

1 **Clinical performance of SARS-CoV-2 IgG antibody tests and potential protective immunity**

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24 **Abstract**

25 As the current SARS-CoV-2 pandemic continues, serological assays are urgently needed for rapid
26 diagnosis, contact tracing and for epidemiological studies. So far, there is little data on how
27 commercially available tests perform with real patient samples and if detected IgG antibodies
28 provide protective immunity. Focusing on IgG antibodies, we demonstrate the performance of two
29 ELISA assays (Euroimmun SARS-CoV-2 IgG & Vircell COVID-19 ELISA IgG) in comparison to one lateral
30 flow assay ((LFA) FaStep COVID-19 IgG/IgM Rapid Test Device) and two in-house developed assays
31 (immunofluorescence assay (IFA) and plaque reduction neutralization test (PRNT)). We tested follow
32 up serum/plasma samples of individuals PCR-diagnosed with COVID-19. Most of the SARS-CoV-2
33 samples were from individuals with moderate to severe clinical course, who required an in-patient
34 hospital stay.

35 For all examined assays, the sensitivity ranged from 58.8 to 76.5% for the early phase of infection
36 (days 5-9) and from 93.8 to 100% for the later period (days 10-18) after PCR-diagnosed with COVID-
37 19. With exception of one sample, all positive tested samples in the analysed cohort, using the
38 commercially available assays examined (including the in-house developed IFA), demonstrated
39 neutralizing (protective) properties in the PRNT, indicating a potential protective immunity to SARS-
40 CoV-2. Regarding specificity, there was evidence that samples of endemic coronavirus (HCoV-OC43,
41 HCoV-229E) and Epstein Barr virus (EBV) infected individuals cross-reacted in the ELISA assays and
42 IFA, in one case generating a false positive result (may giving a false sense of security). This need to
43 be further investigated.

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45 **Background**

46 SARS-CoV-2 is a new Coronavirus, belonging to the group of betacoronaviruses, which emerged in
47 December 2019 in Wuhan, China. It is the causative agent of an acute respiratory disease known as
48 coronavirus disease 2019 (COVID-19). The spectrum of clinical signs can be very broad and
49 asymptomatic infections are reported. The virus has rapidly spread globally. On 11 March 2020 the
50 World Health Organization (WHO) declared COVID-19 as a pandemic. Nucleic acid amplification
51 testing (NAT) is the method of choice in the early phase of infection (1). However, to acquire
52 knowledge about the seroprevalence of SARS-CoV-2 and to test for (potential) individual immunity,
53 there is an increasing demand in the detection of antibodies – especially of IgG antibodies.
54 Convalescent plasma may be used for therapeutic or prophylactic approaches as vaccines and other
55 drugs are under development (2). For all these purposes, sensitive and especially highly specific
56 antibody assays are needed. The spike (S) protein of SARS-CoV-2 has shown to be highly
57 immunogenic and is the main target for neutralizing antibodies (3). Currently there are many S
58 protein based commercially or in-house developed assays available, but there is limited data on how
59 these tests perform with clinical samples and if the detected IgG antibodies provide protective
60 immunity. This study aims to provide a quick overview on some of these assays (two commercial
61 available ELISA, an LFA, an IFA and a PRNT, focusing on the detection and neutralization capacity of
62 IgG antibodies in follow up serum or plasma samples of individuals with PCR-diagnosed infections
63 with SARS-CoV-2. To assess potential cross-reactivity, we examined defined follow-up samples of
64 individuals infected with endemic coronaviruses and other infectious diseases.

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66 **Materials and methods**

67 **Serum and plasma samples**

68 We collected follow up serum or plasma samples (in the following simply stated as samples) from
69 individuals with PCR-diagnosed infections with SARS-CoV-2 (n=33) at different time points (table 1).
70 Most of these individuals had a moderate to severe clinical course and required an in-patient hospital
71 stay at the intensive care unit. Additionally, follow up samples of recent PCR-diagnosed infections
72 with SARS-CoV (3 patients from the 2003 outbreak), HCoV-OC43 (n=4), HCoV-HKU1 (n=1), HCoV-
73 NL63 (n=2), HCoV-229E (n=4) and recent serological/PCR-diagnosed infections with acute EBV (n=4,
74 three serologically EBV-VCA-IgM positive and one PCR- and serologically EBV-VCA-IgM positive) and
75 acute CMV (n=3) (all serologically IgM and PCR-positive) were collected. The samples of individuals
76 infected with endemic human coronavirus, CMV and EBV were used to assess potential cross
77 reactivity and the risk of potential false positive results.

