1 **Inference of SARS-CoV-2 spike-binding neutralizing antibody titers in sera from**
2 **hospitalized COVID-19 patients by using commercial enzyme and**
3 **chemiluminescent immunoassays**

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17 **Keywords:** SARS-CoV-2, COVID-19, neutralizing antibodies, Enzyme-linked
18 immunosorbent assay, chemiluminescent immunoassays.

19 **Running Title:** Serum neutralizing antibody titers predicted by commercial SARS-
20 CoV-2 immunoassays.

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22

23 ABSTRACT

24 **Background:** Whether antibody levels measured by commercially-available enzyme or
25 chemiluminescent immunoassays targeting the SARS-CoV-2 spike (S) protein can act
26 as a proxy for serum neutralizing activity remains to be established for many of these
27 assays.

28 **Objectives:** To evaluate the degree of correlation between neutralizing antibodies
29 (NtAb) binding the SARS-CoV-2 Spike (S) protein and SARS-CoV-2-S-IgG levels
30 measured by four commercial immunoassays in sera drawn from hospitalized COVID-
31 19 patients.

32 **Patients and Methods:** Ninety sera from 51 hospitalized COVID-19 patients were
33 assayed by a pseudotyped virus neutralization assay, the LIAISON SARS-CoV-2 S1/S2
34 IgG, the Euroimmun SARS-CoV-2 IgG ELISA, the MAGLUMI 2019-nCoV IgG and
35 the COVID-19 ELISA IgG assays.

36 **Results:** Overall, the results obtained with the COVID-19 ELISA IgG test showed the
37 highest agreement with the NtAb assay (κ , 0.85; 95% CI, 0.63-1). The most sensitive
38 tests were the pseudotyped virus NtAb assay and the COVID-19 ELISA IgG assay
39 (92.2% for both). Overall, the degree correlation between antibody titers resulting in
40 50% virus neutralization (NtAb₅₀) in the pseudotyped virus assay and SARS-CoV-2 IgG
41 levels was strong for the Euroimmun SARS-CoV-2 IgG ELISA (Rho=0.73) and
42 moderate for the remaining assays (Rho=0.48 to 0.59). The kinetic profile of serum
43 NtAb₅₀ titers could not be reliably predicted by any of the SARS-CoV-2 IgG
44 immunoassays.

45

46 **Conclusions:** the suitability of SARS-CoV-2-S-IgG commercial immunoassays for
47 inferring neutralizing activity of sera from hospitalized COVID-19 patients varies
48 widely across tests and is influenced by the time of sera collection after the onset of
49 symptoms.

50 **1. Background**

51 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a prototypical
52 *Sarbecovirus*, causes Coronavirus disease 2019 (COVID-19) and is associated with
53 significant morbidity and mortality [1,2]. SARS-CoV-2 neutralizing antibodies (NtAb)
54 presumably play a pivotal role in preventing infection and may promote virus clearance
55 [3,4]. In support of these assumptions, passive transfer of two mAbs blocking SARS-
56 CoV-2 interaction with angiotensin-converting enzyme II receptor as monotherapy
57 protected rhesus macaques from infection [5]. Moreover, transfusion of plasma from
58 immune individuals with high NtAb titers seemed to be associated with improved
59 clinical outcomes in critically ill COVID-19 patients [6,7]. Virus neutralization assays,
60 either using live native SARS-CoV-2 virus, engineered SARS-CoV-2 pseudotyped
61 viruses or replication-competent SARS-CoV-2 chimeric viruses [8-10] are cumbersome,
62 require specialized facilities, and are time consuming. A large number of enzyme-linked
63 (ELISA) or chemiluminescent immunoassays (CLIA) detecting antibodies that bind
64 SARS-CoV-2 structural proteins have been commercialized [11]. Whether antibody
65 levels measured by these serological assays can be used as a proxy for serum
66 neutralizing activity is a relevant issue, which has been dealt with in several peer-
67 reviewed or preprint studies [12-20], but demands further investigations.

