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Initial characterisation of commercially available ELISA tests and the immune response of the clinically correlated SARS-CoV-2 biobank "SERO-BL-COVID-19" collected during the pandemic onset in Switzerland

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Abstract

Background To accurately measure seroprevalance in the population, both the expected immune response as well as the assay performances have to be well characterised. Here, we describe the collection and initial characterisation of a blood and saliva biobank obtained after the initial peak of the SARS-CoV-2 pandemic in Switzerland.

Methods Two laboratory ELISAs measuring IgA & IgG (Euroimmun), and IgM & IgG (Epitope Diagnostics) were used to characterise the biobank collected from 349 re- and convalescent patients from the canton of Basel-Landschaft.

Findings The antibody response in terms of recognized epitopes is diverse, especially in oligosymptomatic patients, while the average strength of the antibody response of the population does correlate with the severity of the disease at each time point.

Interpretation The diverse immune response presents a challenge when conducting

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epidemiological studies as the used assays only detect ∼90% of the oligosymptomatic cases. This problem cannot be rectified by using more sensitive assay setting as they concomitantly reduce specificity.

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Keywords: COVID-19, SARS-CoV-2, Serology, Biobank, Immune response, oligosymptomatic patients, ELISA

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1. Introduction

Effective host responses to viral infections, including those to coronaviruses, are driven by adaptive immunity [1]. For endemic or previously emerging coronaviruses, the antibody response has been correlated with protection from re-infection for a varying period of time. In SARS-CoV-2 infections, most studies on antibody kinetics are based on severe or hospitalized patients, even though subclinical or even oligosymptomatic patients represent the majority of cases. Less severe cases of SARS-CoV-2 (as well as other endemic CoVs) are associated with lower antibody responses, and therefore pose a challenge for accurate detection using serological assays.

However, one of the most important correlates of immunological protection is the presence of neutralizing antibodies, which is preferably measured by using functional assays with replication competent virus [2]. For COVID-19 patients, such assays are time consuming and must be conducted in Biosafety Level 3 facilities, which renders them infeasible for wide-scale testing. Some alternative functional assays are based on pseudotyped or chimeric viral particles [3], but these reagents are neither trivial to produce nor do they scale to large sample sizes. The most feasible alternative assays are therefore binding assays, such as enzyme-linked immunosorbent assays (ELISA), and preferably report on the quantity of antibodies binding to neutralizing epitopes such as the receptor binding domain (RBD) of the spike protein [4].

Current clinically approved ELISA tests either bind to the nucelocapsid (NCP) protein or (part of) the spike protein (which includes the RBD). Both of these proteins, but especially the NCP, are known to generate a strong host immune response in other beta-coronaviruses [5, 6]. In contrast, nearly all neutralising antibodies against SARS-CoV-2 discovered to date bind to the small RBD portion of the spike protein [7, 8], and currently available commercial assays indeed insufficiently predict neutralisation [2]. The

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26 presence and characterization of antibody responses in COVID-19 patients by serological
27 assays has been described in several reports [9, 10, 11, 12, 13, 14]; a key observation is
28 that across many patients, antibodies are detected at ~ 10 days post-onset of symptoms.
29 The performance of assays is characterized by their sensitivity and specificity [15].
30 To-date, most commercial ELISA performance validations were obtained from biobanks
31 relying on hospitalised patients; this positive patient cohort will likely have higher anti-
32 body levels than milder, non-hospitalized patients [16]. This is also observed for SARS-
33 CoV-2 infections [17]. It is thus unclear if the available tests are sufficiently sensitive to
34 also detect oligosymptomatic cases.
35 Here, we describe the collection and initial analysis of a blood biobank representa-
36 tive for the observed COVID-19 symptomatic range in the population of Switzerland.
37 The positive cohort in this biobank consists of 341 samples obtained from participants
38 determined to have SARS-CoV-2 (PCR-positive test) in various symptomatic and post-
39 symptomatic stages. The negative cohorts include 115 samples obtained from PCR-
40 negative tested participants, and 150 samples of blood donors from the 2016/17 flu
41 season. The distribution in age and disease severity in this biobank is similar to that
42 reported for other areas in Western Europe. For each sample, we measured the antibody
43 response toward the NCP and S1 proteins using the ELISA tests for IgM and IgG from
44 Epitope Diagnostics and IgA and IgG from Euroimmun, and characterized the perfor-
45 mance of these assays. The specificity of both the Euroimmun IgG and the Epitope
46 Diagnostics IgM assays was close to 100%, while the other two tests showed specificities
47 of $\sim 96\%$ and lower. The sensitivity of the IgA and IgG tests was only sufficient to detect
48 $\sim 90\%$ of the cases, while the IgM test only detected $\sim 50\%$. Previous studies reported
49 a low and late IgM response, especially in less severe COVID-19 patients [10, 11], which
50 might partially explain the low IgM test sensitivity. Taken together, our data indicates
51 that the immune response in oligosymptomatic patients is diverse and ill captured with
52 the two employed serological assays.