78 **Lateral flow assay**

79 The FasStep (COVID-19 IgG/IgM) rapid test cassettes (COV-W32M, Assure Tech (Hangzhou) Co., Ltd,
80 China) were used according to the manufacturer's recommendation. We have no details on the used
81 antigen component. 10 µl serum and two drops of sample buffer were applied to the sample well.
82 Test results were visually evaluated after 10 minutes.

83 **ELISA**

84 The CE certified versions of the Euroimmun SARS-CoV-2 IgG ELISA (Euroimmun, Lübeck, Germany)
85 and Vircell COVID-19 ELISA IgG (Vircell Spain S.L.U., Granada, Spain) were used, in an identical
86 manner, according to the manufacturer's recommendation. Both ELISAS use SARS-CoV-2
87 recombinant antigen from spike glycoprotein (S protein) and the Vircell ELISA additionally
88 Nucleocapsid (N protein). Samples were diluted 1:101 or 1:20, respectively, in sample buffer and
89 incubated at 37° for 60 min in a 96-well microtiter plate followed by each protocols washing and
90 incubation cycles, including controls and required reagents. Optical density (OD) was measured for

91 both assays at 450 nm using a Virclia microplate reader (Vircell Spain S.L.U., Granada, Spain). The
92 signal-to-cut-off ratio was calculated and values expressed according to each manufacturer's
93 protocol.

94 **Immunofluorescence assay (IFA)**

95 For an immunofluorescence assay Vero cells (african green monkey, ATCC CCL-81 (American Type
96 Culture Collection, Manassas, Virginia, USA)) were infected with SARS-CoV-2 and harvested two days
97 post infection. Briefly, cells were trypsinized and washed once with PBS before transferred onto a 10-
98 well diagnostic microscope slides. After drying, cells were fixated with 100% ethanol for 10 minutes.
99 Patient samples were diluted in sample buffer (Euroimmun AG, Lübeck, Germany) in a dilution of
100 1:50 and 30 µl applied per well. The slides were incubated at 37°C for 1 hour and washed three times
101 with phosphate-buffered saline (PBS)-Tween (0.1%) for 5 minutes. 25 µl of goat-anti human
102 fluorescein-labeled IgG conjugate was used as secondary antibody. The slides then were incubated
103 for 30 minutes and washed three times with PBS-Tween for 5 minutes. The microscopic analysis was
104 performed by 200-fold magnification using a Leica DMLS fluorescence microscope (Leica
105 Mikrosysteme Vertrieb GmbH, Wetzlar Germany).

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107 **Plaque reduction neutralization test (PRNT)**

108 To test for neutralizing capacity of SARS-CoV-2 specific antibodies, Caco-2 cells (human colon
109 carcinoma cells, ATCC DSMZ ACC-169 (American Type Culture Collection, Manassas, Virginia, USA))
110 were seeded on a 96-well plate 3-5 days prior infection. 2-fold dilutions of the test sera beginning
111 with a 1:10 dilution (1:10; 1:20; 1:40; 1:80; 1:160; 1:320; 1:640 and 1:1280) were made in culture
112 medium (Minimum essential medium, MEM; Gibco, Dublin, Ireland) before mixed 1:1 with 100
113 TCID₅₀ (Tissue culture infectious dosis 50) of reference virus (SARS-CoV-2 FFM1 isolate). FFM1 was
114 isolated from a patient at University Hospital Frankfurt who was tested positive for SARS-CoV-2 by
115 PCR. Virus-serum mixture was incubated for one hour at 37°C and transferred onto the cell

116 monolayer. Virus related cytopathic effects (CPE) were determined microscopically 48 to 72 hours
117 post infection. To determine a potential neutralizing ability of patient serum, CPE at a sample dilution
118 of 1:10 is defined as non-protective while a CPE at a dilution of >1:20, is defined as protective.

