Clinical And Analytical Performance Of An Automated Serological Test That Identifies S1/S2 Neutralizing IgG In Covid-19 Patients Semiquantitatively.

Fabrizio Bonelli[a#], Antonella Sarasini[b], Claudia Zierold[a], Mariella Calleri[a], Alice Bonetti[b,c], Chiara Vismara[d], Frank Blocki[a], Luca Pallavicini[a], Alberto Chinali[a], Daniela Campisi[d], Elena Percivalle[b], Anna Pia DiNapoli[b,c], Carlo Federico Perno[d], Fausto Baldanti[b,c]

[a]DiaSorin, S.p.A., Saluggia, Italy

[b]Molecular Virology Unit, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia, Italy

[c]Department of Clinical, Surgical Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy

[d]Department of Laboratory Medicine, ASST Niguarda Hospital, University of Milan, Milan, Italy.

Running title: Automated Test For Covid-19 Neutralizing IgG Detection.

[#]**Corresponding author:** Fabrizio Bonelli, fabrizio.bonelli@diasorin.it

Abstract word count: 246

Text word count: 3188

ABSTRACT

BACKGROUND. In the Covid-19 pandemic, highly selective serological testing is essential to define exposure to SARS-CoV-2 virus. Many tests have been developed, yet with variable speed to first result, and of unknown quality, particularly when considering the prediction of neutralizing capacity.

OBJECTIVES/METHODS. The LIAISON® SARS-CoV-2 S1/S2 IgG assay was designed to measure antibodies against the SARS-CoV-2 native S1/S2 proteins in a standardized automated chemiluminescent assay. Clinical and analytical performance of the test were validated in an observational study using residual samples (>1500) with positive or negative Covid-19 diagnosis.

RESULTS. The LIAISON® SARS-CoV-2 S1/S2 IgG assay proved highly selective and specific, and offers semiquantitative measures of serum or plasma levels of anti-S1/S2 IgG with neutralizing activity. The diagnostic sensitivity was 91.3% and 95.7% at >5 or ≥15 days from diagnosis respectively, and 100% when assessed against a neutralizing assay. The specificity ranged between 97% and 98.5%. The average imprecision of the assay was <5 % coefficient of variation. Assay performance at 2 different cut-offs was evaluated to optimize predictive values in settings with different % disease prevalence.

CONCLUSIONS. The automated LIAISON® SARS-CoV-2 S1/S2 IgG assay brings efficient, sensitive, specific, and precise serological testing to the laboratory, with the capacity to test large amounts of samples per day: first results are available within 35 minutes with a throughput of 170 tests/hour. The test also provides a semiquantitative measure to identify samples with neutralizing antibodies, useful also for a large scale screening of convalescent plasma for safe therapeutic use.

IMPORTANCE

With the worldwide advance of the COVID-19 pandemic, efficient, reliable and accessible diagnostic tools are needed to support public health officials and healthcare providers in their efforts to deliver optimal medical care, and articulate sound demographic policy. DiaSorin has developed an automated serology based assay for the measurement of IgG specific to SARS CoV-2 Spike protein, and tested its clinical performance in collaboration with Italian health care professionals who provided access to large numbers of samples from infected and non-infected individuals. The assay delivers excellent sensitivity and specificity, and is able to identify samples with high levels of neutralizing antibodies. This will provide guidance in assessing the true immune status of subjects, as well as meeting the pressing need to screen donors for high titer convalescent sera for subsequent therapeutic and prophylactic use.

INTRODUCTION

SARS-CoV-2, the virus responsible for the Covid-19 pandemic, has spread at an alarming rate since the first case tracked back to mid-November of 2019 in Wuhan China (1). Contraction and subsequent transmission accrues most prevalently from community exposure, non-human exposure, or amongst relatives living in proximity to symptomatic or asymptomatic infected individuals. Due to the lack of readily available diagnostics, inferior means of infection control, or the inability to triage and isolate both acute and suspected cases consequent to space limitations, the Covid-19 pandemic, in placing increasingly excessive demands on the global healthcare network, has unveiled a number of critical limitations (2).

