-AB validation set and consisted of serum from 205 infected and 72 SARS-CoV-2 naïve donors. A All samples which were classified differently between the four antibody tests and are shown in a heatmap in Figure 2. Overall, MULTICOV-AB outperformed all three commercial assays in sensitivity and one commercial assay in specificity, which was below 100%.

A more detailed description of clinical MULTICOV-AB validation has already been published in Becker, Strengert et al. “Exploring beyond Exploring beyond clinical routine SARS-CoV-2 serology using MultiCoV-Ab to evaluate endemic coronavirus cross-reactivity” (Nature Communications *(2021)12:1152).*

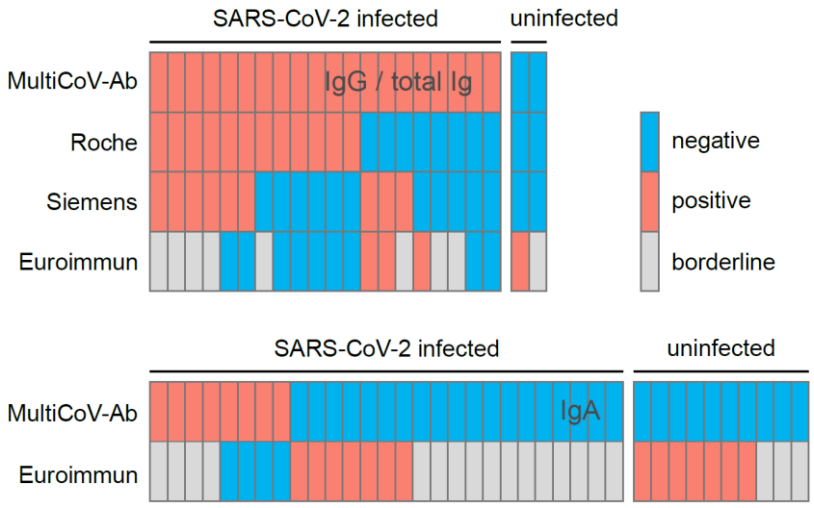

Figure 2: Sample set of 205 infected and 72 naïve donors was used to compare assay performance of the MultiCoV-Ab using Spike Trimer and RBD antigens with commercially available single analyte SARS-CoV-2 IVD assays which detect total Ig (Elecsys Anti-SARS-CoV-2 (Roche); ADVIA Centaur SARS-CoV-2 Total (COV2T; Siemens Healthineers)) or IgG (Anti-SARS-CoV-2-ELISA - IgG or IgA.SARS-CoV-2 infection status of samples based on PCR diagnostic is indicated as SARS-CoV-2 positive or negative. Antibody test results were classified as negative (blue), positive (red), or borderline (gray) as per the manufacturer's definition. Only samples with divergent antibody test results are shown. (This figure is published as Figure 1b of *Becker, Strengert et al., Nature Communications (2021)12:1152*).

Overall, the developed multiplex serological assay MULTICOV-AB to detect SARS-CoV-2 exposure, showed excellent clinical performance characteristics, which makes it not only suitable for screening of large sample cohorts to accurately determine the seroconversion rate in the general population but also to better understand the role of the humoral immune response in manifestation and progression of COVID-19.