119 **Results**

120 In the early phase of infection, from days 5-9 after PCR-confirmed infection with SARS-CoV-2, the in-
 121 house developed IFA and PRNT showed a sensitivity of 76.5% (13/17), the Vircell ELISA a sensitivity of
 122 70.6% (12/17), the Assure Tech Rapid Test a sensitivity of 62.5% (10/16) and the Euroimmun ELISA a
 123 sensitivity of 58.8% (10/17). For the later period from days 10-18, the Euroimmun ELISA and Assure
 124 Tech Rapid Test showed a sensitivity 93.8% (15/16), the Vircell ELISA, IFA and PRNT of 100% (16/16) -
 125 (TABLE 1). For selected samples (SARS-CoV samples from the 2003 outbreak excluded, TABLE S2), the
 126 Euroimmun ELISA showed a specificity of 95.7%, generating a borderline result for the HCoV-OC43
 127 sample, the Vircell ELISA of 95.2%, generating a positive result for HCoV-229E sample and the in-
 128 house developed IFA of 100% (an unspecific result for one EBV sample was excluded). Including the
 129 three SARS-CoV samples from the 2003 outbreak, the Euroimmun ELISA showed a specificity of
 130 96.2% (not generating any cross-reactive results for the SARS-CoV samples), the IFA of 86.4% and the
 131 Vircell ELISA of 83.3% (both assays generating positive results for all three SARS-CoV samples). The
 132 Assure Tech Rapid Test did not generate any false positive results for the tested samples. None of the
 133 other tested samples cross-reacted in terms of generating borderline or false positive results.

134 TABLE 1 – Sensitivity and specificity of the examined SARS-CoV-2 IgG assays from days 5-9 and days 10-18.

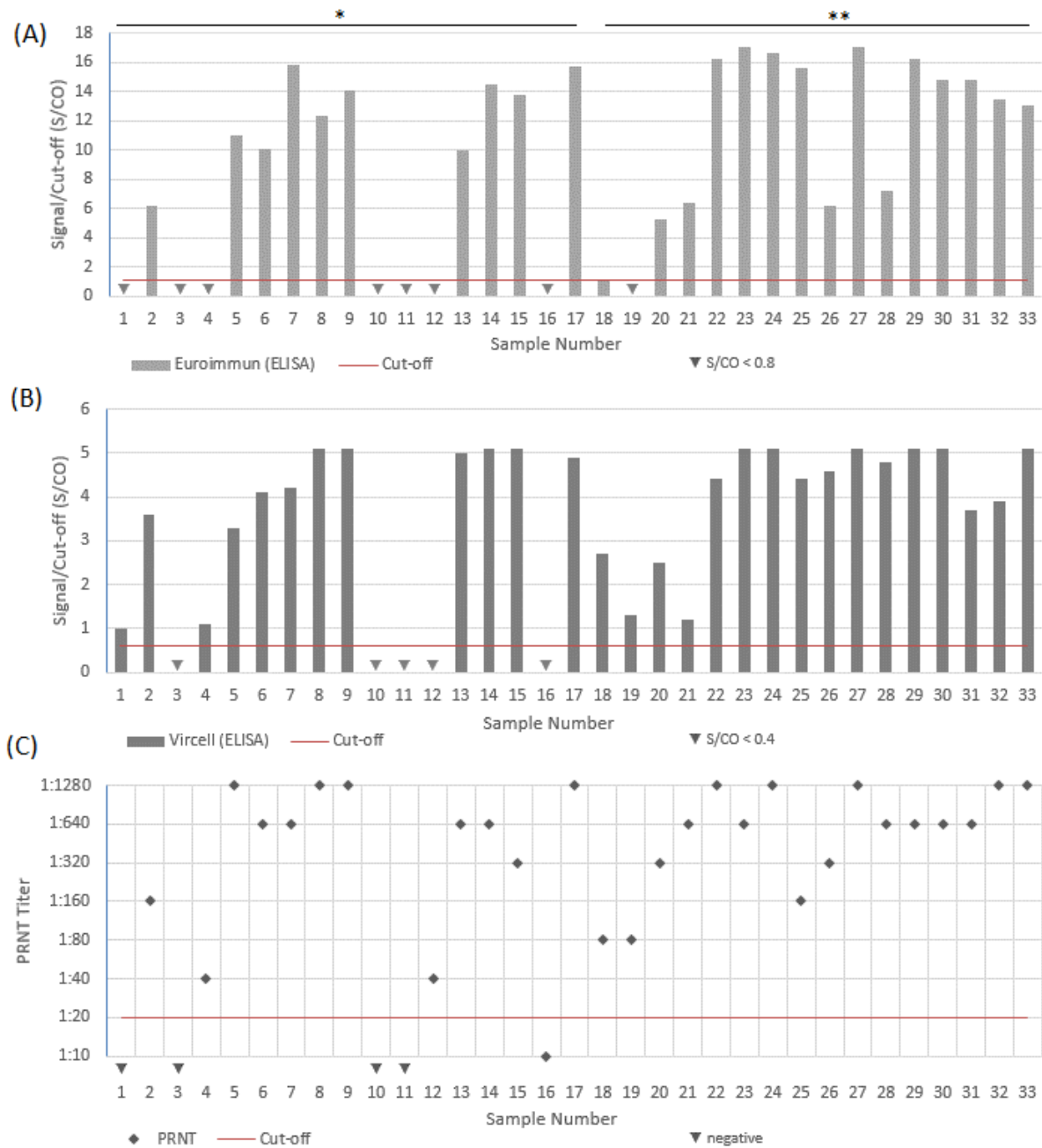
Company	Days after confirmed SARS-CoV-2 PCR			
	5-9 (days)	10-18 (days)		
		sensitivity (%)	specificity (%)	specificity (%) incl. SARS-CoV (2003)*
Euroimmun (ELISA)	58.8 (10/17)	93.8 (15/16)	95.7 (22**/23)	96.2 (25/26)
Vircell (ELISA)	70.6 (12/17)	100 (16/16)	95.2 (20/21)	83.3 (20/24)
IFA (in-house)	76.5 (13/17)	100 (16/16)	100 (19/19)***	86.4 (19/22)
Assure Tech (Rapid test)	62.5 (10/16)	93.8 (15/16)	100 (13/13)	-
PRNT (in-house)	76.5 (13/17)	100 (16/16)	-	-