No safe vaccines have been developed for SARS-CoV infections to date, and the lack of currently available effective antiviral therapies, in spite of years of ongoing research, are certainly hampering efforts to combat this pandemic. In addition, the current molecular-based diagnostic tools utilized to diagnose infection, though serving adequately as the only means available, are not suitable for mass screening, and though many serological assays have been developed, no scientific data are available to authenticate their effectiveness. Finally, once the World Health Organization (WHO) recognized the SARS-CoV-2 outbreak as a Public Health Emergency of International Concern on January 30, 2020, efforts have been hampered internationally, nationally, and locally by a lack of coordinated guidance to properly inform public policy makers, and a lack of ready access to accurate and rapid testing.

Understanding the efficiency of community transmission of SARS-CoV-2, including the contribution of mild or asymptomatic cases, represents a knowledge gap

(1). As of May 5, 2020, global cases have surpassed 3 million, with 254,592 registered deaths (3). Global mortality consequent to SARS-CoV-2 infection is running at 7.0% with national rates for France, Italy, Spain, United Kingdom, Germany, and the United States running at 14.9%, 13.7%, 11.7%, 15.0%, 4.2%, and 5.8% (4).

In view of these daunting numbers, effective, sensitive and specific means for the identification and laboratory confirmation SARS-CoV-2 infection are urgently needed. In response to these needs, DiaSorin has developed a highly sensitive, specific, automated and contained chemiluminescence serological assay for the detection of SARS-CoV-2 Spike protein-specific antibodies with neutralizing potential from serum or plasma, to be used in diagnostic, epidemiological and vaccine evaluation studies. Specifically, it is envisioned that the test may be used to: 1) screen infected health care workers and the general population for recovery and/or past exposure; 2) epidemiological studies characterizing the demographics of viral spread and the efficacy of containment measures directed towards SARS-CoV-2 at the local, national, and international level; 3) screen convalescent sera for both therapeutic and prophylactic use, and 4) evaluate vaccine effectiveness in clinical studies.

RESULTS

Sample characteristics.

Clinical assessment of the LIAISON® SARS-CoV-2 S1/S2 IgG assay was performed using various sample groups (Figure 1). All the patient groups categorized as positive for Covid-19 were significantly different from the negative groups at $P<0.0001$ as determined by a pairwise t-test comparison with Bonferroni multiplicity adjustment. Median S1/S2 IgG levels were 96.3 AU/mL (95% CI 85.8 to 108.0 AU/mL, N=64), 28.6

AU/mL (95% CI 10.6 to 45.1 AU/mL, N=67), and 15.5 AU/mL (95% CI 5.7 to 32.2 AU/mL, N=80) for intensive care unit (ICU) patients, hospitalized patients, and RT-PCR-positive patients, respectively. Median levels of negative samples were 2.3 AU/mL (95% CI 2.2 to 2.4 AU/mL, N=1140), 2.4 AU/mL (95% CI 2.1 to 2.9 AU/mL, N=50), and 2.2 AU/mL (95% CI 1.8 to 4.6 AU/mL, N=10) for pre-Covid-19, RT-PCR negative, and other coronavirus (non-SARS-Cov-2) subjects, respectively. In addition, the ICU patient group had significantly higher levels of S1/S2 IgG compared to hospitalized patient group ($P<0.0001$). Table 1 further dissects the temporal component of distribution with early samples having low levels of S1/S2 IgG presenting a low positive predictive agreement to RT-PCR at time ≤5 days from diagnosis (33.3%), increasing to 95.7% at ≥15 days from diagnosis.