68 **2. Objectives**

We focused on evaluating the degree of correlation between NtAb binding the SARS-CoV-2 S protein, which is known to elicit the most potent antibodies for virus neutralization [21,22], and SARS-CoV-2 IgG levels measured by four commercially-available semiquantitative immunoassays targeting the S protein in sera drawn from hospitalized COVID-19 patients.

3. Study design

3.1. Serum specimens and patients

A total of 90 sera from 51 non-consecutive patients with laboratory-confirmed SARS-CoV-2 infection by RT-PCR [23,24] that were admitted to Hospital Clínico Universitario of Valencia between March 5 to April 30, 2020, were included in the current study. The Demographic, clinical, and laboratory data of these patients are displayed in Table 1. Forty-one sera were obtained within the first two weeks (< day 15) after the onset of symptoms, at a median of 11 days (range, 5-14 days), and 49 afterward ($\geq$ 15 days, at a median of 23 days; range, 15-41 days). Sequential specimens were available from 20 out of the 51 patients (median 3 specimens per patient; range 2 to 6). This study was approved by the Research Ethics Committee of Hospital Clínico Universitario INCLIVA (March 2020).

3.2. SARS-CoV-2 Neutralizing antibody assay

A Green fluorescent protein (GFP) reporter-based pseudotyped virus neutralization assay using a non-replicative vesicular stomatitis virus (VSV lacking the G protein) backbone coated with the SARS-CoV-2 spike (S) protein was used for neutralization assays on Vero cells, as previously described [24]. Briefly, sera were heat-inactivated for 30 minutes at 56°C then brought to an initial 10-fold dilution, followed by four 4-fold dilutions in duplicate. Each dilution was mixed with an equal volume containing

93 1,250 plaque-forming units of the VSV-S virus and incubated at 37°C for 1 h.
 94 Subsequently, the mixture was added to Vero cells and incubated for 18 hours, after
 95 which GFP expression was measured using a live cell microscope system (IncuCyteS3;
 96 Sartorius). All sera which did not reduce viral replication by 50% at 1/20 dilution were
 97 considered non-neutralizing and were arbitrarily assigned a value of 1/10. All sera that
 98 did not result in >70% recovery of GFP signal at the highest antibody dilution were
 99 retested using 5-fold dilutions ranging between 100 and 12,500-fold. Finally, the
 100 antibody dilution resulting in 50% virus neutralization (NtAb₅₀) was calculated using
 101 the drc package (version 3.0-1) in R via a 2 parameter logistic regression model (LL.2
 102 model).

103 **3.3. Commercial SARS-CoV-2 IgG immunoassays**

104 Four commercially-available semiquantitative immunoassays were used in the current
 105 study. Performance and interpretation of results were done in accordance with the
 106 respective manufacturer's instructions. The LIAISON SARS-CoV-2 S1/S2 IgG
 107 chemiluminescent assay (DiaSorin S.p.A., Saluggia, Italy) detects IgG antibodies
 108 directed against a recombinant S protein (S1/S2). Samples displaying < 12.0 AU/mL are
 109 considered negative, those ranging between 12.0 to 15.0 AU/mL are undetermined and
 110 those > 15 AU/mL are deemed as positive. The Euroimmun SARS-CoV-2 IgG ELISA
 111 (Euroimmun, Lübeck, Germany) uses a recombinant S1 domain of the S protein as a
 112 target. Results are expressed as a ratio, calculated by dividing the optical densities of the
 113 sample by those of an internal calibrator provided with the test kit. The cut-off index
 114 (COI) for samples to be considered positive was ≥ 1.1 and inconclusive from 0.8 and
 115 1.09. The MAGLUMI 2019-nCoV IgG is an indirect CLIA for assessment of IgG
 116 antibodies against SARS-CoV-2 S and nucleocapsid (N) proteins on the fully automated

MAGLUMI analyzers (SNIBE – Shenzhen New Industries Biomedical Engineering Co., Ltd, Shenzhen, China). A test result ≥ 1.10 AU/mL is considered positive. The COVID-19 ELISA IgG (Vircell Spain S.L.U., Granada, Spain) is an enzyme immunoassay that detects IgGs targeting the S1 and N proteins. Sera displaying antibody indices (AI) < 1.4 are considered negative, those between $1.4 < 1.6$ inconclusive, and those > 1.6 positive. For all the assays, inconclusive results were considered as positives for analysis purposes.