53 2. Materials & methods

54 *2.1. Ethics statement*

55 This study is part of the project 'COVID-19 in Baselland Investigation and Validation
56 of Serological Diagnostic Assays and Epidemiological Study of Sars-CoV-2 specific Anti-
57 body Responses (SERO-BL-COVID-19)' approved by the ethics board "Ethikkommission

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58 Nordwest- und Zentralschweiz (EKNZ)", Hebelstrasse 53, 4056 Basel representative of
59 Swissethics under the number (2020-00816).

60 Every participant has received a written informed consent at least 24 hours before
61 participating in this study (attached original document in German language). The par-
62 ticipants had to sign the written informed consent and needed to show it in order to be
63 given access to the test facility. The participants could withdraw their participation at
64 any time without stating any reason.

65 *2.2. Collection of samples*

66 *2.2.1. Blood Collection*

67 Venous blood was taken by puncturing a disinfected cubital or similar area using a
68 BD safety-lock system into a vacutainer. In total, 10–12 mL each for EDTA-blood serum
69 was taken. The blood collection was performed by a medical assistant or nurse. After
70 blood collection, the samples were either transferred to the diagnostic lab or directly
71 processed on site in the make-shift laboratory.

72 *2.2.2. Saliva Collection*

73 Saliva was collected non-invasively using the dedicated Salivette tubes (Sarstedt
74 Cat. # 51.1534). In short, the participant delivered saliva into an adsorbent filter, which
75 was then placed by the participant in the Salivette tube. After handover to the medical
76 staff, the saliva was centrifuged on site at 4°C using 1,000× g for 2 min to remove cells
77 and debris. The tube was then rapidly frozen using a salted ice-water bath and stored
78 at −20°C before transporting to the lab on dry ice and storage at −80°C until further
79 use.

80 *2.2.3. Plasma and PBMC isolation and cell cryopreservation*

81 Density gradient separation was used in peripheral blood mononuclear cell (PBMC)
82 isolation. 12 mL of fresh donor blood was received in 3× 4 mL plastic whole blood tubes
83 with spray-coated K_2EDTA BD Vacutrainer™ (Becton Dickinson, Cat. # 367844). The
84 whole blood was diluted in 1:1 ratio with 12 mL of PBS (w/o Ca^{2+} and Mg^{2+}). The
85 total volume of diluted blood (24 mL) was gently and slowly layered on 14 mL of Ficoll
86 Lymphoprep^{TM} (STEMCELL, Cat. # 07861). Samples were centrifuged at 400× g 40
87 min, 22°C, no brakes. 14 mL of plasma was transferred in a 15 mL conical tube and
88 stored at 4°C. The layer of mononuclear cells was aspirated and transferred in a 50
89 mL conical tube containing 25 mL of PBS (w/o Ca^{2+} and Mg^{2+}). Cells were washed

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90 300× g 8 min, 22°C, with brakes. Washing was repeated with an additional 25 mL of
91 PBS (w/o Ca^{2+} and Mg^{2+}). Mononuclear cells were subsequently resuspended in 1 mL
92 of freezing media (heat inactivated FBS supplemented with 10% DMSO) and aliquoted
93 into two 1.5 mL cryogenic tubes (Nalgene System, Thermo Scientific, Cat. # 5000-1020).
94 The cryogenic tubes were put into freezing containers Mr.Frosty™ (Thermo Scientific,
95 Cat. # 5100-0001) and the containers were immediately placed into an −80°C freezer
96 for 24 hrs, and then transferred into a liquid nitrogen tank.

97 *2.2.4. Point of care validation*

98 To perform the point of care test (POCT) validation, a capillary blood sample was
99 taken from each subject by puncturing the end of a finger and taking the blood with
100 a micro pipette. Immediately after collecting, the blood was put in the lateral flow
101 chamber of the POCT and after 15 minutes the result was visually scored as positive
102 or negative by the medical assistant. Additionally, the tests were imaged using a Nikon
103 D5000 camera.

104 *2.2.5. Blood donor cohort*

105 Samples from nonremunerated blood donors originate from the Swiss cantons of Thur-
106 gau, Basel, Bern, Waadt and Geneva, and were taken during the pre-pandemic period
107 16th and 17th December 2016. These samples were frozen as EDTA plasmas on mi-
108 crotiterplates for −20°C.

109 *2.3. ELISA analysis*

110 The following four commercially available immunoassays were characterized in the
111 study: the Anti-SARS-CoV-2-ELISA-IgA (Euroimmun AG, Lübeck, # EI 2606-9601 A),
112 the Anti-SARS-CoV-2-ELISA-IgAG (Euroimmun AG, Lübeck, # EI 2606-9601 G), the
113 EDI Novel Coronavirus COVID-19 IgM ELISA kit (Epitope Diagnostics, Inc., # KT-
114 1033) and the EDI Novel Coronavirus COVID-19 IgG ELISA kit (Epitope Diagnostics,
115 Inc., # KT-1032). All ELISA kits were CE and IVD labeled.