Clinical Performance

A receiver operating characteristic analysis was fitted to determine the best cut point supporting positive diagnoses. The maximum Youden index occurs at a cut point of 9.4 (sensitivity / specificity of 95% / 97% respectively), with an area under the curve of 0.980 (95% CI 0.960-0.990, $p<0.0001$, Figure 2).

Clinical performance of the LIAISON® SARS-CoV-2 S1/S2 IgG assay is shown in Table 2. The sensitivity was determined by investigating 211 samples collected longitudinally over the course of time from 84 patients at admission and thereafter variably up to 36 days. Infection with SARS-CoV-2 was confirmed by a positive RT-PCR test at the early phase of infection at time of diagnosis. Logarithmic values of SARS-Cov-2 S1/S2 IgG are plotted over time with a fitted curve (Figure 3), projecting estimations of 5 days for an average sample to reach 15 AU/mL, with 92.9% of the

samples exceeding the 15 AU/mL threshold by 5 or more days post diagnosis. Table 2 compares the sensitivities and specificities consequent to higher cut-off of 15 AU/mL as currently suggested by the manufacturer's instructions for use. Diagnostic sensitivity with the lower cut-off is calculated at 33.3% for the early samples (≤ 5 days after diagnosis) and 91.3% for samples collected >5 days post diagnosis. Diagnostic sensitivity with the higher cut-off drops to 22.6%% for the early samples (≤ 5 days after diagnosis) and 88.2% for samples collected >5 days post diagnosis. Conversely, specificity (from the testing of 1140 stored residual samples from laboratory routine collected before the Covid-19 outbreak) increased slightly from 97.1% to 98.5% at the lower and higher cut-offs, respectively. Specificities evaluated using all negative samples were 97.0% and 98.1%. The importance of choosing a cut-off that provides higher sensitivity (9 AU/mL) versus one that provides lower sensitivity but higher specificity (15 Au/mL) is influenced by the disease prevalence, reflected in positive and negative predictive values (PPV and NPV). When the intent is to use the assay for screening, a higher threshold may be desirable, whereas, in a high prevalence environment such as hospitals caring for high numbers of Covid-19 subjects, when the test is used for aid in diagnosis, the lower threshold 9 AU/mL may be preferred (Table 3).

Comparison to samples with neutralizing titer.

Comparison to a neutralization (NT) assay was evaluated by testing 304 samples collected during the outbreak from subjects whose NT assay results were available: 180 were NT assay-negative, and 124 were NT assay-positive (titer >1:40). Positive agreement was 94.4% (95% CI 88.8% - 97.2%) and negative agreement was 97.8%

(95% CI 94.1% - 99.1%). The relationship between the LIAISON® SARS-CoV-2 S1/S2 IgG assay and NT assay-negative or NT assay-positive samples portrays a nearly complete separation between the 2 groups with medians of 2.4 AU/mL (95% CI 2.2 to 2.6 AU/mL), and 61.8 AU/mL (95% CI 50.3 to 70.7 AU/mL), respectively (Figure 4). In Figure 5A, the LIAISON® SARS-CoV-2 S1/S2 IgG assay's measurements were separated into 3 semi-quantitative groups (<40 AU/mL, 40-80 AU/mL, and >80 AU/mL) and related to NT assay titers ≥1:160, which is the threshold recommended by the FDA guidelines for use in convalescent blood transfusion. 39% (17/43), 56% (24/43), and 87% (33/38) of the samples, respectively, had NT assay titers ≥1:160 (5). Furthermore, since the FDA guidelines also admit NT assay titers of ≥1:80 as acceptable, additional leeway is granted towards use of the LIAISON® SARS-CoV-2 S1/S2 IgG assay to pre-screen or assess blood donor samples for potential convalescent plasma/serum therapy: 92% (35/38) and 79% (34/43) of the > 80 AU/mL and 40-80 AU/mL groups, respectively, had NT assay titers ≥1:80 (Figure 5B).