3.4. Definitions

Here, NtAb titers $\geq 1/160$ were considered high as this is the minimum NtAb₅₀ titer of plasma from COVID-19 convalescent individuals recommended by the FDA for therapeutic use [25]. Trajectories of antibody titers/levels were categorized as ascendant, descendant, fluctuating, or constant. To this end, variations of antibody levels $> 10\%$ across sequential specimens were deemed to be relevant, as the intra-run coefficient of variation was below this figure for all the immunoassays (all samples from a given individual were assayed in the same run).

3.5. Statistical methods

Test performances were evaluated for their sensitivity with the associated 95% confidence interval (CI). Cohen's Kappa (κ) statistic was used to evaluate the qualitative agreement between immunoassays. Spearman's rank test was used to assess the correlation between continuous variables using the entire dataset. To identify the optimal SARS-CoV-2 IgG levels measured by commercial immunoassays predicting NtAb₅₀ titers $\geq 1/160$, receiver operating characteristic (ROC) curve analysis was performed. Two-sided exact *P*-values are reported, and a *P*-value < 0.05 is considered statistically significant. The analyses were performed using SPSS version 20.0 (SPSS,

Chicago, IL, USA). Kinetics of antibody titers/levels were categorized as ascendant (an increase of antibody levels >10% compared to previous sampling point), descendant (any decrease), fluctuating, or constant.

4. Results

4.1. Patients characteristics

All 51 patients presented with pneumonia and imaging or laboratory findings compatible with COVID-19 and were hospitalized in either the pneumology ward (n=27) or the intensive care unit (ICU; n=24). As shown in Table 1, most patients (69%) had one or more co-morbidities and displayed high serum levels of several pro-inflammatory biomarkers at the time of serological testing. Four ICU patients eventually died.

4.2. Agreement between NtAb and SARS-CoV-2 IgG immunoassays results

Qualitative results returned by immunoassays were evaluated either considering the entire data set or grouping sera according to the time of sampling after the onset of symptoms (<15 days or ≥15 days) (Table 2). As shown in Table 3, overall, results provided by the COVID-19 ELISA IgG test best matched those obtained with the NtAb assay (κ , 0.84; 95% CI, 0.63-1), followed by those of the Euroimmun SARS-CoV-2 IgG ELISA (κ , 0.52; 95% CI, 0.22-0.81), LIAISON SARS-CoV-2 S1/S2 IgG (κ , 0.5; 95% CI, 0.2-0.78) and MAGLUMI 2019-nCoV IgG (0.4; 95% CI, 0.2-0.77). The same trend was observed when sera collected either < 15 days or ≥ 15 days after the onset of symptoms were analyzed separately. Notably, the concordance between results returned by the NtAb assay and the COVID-19 ELISA IgG was 100% for sera obtained at ≥ 15 days following symptom onset.

164 **4.3. The sensitivity of NtAb and SARS-CoV-2 IgG immunoassays**

165 Overall, the most sensitive tests were the GFP reporter-based pseudotyped virus
166 neutralization assay and the COVID-19 ELISA IgG, followed by the MAGLUMI 2019-
167 nCoV IgG, the Euroimmun SARS-CoV-2 IgG ELISA and the LIAISON SARS-CoV-2
168 S1/S2 IgG (Table 4). Differences in sensitivity were more apparent when sera were
169 collected early after the onset of symptoms (< 15 days) were analyzed independently,
170 and these tended to decrease in sera obtained at a later time point (Table 4).