116 To enable a quantitative comparison between ELISA experiments, we calculated fold
117 changes in OD relative to the assay- and run-specific cut-off values ($OD_{sample}/OD_{cut\text{-}off}$),
118 where $OD_{cut\text{-}off} = 1.1 \times OD_{cal}$ for both Euroimmun ELISAs, $OD_{cut\text{-}off} = (1.1 + 0.18) \times OD_{NC}$
119 for EDI IgG and $OD_{cut\text{-}off} = (1.1 + 0.10) \times OD_{NC}$ for EDI IgM, where OD_{cal} and OD_{NC}
120 are calibration respectively average negative control values as described by the manufac-
121 turers. Note that fold changes are not comparable between Euroimmun and EDI ELISA

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122 test kits because the assays show large differences in dynamic range and saturation.
123 Detailed calculation can be found in the supplementary methods.

124 *2.4. Statistical analysis*

125 Patient data and results of POCTs were originally stored in the REDCap database
126 system of the Canton Hospital Basel-Landschaft. Results from ELISA tests were entered
127 into Excel worksheets. All data were preprocessed and a common database created
128 using in-house scripts in R [18]. Statistical analysis and creation of figures and tables
129 was carried out using R; binomial confidence intervals are 95%-Clopper-Pearson intervals
130 calculated using exactci() from package PropCIs [19]. A refresher for the calculation of
131 specificity and sensitivity calculation can be found in the supplementary methods.

132 *2.5. Role of the funding source*

133 The sponsor had no role in study design; in the collection, analysis, and interpretation
134 of data; in the writing of the report; or in the decision to submit the paper for publication.

135 3. Results

136 *3.1. Study design & cohorts*

137 The goal of our study design was to collect a representative cohort of COVID-19
138 disease manifestation during the first wave of COVID-19 in the canton Basel-Landschaft,
139 Switzerland. During the initial phase of the pandemic, only people in risk groups showing
140 symptoms were tested; later, testing was extended to all people showing symptoms and
141 5311 people had been tested in the canton at the beginning of study recruitment, with
142 802 (15.1%) positive and 4509 (84.9%) negative PCR test results. The cases were mostly
143 observed close to or in areas with a high frequency of commuting to the city of Basel,
144 but the ratio of positive tests showed no apparent bias for or against rural communities.
145 All RT-PCR-tested individuals were eligible for participation except when they were
146 <18 years of age, had a severely compromised immune system, were hospitalized at
147 the time of sample collection, or were deceased. From these, 349 positive individuals
148 committed to participating in the study, and 111 negative individuals were randomly
149 selected. We aimed for sufficient sample size for two positive cohorts: an 'acute' cohort
150 with diagnosed COVID-19 up to 12 days before study entry, and a 'convalescent' cohort
151 with more than 12 days between positive diagnosis and study entry.

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152 Individuals were continuously recruited during a 2 week window from 11. April
153 2020 to 22. April 2020 and visited the 'Abklärungsstation COVID-19' in Münchenstein,
154 Switzerland. The medical history and the status were recorded in a doctors interview,
155 the vital parameters were acquired, and saliva and blood samples were collected. All
156 participants of the positive cohort were guided through the building, while the negative
157 cohort was examined in a make-shift field hospital erected next to the building to mini-
158 mize the danger of infection with COVID-19. Participant characteristics are summarized
159 in Table 1.

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Cohort	PCR pos, $\leq$ 7d (N = 31)	PCR pos, $>$ 7d & $\leq$ 12d (N = 46)	PCR pos, $>$ 12d (N = 272)	PCR neg, $>$ 5d (N = 111)
Sex				
female	17 (55%)	25 (54%)	130 (48%)	63 (57%)
male	14 (45%)	21 (46%)	142 (52%)	48 (43%)
Age				
years, median (range)	45 (21-80)	51 (20-80)	51.5 (17-93)	48 (19-87)
Weight				
kg, median (range)	73 (51-110)	72 (40-109)	76 (49-135)	73 (47-130)
Height				
cm, median (range)	173.5 (157-187)	172 (150-191)	173 (72-198)	172 (149-195)
SpO2				
%, median (range)	98 (93-99)	98 (88-99)	98 (85-99)	98 (95-99)
Total days ill				
0–5 days	6 (19%)	1 (2%)	28 (10%)	—
5–10 days	15 (48%)	23 (50%)	146 (54%)	—
10–14 days	10 (32%)	22 (48%)	98 (36%)	—

Table 1: Characteristics of patients included in the study.