Analytical Performance

The LIAISON® SARS-CoV-2 S1/S2 assay was evaluated for intra-assay imprecision using 6 samples with moderate, low, or negative S1/S2 IgG levels. The average intra-assay imprecision was 2.8 %CV (range 2.0-3.4%CV), and total-assay imprecision averaged 3.2%CV (range 2.7-3.9%CV) (Table 5).

Cross-reactivity with other coronaviruses was tested against 10 patient samples positive by their respective RT-PCR tests to other coronaviruses that maintained negative NT assay results by SARS-Cov-2. Their LIAISON values ranged from 1.81 to 7.09 AU/mL, with an average of 3.45 AU/mL which falls far below both cut points of 9

and 15 AU/mL indicating the absence of cross-reactivity with the other coronaviruses tested (Table 6). Additionally, cross-reactivity was assessed in samples from patients with conditions caused by other viruses, other organisms, or with atypical immune system activity. As shown in Table 7, 3 out of 160 assessed specimens (1.9%) resulted positive with the LIAISON® SARS-CoV-2 S1/S2 IgG assay. Potentially interfering substances such as triglycerides (3000 mg/dL), cholesterol (400 mg/dL), hemoglobin (1000 mg/dL) conjugated and unconjugated bilirubin (40 mg/dL), acetaminophen (500 mg/mL and ibuprofen (500 mg/mL) showed no interference at the indicated concentrations. The LIAISON® SARS-CoV-2 S1/S2 IgG assay demonstrated a negative bias up to a 16% in S1/S2 IgG-positive specimens with biotin concentrations above 3500 ng/mL, a concentration 15-fold higher than that induced following ingestion of a 20 mg/day biotin supplement (6).

DISCUSSION

SARS-Cov-2 is a single strand, positive sense RNA virus that is most closely related to SARS-CoV and other B lineage members of the β genogroup of coronaviruses (7, 8). Other readily recognized members of the coronavirus family include the extremely virulent MERS CoV, and the less virulent OC43, HKU1, 229E and NL63, HCoVs more commonly associated with the common cold in adults. Coronavirus RNA encodes for four major categories of structural protein including Membrane, Envelope, Nucleocapsid and Spike that are referred to as M, E, N and S, respectively. While N protein elicits cell mediated immunity attributable to two predominant CD8 T cell epitopes (9), of the remaining three structural proteins, S protein is widely

197 recognized as that most specific with regard to generating protective, neutralizing
198 antibodies (10, 11).

199 The specificities reported from *in vitro* diagnostic immunoassays (12-14) are
200 impacted greatly by the fidelity of preservation of both linear and conformational
201 epitopes of the given analyte being measured for presentation to specific
202 immunoglobulins within a patient's serum sample (here SARS-Cov-2 S1/S2). Specificity
203 is frequently significantly compromised by the casual manner in which most ELISA
204 assays are fabricated. This is consequent to the generally accepted means of passive
205 adsorption of the target analyte protein to the plastic or nylon surface of microtiter
206 plates. This procedure induces significant structural deformation and denaturation, with
207 the consequent loss of native conformation, as well as occlusion of access to both
208 conformational and linear epitopes beneath the protein stuck to the plastic titer plate's
209 surface. As explained in Materials and Methods, our system allows for optimal
210 maintenance of Spike protein conformation. Consequently, the LIAISON® SARS-CoV-2
211 S1/S2 IgG assay rendered no false positive results from NT assay-negative, RT-PCR-
212 positive samples for related coronavirus members, and its performance is sensitive,
213 specific, and precise as evaluated in >1500 samples.