171 **4.4. Correlation between NtAb₅₀ titers and IgG levels measured by SARS-CoV-2** 172 **IgG immunoassays**

173 NtAb₅₀ titers ranged from $\leq 1/20$ (undetectable) to 1/12,500. As shown in Figure 1,
174 overall, the correlation between NtAb₅₀ titers and SARS-CoV-2 IgG levels, as returned
175 by the corresponding immunoassay, was strong for the Euroimmun SARS-CoV-2 IgG
176 ELISA (Rho=0.73) and moderate (Rho=0.48 to 0.59) for the remaining platforms. In all
177 instances, correlations were positive and statistically significant. The degree of
178 correlation between NtAb₅₀ titers and SARS-CoV-2 IgG levels was substantially better
179 for sera collected within the first two weeks after the onset of symptoms (Rho=0.54-
180 0.80) than for those obtained afterward (Rho=0.30-0.52), irrespective of the commercial
181 immunoassay employed.

182 **4.5. Inference of high NtAb₅₀ titers by using SARS-CoV-2 IgG immunoassays**

183 Seventy-four out of 90 sera displayed high NtAb₅₀ titers ($\geq 1/160$). ROC analysis was
184 performed to determine the IgG levels measured by commercial immunoassays that
185 predict NtAb₅₀ titers of such a magnitude (Figure 2). Specificity was prioritized at the
186 expense of sensitivity for threshold selection. The data are shown in Table 5. The best
187 combination of specificity, sensitivity, and predictive values was achieved by the

188 LIAISON SARS-CoV-2 S1/S2 IgG threshold, with minimal differences across the
189 remaining platforms.

190 **4.6. Longitudinal follow-up of NtAb₅₀ titers and SARS-CoV-2 IgG levels measured** 191 **by immunoassays**

192 We selected patients (n=11) with 3 or more sera available for kinetic studies. Kinetics of
193 NtAb₅₀ titers and SARS-CoV-2 IgG levels determined by commercial immunoassays
194 varied notably across patients and were dependent on the immunoassay considered.
195 Concordance between trajectories of NtAb₅₀ titers and SARS-CoV-2 IgG levels was
196 highest when using the COVID-19 ELISA IgG (6 out of 11 patients) assay, followed by
197 MAGLUMI 2019-nCoV IgG (5 out of 11), and LIAISON SARS-CoV-2 S1/S2 IgG and
198 Euroimmun IgG (both 3 out of 11) assays. Representative cases of concordant and
199 discordant trajectories are depicted in Figure 3.

200 **5. Discussion**

201 A major role in preventing SARS-CoV-2 infection and blunting virus spread in
202 COVID-19 patients is predicted for NtAb [3-5]. Quantitation of serum NtAb titers in
203 plasma from convalescent COVID-19 patients is recommended for optimal selection of
204 specimens for passive transfer therapies; the FDA recommends the use of plasma with
205 NtAb₅₀ levels $\geq 1/160$ [22]. NtAb assays, either using SARS-CoV-2 strains, pseudotyped
206 viruses or chimeric viruses are technically demanding, require specialized facilities
207 (biosafety level 3 for live SARS-CoV-2 or level 2 for the pseudotyped viruses), and
208 cannot be implemented in routine practice. Thus, it is of interest to find alternative
209 methods to obtain reliable information regarding the neutralizing activity of sera that are
210 simple, fast, high-throughput assays, and commercially available. Here, we compared

the performance of a GFP reporter-based pseudotyped virus neutralization assay (VSV-SARS-CoV-2 S) and four commercial immunoassays targeting the SARS-CoV-2 S protein for the diagnosis of COVID-19 and assessed the extent to which NtAb₅₀ titers and SARS-CoV-2 IgG levels measured by these assays correlate. Prior studies assessing this relationship have been published for the Euroimmun SARS-CoV-2 IgG ELISA and LIAISON SARS-CoV-2 S1/S2 IgG [12,13,15,18-20], but not, to the best of our knowledge, for the MAGLUMI 2019-nCoV IgG and COVID-19 ELISA IgG assays. It must be highlighted that antibody titers measured by the NtAb assay used in the current study strongly correlate with those quantitated by assays using wild type SARS-CoV-2 [26].