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160 Distribution of ages and gender for the positive cohort are similar to the age and gen-
161 der structure of the canton, except for the age between 40–65 which are over-represented
162 and >80 which are under-represented (Table 2 and Supplementary Figure 1). From the
163 349 positive participants, 35 (10%) were bedridden during the acute disease, 62 (18%)
164 required help for their daily activities, while 244 (72%) had no restrictions. Similar dis-
165 tributions are reported elsewhere. All age groups were affected equally, however severe
166 cases were more pronounced in the older population. Increasing disease severity cor-
167 related with the experienced symptoms; bedridden cases suffered approximately 10 or
168 more days, cases requiring help ∼10 days, and the oligosymptomatic cases between 5 to
169 15 days.

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	Age groups						
	15–19*	20–29	30–39	40–49	50–64	65–79	80+
Overall	5 (1.1%)	55 (12%)	64 (13.9%)	99 (21.5%)	159 (34.6%)	68 (14.8%)	10 (2.2%)
Severity of illness							
Not ill	1 (20%)	13 (24%)	23 (36%)	24 (24%)	29 (18%)	18 (26%)	3 (30%)
No restrictions	4 (80%)	36 (65%)	34 (53%)	53 (54%)	91 (57%)	28 (41%)	4 (40%)
Help needed	0 (0%)	6 (11%)	6 (9%)	18 (18%)	22 (14%)	9 (13%)	1 (10%)
Bedridden	0 (0%)	0 (0%)	1 (2%)	4 (4%)	17 (11%)	13 (19%)	2 (20%)
Total days ill							
0–5 days	0 (0%)	7 (17%)	5 (12%)	7 (9%)	11 (8%)	5 (10%)	0 (0%)
5–10 days	3 (75%)	19 (45%)	24 (59%)	39 (52%)	72 (55%)	24 (48%)	3 (43%)
10–15 days	1 (25%)	16 (38%)	12 (29%)	29 (39%)	47 (36%)	21 (42%)	4 (57%)
Baselland (2019)	13924 (5.6%)	30882 (12.4%)	35666 (14.3%)	39190 (15.7%)	65017 (26.1%)	45175 (18.1%)	19054 (7.7%)

Table 2: Age structure of study cohort stratified by severity and by duration of symptoms. Last row shows distribution of ages 15+ in canton of Basel-Land in 2019 for reference. *Note that our study cohort does not include minors with less than 18 years of age.

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170 We use two negative cohorts; (i) samples from 150 2016/17 influenza period blood
171 donors, and (ii) our negative cohort. These cohorts should strengthen the specificity
172 calculations, but also help to address cross-reactivity to viruses currently in circulation.
173 However, the PCR test is prone to false negatives, and we therefore expect to find a small
174 number of incorrectly diagnosed individuals. In line with the reported false negative
175 rate of 15–25% [20, 21], we identified four individuals with negative PCR results but
176 seroconverted in both Euroimmun IgG and IgA and Epitope Diagnostics (EDI) IgG. We
177 consider these four individuals as false negative PCR results and removed them from the
178 dataset. All results are only influenced marginally by this step.

179 *3.2. Performance characteristics of ELISA tests*

180 *3.2.1. Sensitivity & specificity*

181 We performed the EDI and Euroimmun assays according to the manufacturer's in-
182 structions on all samples. The obtained data were normalized (see Material and Methods
183 section) to make results comparable between experiments. The reported fold-changes are
184 defined as the ratio between measured OD and the classification cut-off OD specified by
185 the manufacturer (Figure 1 & Supplementary Figure 2). Positive/negative classification
186 was performed according to manufacturer's instructions, and the assay performance was
187 calculated therefrom.

188 We used a patient's assignment to the PCR-positive or PCR-negative cohort together
189 with the corresponding ELISA test result for calculating performance characteristics for
190 all four assays. The values considering all data are shown in the top of Table 3. We find
191 specificities of the IgG-based assays of about 98%-99%, while IgA shows considerably
192 lower and IgM considerably higher specificity overall. To determine potential cross-
193 reactivity of the assays, we separately calculated the specificity from the 150 samples
194 of the 2016 blood donor cohort (Table 3 (bottom)). Nevertheless, the histogram of all
195 assays and data show overlap between the cohorts (Supplementary Figure 3).