214 The use of convalescent serum to treat subjects with acute SARS-Cov-2 has
215 growing appeal for meeting the immediate challenges being imposed upon increasingly
216 stressed health care systems, in light of the premature status of vaccines, and limited
217 availability of effective anti-viral therapeutic regimens (15-19). The LIAISON® SARS-
218 CoV-2 S1/S2 IgG assay was designed to detect IgG with neutralizing potential, and is
219 shown here to have very good sensitivity and specificity in identifying samples with

positive neutralization titers. Furthermore, if used in a semi-quantitative manner, higher LIAISON units are indicative of higher NT assay titers, and provide a pre-screen tool to assess large numbers of samples. While neutralization tests provide the recognized benchmark, they are not practical for implementation on a large scale screening basis, due to requirements for high biosecurity containment laboratories, and the need for highly trained personnel to execute labor-intensive protocols. With our system, clear separation of NT assay-negative samples from NT assay-positive samples was achieved. In fact, with 40-80 AU/mL levels measured by the LIAISON® SARS-CoV-2 S1/S2 IgG assay, the probability to have neutralization titers ≥1:80 and ≥1:160 was 79% and 56%, while with >80 AU/ml the probability of having neutralization titers ≥1:80 and ≥1:160 was 92%, and 87%, respectively. This may be useful for the efficient screening of convalescent plasma for safe therapeutic use.

The LIAISON® SARS-CoV-2 S1/S2 IgG assay's sensitivity increases significantly as the immune response matures, as one would expect for an IgG-based serology assay's assessment of a host response to viral infection (Table 1 & 2, and Figure 3). Here, sensitivities of 33.3% at <5 days but >91% at ≥5 days post admission on samples from 104 Italian patients whose RT-PCR tests were positive at the time of diagnosis are reported. Serology tests are now being utilized to gain an initial assessment of infection prevalence with reported numbers of ~20% and ~3% from New York and California, respectively (19). In California, a negative test with the LIAISON assay would have an accompanying NPV of >99.5%, and in NYC a NPV of >97.5%, regardless of cut-off, indicating that staying at home and avoiding exposures would be the best option. However, a positive test presents a quite different story, whereby California's

PPV of 80% derived from the higher cut-off, though overall less sensitive, would provide a positive test result, affording more confidence of the subject's true positivity, while the lower cut-off would present some ambiguity as regards any subject's real level of protection (PPV of 62%). In New York, however, regardless of the cut-off, the PPV of 89-95% affords a much greater degree of confidence that an individual would have protective levels of antibody. When testing in a hospital setting, the lower cut-off may be preferable to ensure a higher NPV, even though the overall specificity may be decreased.

In conclusion the automated LIAISON® SARS-CoV-2 S1/S2 IgG assay brings efficient, sensitive, specific, and precise serological testing to the laboratory. Further, the assay is amenable for semi-quantitative efficient pre-screening of samples for neutralizing antibody content, to be used in convalescent plasma therapy.

MATERIALS AND METHODS

Assay format. The LIAISON® SARS-CoV-2 S1/S2 IgG chemiluminescent assay is a recently developed assay from DiaSorin designed to detect IgG antibodies in the serum or plasma of subjects and patients exposed to the SARS-CoV-2 virus. The assay consists of paramagnetic microparticles (PMPs) coated with S1 and S2 fragments of the viral surface Spike protein (8). Recombinant fusion antigens were expressed in human cells (HEK-293) to ensure proper folding, oligomer formation and glycosylation, providing capture moieties more similar to the viral Spike proteins, as processed by natural cellular cleavage (20-22): this distinguishes the DiaSorin CLIA from commonly used ELISAs where the antigens are presented on plastic plate surfaces, and are

susceptible to significant denaturation consequent to passive adsorption to these hydrophobic surfaces (23, 24). Distally biotinylated-S1 and biotinylated-S2 proteins were tethered to the surface of paramagnetic particles coated with streptavidin to assure optimal presentation of both S1 and S2 for access and recognition by specific immunoglobulin within pathologic serum samples.