135 Details on tested samples see TABLE S1 and S2; *including follow up samples of SARS-CoV (2003 outbreak), which is closely
 136 related to SARS-CoV-2; **one “borderline” result; *** one unspecific result was excluded-, not examined.

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138 The signal-to-cut-off (S/CO) ratios of the Euroimmun and Vircell ELISA and the corresponding PRNT
139 titers for the tested samples are shown in FIG 1. In samples 3, 10 and 11, none of the examined
140 assays (including the IFA and Assure Tech Rapid Test), detected SARS-CoV-2 antibodies. In sample 1,
141 only the Vircell ELISA, in sample 4 and 19 only the Vircell ELISA and PRNT (including the IFA) detected
142 antibodies. In samples 12 and 16, only the PRNT (and IFA) detected antibodies (in sample 16 with a
143 titer <1:10). With exception of sample 1, all with the ELISA positive tested samples were also positive
144 tested with the IFA. In the detection of antibodies, the IFA performed like the PRNT on all examined
145 samples. All with the commercially available assays positive tested samples (except of sample 1)
146 showed neutralizing properties in the PRNT (titer >1:20), indicating a potential protective immunity.

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 149 FIG 1 – Results of the for sensitivity tested samples in the ELISA assays and PRNT; (A) Euroimmun ELISA Signal/Cut-off
 150 (S/CO) ratio of tested samples; (B) Vircell ELISA Signal/Cut-off (S/CO) ratio for tested samples; (C) PRNT Titer for tested
 151 samples. *Days 5-9 /**Days 10-18 after confirmed SARS-CoV-2 PCR.

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153 **Discussion**

154 In terms of sensitivity, our data are consistent with previously published data. In a study from Liu et
155 al., using an rS-based ELISA assay, the group found SARS-CoV-2 IgG antibodies in less than 60% of the
156 samples from days 6-10 after disease onset. The sensitivity increased to >90% in samples from days
157 16-20 (4). In a study from Wölfel et al., using an in-house developed IFA, the group found
158 seroconversion in all examined follow-up serum samples of COVID-19 patients by day 14 after onset
159 of symptoms. The samples were further analyzed via PRNT, all showed neutralization activity against
160 SARS-CoV-2 (5).