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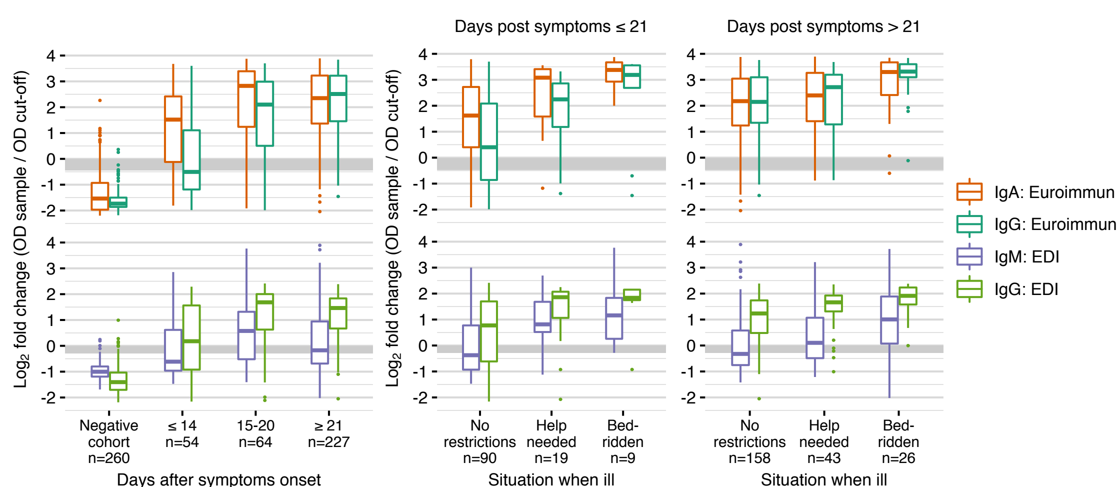

Figure 1: **Overview on ELISA results.** Epitope Diagnostics (EDI) and Euroimmun assays were performed with serum from 607 individuals. The negative cohort consists of 150 serum samples collected during the 2016/17 influenza period and 110 serum samples from PCR-negative individuals from 2020. Positive cases are stratified by days post symptoms onset (left) and additionally stratified by disease severity (right). Fold changes are defined as the ratio between measured OD and the classification cut-off OD specified by the manufacturer. Grey area indicates the range where serum samples are classified as uncertain; samples above (below) this area are classified as positive (negative).

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	EDI				Euroimmun		
	Serum		Plasma		Serum		
	IgM	IgG	IgM	IgG	IgA	IgG	IgG 1.5×
TP	161	287	176	280	304	291	272
FP	3	6	11	12	22	2	0
TN	257 (2)	254 (7)	100 (11)	99 (13)	238 (12)	258 (2)	260 (0)
FN	184 (35)	58 (16)	161 (36)	57 (14)	41 (15)	54 (12)	73 (0)
Se [CI], %	46.7 [41.3, 52.1]	83.2 [78.8, 87]	52.2 [46.7, 57.7]	83.1 [78.6, 86.9]	88.1 [84.2, 91.3]	84.3 [80.1, 88]	78.8 [74.1, 83]
Sp [CI], %	98.8 [96.7, 99.8]	97.7 [95, 99.1]	90.1 [83, 94.9]	89.2 [81.9, 94.3]	91.5 [87.5, 94.6]	99.2 [97.2, 99.9]	100 [98.6, 100]
	2016 blood donors						
TN	150 (0)	147 (3)	—	—	136 (7)	150 (2)	150 (0)
FP	0	3	—	—	14	0	0
Sp [CI], %	100 [97.6, 100]	98 [94.3, 99.6]	—	—	90.7 [84.8, 94.8]	100 [97.6, 100]	100 [97.6, 100]

Table 3: Top: Number true positive (TP), false positive (FP), true negative (TN), false negative (FN), sensitivity (Se) and specificity (Sp) for Epitope Diagnostics (EDI) serum and plasma samples and Euroimmun ELISA serum samples for all cohorts. All samples with uncertain result were considered negative for the analysis (number of uncertain samples shown in brackets). Column IgG 1.5× for Euroimmun corresponds to using a threshold of 1.5 instead of 1.1 for the OD-ratio. Bottom: specificity based on serum samples of negative 2016 blood donor cohort only.

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196 The IgG- and IgA-based assays show overall sensitivities above 87%, while the sen-
197 sitivity of IgM is extremely low at slightly above 50%. However, the different types of
198 antibodies act at different stages of the immune response. We therefore stratified the
199 calculation of assay sensitivities by days after onset of symptoms into three categories:
200 14 days or less, 15 to 20 days, and 21 days or more (Table 4 & Supplementary Fig-
201 ure 4). Sensitivities increase with days after onset of symptoms for both IgG assays from
202 about 50% to 95%. The sensitivity of the IgM assay remains low for all strata, while
203 IgA already shows substantially higher sensitivities than all other assays for less than
204 14 days after onset of symptoms. Specifically, sensitivity is low for the EDI Diagnostics
205 IgM assay, reaching a maximum of 64% for two to three weeks after onset of symptoms.
206 Both IgG assays show overall sensitivities below 90%, but sensitivities increase to 95% or
207 higher three weeks after onset of symptoms. The Euroimmun IgA sensitivity is highest
208 among the four assays for less than two weeks after onset of symptoms with 82% and
209 also reaches about 95% after three weeks or more after onset of symptoms.