The automated assay format consists of a first incubation step (10 minutes) of S1/S2-coated PMPs with patient sample (20 μl of either plasma or serum) in assay buffer to allow the binding of IgG in the sample specific to the antigens, followed by a wash step to remove unbound materials. Next ABEI (N-(4-aminobutyl)-N-ethyl-isoluminol)-labeled polyclonal goat anti-human IgG are added to the PMPs and further incubated for 8 minutes. After a final wash cycle, starter reagents are added and emitted relative light units (RLU) proportional to the sample's anti-S1/S2 IgG levels are converted to arbitrary units (AU/mL) based on a standardized master curve. The automated assay is standardized based on a pool of patient samples with high S1/S2 IgG titers. First results are available within 35 minutes, and the throughput is 170 tests/hour.

Analytical assay performance. A 5 day precision study according to CLSI EP5-A3 guidelines was performed using a panel of 6 plasma samples, prepared by either spiking or diluting as necessary to obtain negative, low positive and moderate positive samples. The panel samples were tested with LIAISON® SARS-CoV-2 S1/S2 IgG assay in 6 replicates per run, 3 runs per day for 5 operating days on one LIAISON® XL Analyzer (N=90).

A cross-reactivity study was performed to evaluate: 1) other SARS viruses (HCoV-229E, HCoV-HKU1, HCoV-OC43, and HCoV-untyped); 2) conditions caused by other viruses that may cause symptoms similar to SARS-CoV-2 infection; 3) infectious diseases caused by other organisms; and 4) conditions that may result in atypical immune system activity. Samples for the evaluation were collected before October 2019, prior to the Covid-19 pandemic. In addition, samples with potentially interfering factors such as triglycerides, hemoglobin, bilirubin, cholesterol, acetaminophen, ibuprofen and biotin were assessed with the LIAISON® SARS-CoV-2 S1/S2 IgG assay.

SARS-CoV-2 microneutralization assay (NT assay). A neutralizing assay described elsewhere was used to determine the neutralization titer against SARS-CoV-2 (Percivalle et al, submitted for publication). Briefly, 50 μl of diluted serum (4-fold serial dilutions from 1:10 to 1:640) were added to an equal volume of viral suspension (tissue culture infectious dose 50 of a SARS-CoV2 strain isolated from a symptomatic patient), incubated, and then combined with Vero-E6 cells. After incubation, the cells were stained with Gram's crystal violet solution. Wells were scored to evaluate the degree of cytopathic effect compared to the virus control. Neutralizing titer was the maximum dilution evidencing a reduction of 90% of cytopathic effect. In this study a titer of ≥1:40 was considered positive. This test was used to confirm positive serological samples used in the clinical studies, and to determine the neutralization effectiveness of the samples for the identification of convalescent donors living in the first Italian Red Zone (Percivalle et al, submitted for publication).

Clinical Samples. This observational study used de-identified fresh or frozen residual samples collected between 2011 and April 2020 at the Policlinico San Matteo in

Pavia, and at the Niguarda Hospital in Milan (Italy). Sample groups included: 1) paired samples (admission and discharge) from patients affected by Covid-19 and hospitalized with moderate symptoms (confirmed by RT-PCR , N=31); 2) sets of samples (admission and follow-ups) from patients affected by Covid-19 and hospitalized in the ICU with severe symptoms (confirmed by RT-PCR, N=16); 3) samples from patients affected by Covid-19 and hospitalized in ICU with severe symptoms (confirmed by RT-PCR, N=21); 4) samples from patients affected by Covid-19 testing positive by RT-PCR (N=37); 5) samples from subjects collected before the outbreak of Covid-19 (lab routine 2011, N=1140); 6) samples from subjects not infected by SARS-CoV-2 but affected by other coronaviruses, i.e. HCoV-229E, HCoV-HKU1, HCoV-OC43, or HCoV-untyped strain (N=10); 7) samples from subjects testing negative by RT-PCR (N=50); 8) samples negative by the SARS-CoV-2 NT assay described in Materials & Methods, collected from subjects during the outbreak (N=180); and 9) NT assay-positive samples collected from subjects during the outbreak (N=124).