161 An important finding of our study is, that (with exception of sample 1) all detected SARS-CoV-2 IgG
162 antibodies in the analyzed cohort, using the commercially available assays examined, demonstrated
163 neutralizing (protective) properties in the PRNT. The screening for SARS-CoV-2 IgG antibodies
164 [especially for potential protective IgG antibodies against the S protein (6)] using ELISA or lateral flow
165 assays is more convenient and practicable than using the hands on- and time-intensive IFA or PRNT,
166 which can only be performed by experienced personnel, and the PRNT, only in a BSL-3 laboratory.
167 ELISA based assays can be automated and used for larger sample sizes. Lateral flow assays can be
168 used by less experienced personnel in a point-of-care setting, generating results in short time. Some
169 samples, however, were only detected with the IFA and PRNT as gold standard. The titer needed for
170 potential protective immunity is not yet (officially) defined. In one study, it is reported, that a
171 individual cleared SARS-CoV-2 without developing antibodies up to 46 days after illness (7). The
172 mechanism of immunity, especially of protective immunity (if applicable) and how long it will last,
173 need to be further investigated. Besides humoral mediated immunity, there is evidence that T-cell
174 mediated immunity plays a role (8). Most of the SARS-CoV-2 samples analysed in this study were
175 from individuals with moderate to severe clinical course, who required an in-patient hospital stay.
176 We have also tested follow-up samples of individuals PCR-diagnosed with COVID-19 with mild or no
177 symptoms at all, IgG antibodies could only be detected after 6 weeks (data not shown). In terms of
178 specificity, cross-reacting antibodies of endemic coronavirus infected individuals or of individuals

179 with other active infectious diseases (e.g. EBV or CMV) are a known phenomenon (9). The examined
180 assays in our study demonstrated a good specificity. Only the Vircell ELISA generated one positive
181 result for one HCoV-229E sample, whereas the Euroimmun ELISA generated only one borderline
182 result for the HCoV-OC43 sample and the IFA an unspecific signal in one EBV sample. For the Assure
183 Tech Rapid Test, no cross-reactions were observed, however, a larger sample size would be needed
184 to get a clearer picture. The cross-reactivity of the SARS-CoV samples from the outbreak of 2003 in
185 the Vircell ELISA and IFA are of less importance as the virus is known to be eradicated. Nonetheless,
186 as a false positive result might give a false sense of security, efforts should be made to further
187 improve the specificity of the available assays. All in our study examined assays are eligible for the
188 detection of SARS-CoV-2 IgG antibodies, indicating a potential protective immunity. Ideally, to get the
189 maximum sensitivity, testing should be performed in the later phase of infection (≥ 10 days) after
190 PCR-confirmation or disease onset of COVID-19. The Vircell ELISA, IFA and PRNT demonstrated the
191 highest sensitivity throughout our study. At the moment, however, the PRNT is still the method of
192 choice for questions regarding potential SARS-CoV-2 immunity and should be performed when
193 available.

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238 **Supplementary Material**

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240 TABLE S1 – For sensitivity tested individual follow-up samples of SARS-CoV-2 PCR-confirmed individuals at different time
241 points and generated results.