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EDI ELISA						
	IgM			IgG		
	≤ 14 days	15–20 days	≥ 21 days	≤ 14 days	15–20 days	≥ 21 days
TP	20	32	109	29	44	214
FN	34 (5)	20 (4)	130 (26)	25 (2)	8 (1)	25 (13)
Se [CI], %	37 [24.3, 51.3]	61.5 [47, 74.7]	45.6 [39.2, 52.2]	53.7 [39.6, 67.4]	84.6 [71.9, 93.1]	89.5 [84.9, 93.1]
Euroimmun ELISA						
	IgA			IgG		
	≤ 14 days	15–20 days	≥ 21 days	≤ 14 days	15–20 days	≥ 21 days
TP	40	46	218	23	42	226
FN	14 (5)	6 (1)	21 (9)	31 (3)	10 (4)	13 (5)
Se [CI], %	74.1 [60.3, 85]	88.5 [76.6, 95.6]	91.2 [86.9, 94.5]	42.6 [29.2, 56.8]	80.8 [67.5, 90.4]	94.6 [90.9, 97.1]

Table 4: Sensitivity of EDI (top) and Euroimmun (bottom) ELISA stratified by days after onset of symptoms. All samples with uncertain result were considered negative for the analysis (number of uncertain samples shown in brackets).

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210 We performed the same analysis stratified by three levels of disease severity—'no
211 restriction', 'help needed', and 'bedridden'—and combined these levels with two levels
212 for days after onset of symptoms: short (≤ 21 days) or long (> 21 days). The resulting
213 sensitivities and specificities and their 95% confidence intervals are given in Table 5. As
214 expected, sensitivities also increase with severity of symptoms for both less and more
215 than three weeks after onset of symptoms, but sample sizes are comparatively small for
216 shorter time and higher severity. Notably, the IgG response is detectable in all samples
217 of the 'bedridden' cohort > 21 days, and is then comparable to the manufacturer's
218 characterisation on this subset.

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	EDI IgM					
	Bedridden		Help needed		No restrictions	
	$\leq$ 21d	$>$ 21d	$\leq$ 21d	$>$ 21d	$\leq$ 21d	$>$ 21d
TP	7	20	15	23	36	60
FN	2 (2)	6 (3)	4 (0)	20 (6)	54 (8)	98 (16)
Se [CI], %	77.8 [40, 97.2]	76.9 [56.4, 91]	78.9 [54.4, 93.9]	53.5 [37.7, 68.8]	40 [29.8, 50.9]	38 [30.4, 46]
	EDI IgG					
	Bedridden		Help needed		No restrictions	
	$\leq$ 21d	$>$ 21d	$\leq$ 21d	$>$ 21d	$\leq$ 21d	$>$ 21d
TP	8	25	17	39	58	140
FN	1 (0)	1 (1)	2 (0)	4 (2)	32 (4)	18 (9)
Se [CI], %	88.9 [51.8, 99.7]	96.2 [80.4, 99.9]	89.5 [66.9, 98.7]	90.7 [77.9, 97.4]	64.4 [53.7, 74.3]	88.6 [82.6, 93.1]
	Euroimmun IgA					
	Bedridden		Help needed		No restrictions	
	$\leq$ 21d	$>$ 21d	$\leq$ 21d	$>$ 21d	$\leq$ 21d	$>$ 21d
TP	9	25	18	40	71	141
FN	0 (0)	1 (0)	1 (0)	3 (2)	19 (6)	17 (7)
Se [CI], %	100 [66.4, 100]	96.2 [80.4, 99.9]	94.7 [74, 99.9]	93 [80.9, 98.5]	78.9 [69, 86.8]	89.2 [83.3, 93.6]
	Euroimmun IgG					
	Bedridden		Help needed		No restrictions	
	$\leq$ 21d	$>$ 21d	$\leq$ 21d	$>$ 21d	$\leq$ 21d	$>$ 21d
TP	7	25	16	42	53	148
FN	2 (0)	1 (1)	3 (0)	1 (0)	37 (7)	10 (4)
Se [CI], %	77.8 [40, 97.2]	96.2 [80.4, 99.9]	84.2 [60.4, 96.6]	97.7 [87.7, 99.9]	58.9 [48, 69.2]	93.7 [88.7, 96.9]

Table 5: Sensitivities stratified by severity of illness. All samples with uncertain result were considered negative for the analysis (number of uncertain samples shown in brackets).

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219 *3.2.2. Reproducibility and linearity*

220 Intra-assay variability was determined by calculating the mean, standard deviation,
221 and coefficient of variation of the measured OD ratios based in 5 replicate measurements
222 of 8 samples with values ranging from at or below the threshold limit to about the 75%
223 quartile in the two IgG and IgA assays (Supplementary Table 5 & Supplementary Fig-
224 ure 6). In general, reproducibility was high with coefficients of variation of 5% or smaller
225 in samples well above the threshold. This variation increased to $\sim$ 10% at or below the
226 threshold. It is trivial but important to state that, at the threshold, this variability can
227 lead to different classification in each repetition. Last, different samples show different
228 variabilities in the two IgG assays, possibly indicating the difference between the binding
229 epitope and assay manufacturing.