Pertinent additional information included sample collection time, days from diagnosis (hospital or ICU admission), and severity of symptoms (mild, moderate, severe). The protocol for this study (de-identified remainders) was determined to be exempt under existing ethics committee regulations.

Diagnosis. Diagnosis was based on results from routine RT-PCR used in the clinical evaluation (25), as well as NT-assay results. Samples not infected by SARS-CoV-2, but affected by other coronaviruses, were classified by sequencing.

Statistical analyses. The statistical program R 3.5 and MedCalc 19.2 were utilized for all analyses presented. A preliminary exploration using Box-Cox

334 methodology suggested that statistical analyses be done on the logarithmic scale due to
335 skewed distribution of the measurements.
336 Data supporting Figures and Tables in this manuscript will be made fully
337 available upon request.
338
339 **ACKNOWLEDGMENTS**.
340 We acknowledge the full financial support from DiaSorin S.p.A. for this clinical
341 trial. Employees of DiaSorin participated in the study design, data collection and
342 interpretation, and in the preparation of the manuscript.
343 We thank the product development team at DiaSorin (Andrea Dal Corso, Lorenzo
344 Querin, Elisa Mazzoleni, Piernatale Brusasca, Elisa Ghezzi, Clara Rossini, Valeria
345 Rigamonti, Massimo Panizzo, Paolo Ingallinella, Davide Zanin), all the clinical
346 coordinators for sample collection and analysis, data collection and interpretation, as
347 well as Prof Douglas Hawkins for assistance with the statistical analyses.
348 Conflict of interest statement: FB, CZ, MC, FAB, LP and AC are employees of
349 DiaSorin the manufacturer of the LIAISON® SARS-CoV-2 S1/S2 IgG test. CFP spoke
350 for, participated at boards of, and/or received research grants from Abbvie, Janssen,
351 Cepheid, Viiv, Gilead, Merck, Lilly, DiaSorin, Elitech. FB spoke at sponsored meetings,
352 participated at boards of, and/or received research grants from Abbott, Qiagen, Roche,
353 Takeda, Viiv, Merck, DiaSorin, Elitech, HuMabs, Biotest, ABL, NTP. AS, AB, CV,DC,
354 EP, and APD have no conflicts of interest to disclose.
355
356

REFERENCES

1. Reusken CB, Broberg EK, Haagmans B, Meijer A, Corman VM, Papa A, Charrel R, Drosten C, Koopmans M, Leitmeyer K. 2020. Laboratory readiness and response for novel coronavirus (2019-nCoV) in expert laboratories in 30 EU/EEA countries, January 2020. Eurosurveillance 25:2000082.
2. Al-Tawfiq JA, Auwaerter PG. 2019. Healthcare-associated infections: the hallmark of Middle East respiratory syndrome coronavirus with review of the literature. Journal of Hospital Infection 101:20-29.
3. Dong E, Du H, Gardner L. 2020. An interactive web-based dashboard to track COVID-19 in real time. The Lancet infectious diseases.
4. Johns Hopkins University, Coronavirus Resource Center. 2020. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE). https://coronavirus.jhu.edu/map.html. Accessed April 18, 2020.
5. U.S. Food and Drug Administration. May 1, 2020. Recommendations for Investigational COVID-19 Convalescent Plasma. https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-exemption-ide-process-cber/recommendations-investigational-covid-19-convalescent-plasma. Accessed May 5, 2020
6. Grimsey P, Frey N, Bendig G, Zitzler J, Lorenz O, Kasapic D, Zaugg CE. 2017. Population pharmacokinetics of exogenous biotin and the relationship between biotin serum levels and in vitro immunoassay interference. International journal of Pharmacokinetics 2:247-256.