Sample Nr.	Day after confirmed SARS-CoV-2 PCR	Euroimmun (ELISA) S/CO	Vircell (ELISA) S/CO	IFA (in-house) qual.	Assure Tech (Rapid Test) qual.	PRNT Titer
1	5	<0.8	1.0	neg.	neg.	neg.
2	6	6.2	3.6	pos.	pos.	1:160
3	6	<0.8	<0.4	neg.	neg.	neg.
4	6	<0.8	1.1	pos.	pos.	1:40
5	6	11	3.3	pos.	pos.	1:1280
6	6	10.1	4.1	pos.	pos.	1:640
7	7	15.8	4.2	pos.	pos.	1:640
8	7	12.3	5.1	pos.	pos.	1:1280
9	7	14.1	5.1	pos.	pos.	1:1280
10	8	<0.8	<0.4	neg.	neg.	neg.
11	8	<0.8	<0.4	neg.	neg.	neg.
12	8	<0.8	<0.4	pos.	neg.	neg.
13	8	10	5	pos.	pos.	1:640
14	8	14.5	5.1	pos.	pos.	1:640
15	8	13.8	5.1	pos.	-	1:320
16	9	<0.8	<0.4	pos.	neg.	1:10
17	9	15.7	4.9	pos.	pos.	1:1280
18	10	1.13	2.7	pos.	pos.	1:80
19	10	neg	1.3	pos.	neg.	1:80
20	10	5.2	2.5	pos.	pos.	1:320
21	10	6.4	1.2	pos.	pos.	1:640
22	10	16.2	4.4	pos.	pos.	1:1280
23	11	17	5.1	pos.	pos.	1:640
24	13	16.6	5.1	pos.	pos.	1:1280
25	13	15.6	4.4	pos.	pos.	1:160
26	14	6.14	4.6	pos.	pos.	1:320
27	14	17	5.1	pos.	pos.	1:1280
28	16	7.2	4.8	pos.	pos.	1:640
29	16	16.2	5.1	pos.	pos.	1:640
30	16	14.8	5.1	pos.	pos.	1:640
31	17	14.8	3.7	pos.	pos.	1:640
32	17	13.4	3.9	pos.	pos.	1:1280
33	18	13	5.1	pos.	pos.	1:1280

242 Euroimmun (S/CO <0.8 = negative, 0.8-<1.1 = equivocal, ≥ 1.1 = positive), Vircell (S/CO <0.4 = neg., 0.4-0.6 = equivocal, >0.6
243 = pos.); pos., positive; neg., negative; -, not tested.

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247 TABLE S2 – For specificity tested follow-up samples of individuals with selected PCR- or serologically-confirmed infections
 248 and generated results.

Sample Nr.	Recently PCR-/serologically-confirmed infected with	Euroimmun (ELISA) S/CO	Vircell (ELISA) S/CO	IFA (in-house) qual.	Assure Tech (Rapid Test) qual.
1	HCOV-OC43	neg.	neg.	neg.	neg.
2	HCOV-OC43	0.9	neg.	neg.	neg.
3	HCoV-OC43	neg.	neg.	neg.	neg.
4	HCoV-OC43	neg.	neg.	neg.	neg.
5	HKU 1	neg.	neg.	neg.	neg.
6	SARS-CoV-1	neg.	2.2	pos.	-
7	SARS-CoV-1	neg.	3.8	pos.	-
8	SARS-CoV-1	neg.	3.9	pos.	-
9	SARS-CoV-2 neg.	neg.	neg.	neg.	-
10	SARS-CoV-2 neg.	neg.	neg.	neg.	-
11	SARS-CoV-2 (neg.)	neg.	neg.	-	-
12	SARS-CoV-2 + Multiplex* neg.	neg.	neg.	neg.	-
13	HCoV-229E	neg.	neg.	neg.	-
14	HCoV-229E	neg.	1.5	neg.	-
15	HCoV 229E + Parainfluenza Virus Type 3	neg.	neg.	neg.	neg.
16	HCoV-229E	neg.	neg.	neg.	neg.
17	HCoV-229E	neg.	neg.	neg.	neg.
18	HCoV-NL63 + Enterovirus/Rhinovirus	neg.	neg.	neg.	neg.
19	HCoV-NL63	neg.	neg.	-	-
20	CMV (+ IgM antibody pos.)	neg.	neg.	neg.	neg.
21	CMV (+IgM antibody pos.)	neg.	neg.	neg.	neg.
22	CMV (+ IgM antibody pos.)	neg.	neg.	-	-
23	EBV-VCA-IgM pos.	neg.	neg.	neg.	neg.
24	EBV (+ -VCA-IgM antibody pos.)	neg.	neg.	neg.	neg.
25	EBV-VCA-IgM antibody pos .	neg.	-	neg.	-
26	EBV-VCA-IgM antibody pos .	neg.	-	unsp.	-

249 Euroimmun (S/CO <0.8 = negative, 0.8-<1.1 = equivocal, ≥ 1.1 = positive); Vircell (S/CO <0.4 = neg., 0.4-0.6 = equivocal, >0.6
 250 = pos.); pos., positive; neg., negative; unsp., unspecific; *Biofire® Filmarray® 20 Target Respiratory Panel (bioMérieux,
 251 Nürtingen, Baden-Württemberg, Germany); -, not tested.

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