230 We investigated the assay linearity using two-fold serially diluted serum samples from
231 5 individuals with high OD values for IgG (Supplementary Figure 11). Both Euroim-
232 mun assays showed a good linearity over a dilution range of 64-fold (2^6 dilutions), while
233 the IgG Epitope Diagnostics (EDI) assay is linear only in a 16-fold (2^4) dilution range.
234 Additionally, both IgG assays show good agreement along the dilutions (Supplementary
235 Figure 10), suggesting that—after normalization and scaling—the IgG assays give com-
236 parable quantitative results. No data of sufficient quality was acquired for a discussion
237 of the IgM assay (Supplementary Figure 11). Additionally, we found that the IgG, but
238 not the IgM, assay from EDI is sensitive to haemolysis (Supplementary Figure 12).

239 *3.3. Kinetics of seroconversion*

240 Our positive cohort can give insight into the kinetics of seroconversion at the pop-
241 ulation level for different antibody types and epitopes and a broad range of disease
242 manifestations. The two ELISA tests employ different epitopes: the EDI test recognizes
243 the SARS-CoV-2 nucleocapsidprotein and uses IgG and IgA while the Euroimmun test
244 recognizes the S1 portion of the spike protein using IgG and IgM.

245 For all antibody types, the strength of the response over time correlated with severity
246 of the symptoms (Figure 2A). We only measured a well discernible IgM response in the
247 "bedridden" group and a slight response in the "help needed" group, while it was largely
248 absent from the "no restriction" group. On the other hand, the IgA and IgG response
249 is measurable in most samples. The average response is higher when "bedridden", while
250 similar antibody response levels are observed for "no restriction" and "help needed".

251 In most viral host responses, the earliest measurable response is IgM followed by IgG

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252 and IgA. Surprisingly, but in line with other reports for SARS-CoV-2 [10, 11] as well
253 as observations for SARS and MERS, all three antibody types reacted within a similar
254 timeframe. The earliest detection, within a week of symptom onset, was for IgA, followed
255 by IgM and IgG both detected from week 2. IgM then peaked 2–3 weeks and IgA 3–4
256 weeks post symptom onset, while no decline for IgG was measured. The temporal order
257 of these peaks can be expected based on the half-life time of the different antibody types
258 in the blood.

259 These observations are further substantiated from observing the responses on the
260 level of the individuals. Most interestingly, we find a robust IgG response against the
261 NCP early on, whereas the response to the spike protein seems to be more prevalent at
262 later time points (Figure 2A). However, neither IgG response is significantly different
263 depending on the severity of the disease. The IgA response against the spike protein is
264 produced rapidly in the absence of an IgG response but subsides at later time points
265 when the IgG response becomes dominant.

266 4. Discussion

267 Here, we describe the collection and initial characterisation of a blood bank from
268 the affected population of the canton Basel-Landschaft, Switzerland. Almost 50% of the
269 people fallen ill with COVID-19 participated in this study and the biobank is therefore
270 exemplary for an outbreak in a western European community. In particular, the COVID-
271 19 severity and symptoms in the biobank samples closely follow the reported distributions
272 elsewhere. We might have slightly biased our cohorts toward less severe cases, by exclud-
273 ing samples from actively hospitalized or deceased patients. Since only 11 cases remained
274 in the hospital at the time of recruitment, this bias is likely small. Overall, we consider
275 the largely representative sample a strength of our biobank, as it allows us to estimate
276 the kinetics of seroconversion and assay performance characteristics on samples closely
277 resembling those expected in population-wide studies for sero-prevalence.

278 We find that both specificity and sensitivity are below the manufacturer's specifica-
279 tions for all four tests, and observe similar sensitivities only for our 'bedridden' group.
280 This indicates that characterizations by the manufacturers are likely based on 'severe'
281 cohorts, which limits their applicability and impairs planning of sero-prevalence studies.
282 We also find that the IgA and IgG assays allow for quantification within a relatively
283 narrow range: the linear range of the Euroimmun assay covers approximately a 64-fold