Here, the overall agreement between qualitative (positive/negative) results returned by the NtAb assay and the four commercially-available immunoassays was fairly good, ranging from 97% for the COVID-19 ELISA IgG assay to 88% for LIAISON SARS-CoV-2 S1/S2 IgG and MAGLUMI 2019-nCoV IgG assays. Concordance across results was better for sera obtained at late times after the onset of symptoms (≥ 15 days), likely reflecting the existence of differences in the kinetics of NtAb and SARS-CoV-2 IgG levels measured by commercial immunoassays early after infection. In line with this finding, the COVID-19 ELISA IgG performed comparably to the NtAb assay in terms of overall sensitivity (92.2%), ranking first across the evaluated commercial immunoassays. As previously reported, the sensitivity of all immunoassays increased after the second week following the onset of symptoms [12,13,15,16, 18,19, 20]. The overall degree of correlation between NtAb₅₀ antibody titers and SARS-CoV-2 IgG levels was strong for the Euroimmun IgG assay but low to moderate for the other commercial immunoassays. The fact that the Euroimmun IgG assay detects antibodies

235 against S1, which contains the RBD domain and concentrates immunodominant
 236 epitopes eliciting high-affinity NtAb antibodies [3,5,21,22] may account for this
 237 finding. IgG antibodies lacking neutralizing activity and recognizing epitopes within S2
 238 and N proteins (additional targets in the remaining immunoassays) may compete with
 239 those binding S1, thus decreasing the degree of correlation. Interestingly, in all cases,
 240 correlations were better for sera collected early after the onset of symptoms than for
 241 those obtained at later times; in line with this, NtAb trajectory could not be inferred in a
 242 large fraction of patients by sequentially monitoring SARS-CoV-2 IgG levels,
 243 regardless of the commercial immunoassay used. However, threshold levels of IgGs
 244 predicting high NtAb₅₀ titers ($\geq 1/160$) with high specificity (93%) could be established
 245 for all immunoassays, although sensitivities were overall poor.

246 In the absence of a reference panel of sera with standard levels of NtAb antibodies,
 247 comparison across studies addressing the above issue is not straightforward, as the
 248 biological characteristics of the neutralization assay, the timing of sera collection and
 249 the clinical severity of COVID-19 patients differ notably between them and may explain
 250 certain discrepancies. Namely, Weidner et al. [12] reported a strong correlation between
 251 IgG levels measured by the LIAISON SARS-CoV-2 S1/S2 IgG and NtAb₅₀ titers
 252 quantified by a wild type SARS-CoV-2 assay ($Rho=0.75$ versus $Rho=0.59$ in the current
 253 study) in sera from convalescent COVID-19 patients. The degree of correlation reported
 254 by Muecksh et al. [20] in a comparable population using an HIV pseudotyped platform
 255 was even higher ($Rho=0.82$). Likewise, GeurtvanKessel et al. [13] found a strong
 256 correlation ($Rho=0.75$) using a live SARS-CoV-2 microneutralization assay and sera
 257 from a mixed patient population in terms of COVID-19 severity. Despite the above-
 258 highlighted differences across studies, our correlation data agree with those of Weidner

et al. [12] and GeurtvanKessel et al. [13] for the Euroimmun IgG assay (Rho 0.75 and 0.76, respectively, versus 0.73 herein) and those of Criscuolo et al. [15] who reported a low correlation between IgG levels measured by the LIAISON SARS-CoV-2 S1/S2 IgG and NtAb₅₀ titers quantified by using a live SARS-CoV-2 platform in sera obtained within 15 days after the onset of symptoms in a small cohort of COVID-19 hospitalized patients. Moreover, Bonelli et al. [18] found that the probability of having NtAb₅₀ titers >1/160, as determined by a SARS-CoV-2 wild type assay, was 92% when LIAISON SARS-CoV-2 S1/S2 IgG levels were >80 AU/ml (90 AU/ml in the current study for a positive predictive value of 98%). Also, in line with a previous report [20], the trajectory of NtAb₅₀ titers could not be predicted by assessing SARS-CoV-2 IgG levels when measured by commercial immunoassays.

In summary, our data indicate that the reliability SARS-CoV-2 IgG commercial immunoassays targeting the S protein to infer neutralizing activity against this protein in sera from hospitalized COVID-19 patients varies widely across tests, and depends upon the time of sera collection after the onset of symptoms.

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Ethical Approval

The current study was approved by the Research Ethics Committee of Hospital Clínico Universitario INCLIVA (March 2020).