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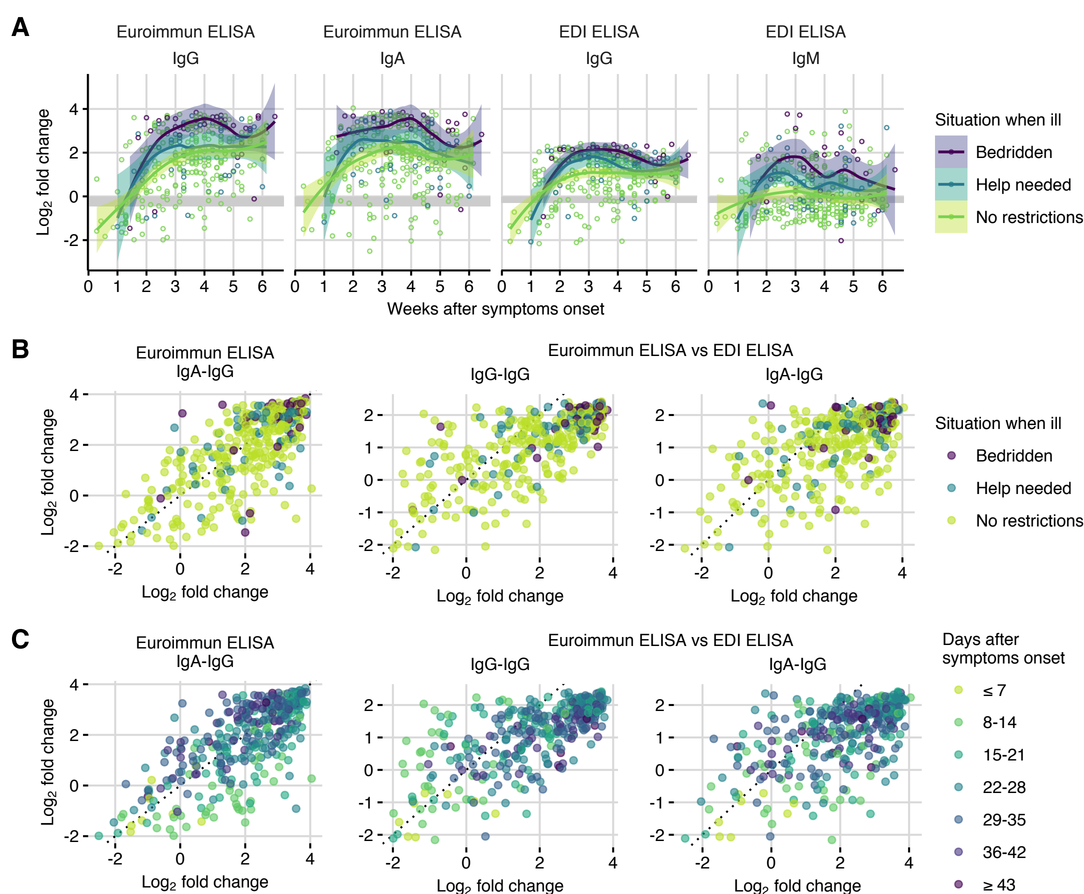

Figure 2: A: Log_2-fold changes in ELISA signal for each assay by time after symptoms onset and by disease severity. Data was fitted using smoothing splines to visualize trends (figure shows fits and 95% confidence intervals). Grey area indicates the range where serum samples are classified as uncertain; samples above (below) this area are classified as positive (negative). B: Scatterplots comparing $\log_2$-fold changes in IgA vs IgG measured by Euroimmun ELISA (left), IgG measured by Euroimmun ELISA vs IgG measured by Epitope Diagnostics (EDI) ELISA (center), and Euroimmun IgA vs EDI IgG (right) by disease severity. C: As in B but with days after onset of symptoms. Fold-changes are defined as the ratio between measured OD and the classification cut-off OD specified by the manufacturer.

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284 dilution range and the EDI assay a 16-fold dilution range. When calculating sensitivities
285 and specificities with our cohort, the IgA test shows the highest sensitivity, followed by
286 the two IgG assays. The poor sensitivity of the IgM test is either due to the absence
287 of a dedicated IgM response [22] in line with previous reports or poor test performance.
288 The highest specificity is achieved with the Euroimmun IgG test. The specificity could
289 be further boosted by a higher cut-off as previously reported, however accompanied by
290 a concurrent drop in sensitivity (Supplementary Tables 14, 15, 16).

291 The average strength of the antibody response, as measured by the ratios, correlates
292 with the severity of the disease at each time point. However, the differences vanish
293 the longer post-symptoms onset the individuals are, and the measured amount in an
294 individual is not predictive for the severity of the disease. Surprisingly, but similar to
295 earlier reports, we measured that the temporal order of the response is shifted. IgA
296 is measured within 1–2 weeks after symptoms onset, followed by the IgG response at
297 week 2–3, while a clear IgM response is absent. Especially at earlier time points, the
298 IgA response is stronger than the IgG response as measured using the S1 spike protein
299 epitope in the Euroimmun assay. To fully understand what the obtained values reflect
300 and how they correlate with diseases and protection from disease, further analyses are
301 planned including measuring the strength to neutralize pseudo-typed viral particles and
302 analysing the individual antibodies by sequencing the PBMCs. However, our current
303 data help to understand the limitations of the current antibody tests on the market and
304 serve as the basis to interpret ongoing studies on other lab assay characterization, POCT
305 performances and PBMC sequencing.

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