Author statement

281 AV: methodology, analysis of data, validation, review & editing; IT: formal analysis,
 282 review and editing; VL: Methodology, investigation; CFG: Methodology, investigation;
 283 EA: resources, project administration, review and editing; RG-R: methodology,
 284 investigation, validation, funding acquisition, review & editing; MJA: methodology,
 285 analysis of data, validation, review & editing; JB: supervision; review & editing; JRD:
 286 Conceptualization, supervision, funding acquisition, review& editing; RG:
 287 Methodology, investigation, validation, funding acquisition, review & editing; DN:
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297 **Conflict of interest**

298 Declarations of interest: none.

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390 **Figure legends**

392 **Figure 1.** Correlation between NtAb₅₀ titers measured by a SARS-CoV-2-S
393 pseudotyped virus neutralization assay and IgG levels measured by commercial
394 SARS-CoV-2 IgG immunoassays in all sera (A), in sera obtained within the first
395 two weeks after the onset of symptoms (<15 days; B), and in sera collected
396 afterward (≥ 15 days; C). Rho and *P* values are shown.

397 **Figure 2.** ROC curve analysis for establishing the optimal SARS-CoV-2 IgG threshold
398 levels that predict the presence of high NtAb₅₀ titers ($\geq 1/160$) in hospitalized
399 patients with COVID-19 for the Euroimmun SARS-CoV-2 IgG ELISA (A),
400 LIAISON SARS-CoV-2 S1/S2 IgG assay (B), MAGLUMI 2019-nCoV IgG (C),
401 and COVID-19 ELISA IgG (D) assays.

402 **Figure 3.** Kinetics patterns of serum NtAb₅₀ titers quantitated by a SARS-CoV-2-S
403 pseudotyped virus neutralization assay and SARS-CoV-2 IgG levels measured
404 by commercial immunoassays in two representative patients displaying
405 concordant (upper panels) or discordant (lower panels) kinetics.

406

A **B** **C**

Table 1. Demographic, clinical and laboratory characteristics of patients with COVID-19

Parameter	Patients
Sex: Male/Female; no. (%)	32 (63)/ 19 (37)
Age; median (range)	53 (21-77)
Days of hospitalization; median (range)	17 (2-67)
Co-morbidities; no. (%)	35 (69)
Number of comorbidities; median (range)	1 (0-5)
Arterial hypertension	23 (45)
Chronic renal disease	2 (4)
Diabetes mellitus	12 (24)
Dyslipidemia	16 (31)
Ischemic cardiovascular disease	4 (8)
Myocardial infarction	2 (4)
Pulmonar disease ^a	7 (14)
Tumor	3 (6)
Laboratory findings; median (range)^a	
CRP (in mg/l)	44 (0.8-273)
Ferritin (ng/ml)	674 (2.5-2986)
Dimer-D (ng/ml)	903 (91-5445)
Lactose dehydrogenase (U/l)	666 (357-1328)
Interleukin-6 (pg/ml)	1012 (4.6-5000)
Total lymphocyte count (*10 ⁹ /L)	1.15 (0.17-3.98)
^a measurements in sera used for serological analyses reported in the current study.	

Table 2. Performance of an antibody neutralization method using a reporter-based pseudotyped virus (vesicular stomatitis virus pseudotyped with the SARS-CoV-2 spike protein) and four commercial SARS-CoV-2 IgG immunoassays for the diagnosis of COVID-19					
Qualitative results/Time of sampling after the onset of symptoms ^a	Antibody assay				
	GFP-VSV-SARS-CoV-2 S pseudotype NtAb test	Euroimmun SARS-CoV-2 IgG ELISA	LIAISON SARS-CoV-2 S1/S2 IgG	MAGLUMI 2019-nCoV IgG	COVID-19 ELISA IgG
Positive (all sera)	83	76	75	77	83
Negative (all sera)	7	14	15	13	7
Positive/<15 days	37	31	30	31	37
Negative/<15 days	4	10	11	10	4
Positive/≥15 days	46	45	45	46	46
Negative/≥15 days	3	4	4	3	3
^a A total of 90 sera were included, of which 41 were collected <15 days after the onset of symptoms and 49 afterwards (≥15 days).					

Table 3. Agreement between the results of a reporter-based pseudotyped virus antibody neutralization method (vesicular stomatitis virus pseudotyped with the SARS-CoV-2 spike protein) and four commercial SARS-CoV-2 IgG immunoassays

Paired results (NtAb assay/commercial immunoassay)	Commercial Immunoassay			
	MAGLUMI 2019-nCoV IgG: all sera/sera <15 days/sera ≥15 days	LIAISON SARS-CoV-2 S1/S2 IgG: all sera/sera <15 days/sera ≥15 days	Euroimmun SARS-CoV-2 IgG ELISA: all sera/sera <15 days/sera ≥15 days	COVID-19 ELISA IgG: all sera/sera <15 days/sera ≥15 days
Positive/Positive	75/30/45	74/29/45	75/30/45	82/36/46
Negative/Negative	5/3/2	6/3/3	6/3/3	6/3/3
Positive/Negative	8/7/1	9/8/1	8/7/1	1/1/0
Negative/Positive	2/1/1	1/1/0	1/1/0	1/1/0

NtAb, neutralizing antibodies.

A total of 90 sera were included, of which 41 were collected <15 days after the onset of symptoms and 49 afterward (≥15 days).

Table 4. Clinical sensitivity of an antibody neutralization method using a reporter-based pseudotyped virus (vesicular stomatitis virus pseudotyped with the SARS-CoV-2 spike protein) and four commercial SARS-CoV-2 IgG immunoassays for the diagnosis of COVID-19

Sera included in the analyses	% Sensitivity of the immunoassay (95% CI)				
	GFP-VSV-SARS-CoV-2 S pseudotype NtAb test	Euroimmun SARS-CoV-2 IgG ELISA	LIAISON SARS-CoV-2 S1/S2 IgG	MAGLUMI 2019-nCoV IgG	COVID-19 ELISA IgG
All sera ^a	92.2 (86.7-97.8)	84.4 (77.0-91.9)	83.3 (75.6-91.0)	85.6 (78.3-92.8)	92.2 (86.7-97.8)
Sera collected < 15 days after the onset of symptoms ^a	90.2 (77.5-96.1)	75.6 (60.7-86.2)	73.2 (58.1-84.3)	75.6 (60.7-86.2)	90.2 (77.5-96.1)
Sera collected ≥15 days since the onset of symptoms ^a	93.9 (83.5-97.9)	91.8 (88.8-96.8)	91.8 (88.8-96.8)	93.9 (83.5-97.9)	93.9 (83.5-97.9)
^a A total of 90 sera were included, of which 41 were collected <15 days after the onset of symptoms and 49 afterwards (≥15 days).					

Table 5. SARS-CoV-2-S- IgG threshold levels measured by four commercial immunoassays for predicting neutralizing antibody titers $\geq 1/160$ measured by a reporter-based pseudotyped virus antibody neutralization method (vesicular stomatitis virus pseudotyped with the SARS-CoV-2 spike protein)

Immunoassay	Threshold	Specificity (CI,95%)	Sensitivity (CI, 95%)	Positive predictive value (CI, 95%)	Negative predictive value (CI, 95%)
Euroimmun SARS-CoV-2 IgG ELISA	8.9 COI	93.8 (81.5-100)	55.4 (44.1-66.7)	97.6 (87.7-99.6)	31.2 (19.9-45.3)
LIAISON SARS-CoV-2 S1/S2 IgG	90.6 AU	93.8 (81.5-100)	67.6 (56.9-78.2)	98.1 (89.7-99.7)	38.5 (24.9-54.1)
MAGLUMI 2019-nCoV IgG	10.9 AU	93.8 (81.5-100)	64.9 (54.0-75.7)	98.0 (89.3-99.6)	36.6 (23.6-51.9)
COVID-19 ELISA IgG	4.1 AI	93.8 (81.5-100)	47.3 (35.9-58.7)	97.2 (85.8-99.5)	27.8 (17.6-40.9)