7. Xia S, Yan L, Xu W, Agrawal AS, Algaissi A, Tseng C-TK, Wang Q, Du L, Tan W, Wilson IA. 2019. A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. Science advances 5:eaav4580.
8. Xia S, Zhu Y, Liu M, Lan Q, Xu W, Wu Y, Ying T, Liu S, Shi Z, Jiang S. 2020. Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. Cellular & Molecular Immunology:1-3.
9. Li T, Xie J, He Y, Fan H, Baril L, Qiu Z, Han Y, Xu W, Zhang W, You H. 2006. Long-term persistence of robust antibody and cytotoxic T cell responses in recovered patients infected with SARS coronavirus. PLoS One 1.
10. Buchholz UJ, Bukreyev A, Yang L, Lamirande EW, Murphy BR, Subbarao K, Collins PL. 2004. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. Proceedings of the National Academy of Sciences 101:9804-9809.
11. Yang Z-y, Kong W-p, Huang Y, Roberts A, Murphy BR, Subbarao K, Nabel GJ. 2004. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. Nature 428:561-564.
12. Grzelak L, Temmam S, Planchais C, Demeret C, Huon C, Guivel F, Staropoli I, Chazal M, Dufloo J, Planas D. 2020. SARS-CoV-2 serological analysis of COVID-19 hospitalized patients, pauci-symptomatic individuals and blood donors. medRxiv.
13. Liu W, Liu L, Kou G, Zheng Y, Ding Y, Ni W, Wang Q, Tan L, Wu W, Tang S. 2020. Evaluation of Nucleocapsid and Spike Protein-based ELISAs for detecting antibodies against SARS-CoV-2. Journal of clinical microbiology.

14. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, Sun R, Wang Y, Hu B, Chen W. 2020. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. Journal of medical virology.
15. Casadevall A, Pirofski L-a. 2020. The convalescent sera option for containing COVID-19. The Journal of clinical investigation 130.
16. Tiberghien P, de Lambalerie X, Morel P, Gallian P, Lacombe K, Yazdanpanah Y. 2020. Collecting and evaluating convalescent plasma for COVID-19 treatment: why and how. Vox sanguinis.
17. Syal K. 2020. COVID-19: Herd Immunity and Convalescent Plasma Transfer Therapy. Journal of Medical Virology.
18. Ahn JY, Sohn Y, Lee SH, Cho Y, Hyun JH, Baek YJ, Jeong SJ, Kim JH, Ku NS, Yeom J-S. 2020. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. Journal of Korean Medical Science 35.
19. Zhao Q, He Y. 2020. Challenges of Convalescent Plasma Therapy on COVID-19. Journal of Clinical Virology.
20. Heald-Sargent T, Gallagher T. 2012. Ready, set, fuse! The coronavirus spike protein and acquisition of fusion competence. Viruses 4:557-580.
21. Belouzard S, Millet JK, Licitra BN, Whittaker GR. 2012. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses 4:1011-1033.
22. Grant OC, Montgomery D, Ito K, Woods RJ. 2020. 3D Models of glycosylated SARS-CoV-2 spike protein suggest challenges and opportunities for vaccine development. bioRxiv.

425 23. Lichtenberg JY, Ling Y, Kim S. 2019. Non-Specific Adsorption Reduction Methods in
426 Biosensing. Sensors 19:2488.
427 24. Jung S-Y, Lim S-M, Albertorio F, Kim G, Gurau MC, Yang RD, Holden MA, Cremer
428 PS. 2003. The Vroman effect: a molecular level description of fibrinogen
429 displacement. Journal of the American Chemical Society 125:12782-12786.
430 25. Corman V, Bleicker T, Brünink S, Drosten C, Zambon M. 2020. Diagnostic detection
431 of 2019-nCoV by real-time RT-PCR. World Health Organization, Jan 17.

432

433

FIGURE LEGENDS

Figure 1: Distribution of SARS-CoV-2 S1/S2 IgG levels in various patient groups. All the patient groups categorized as positive (RT-PCR-positive, hospitalized and ICU) are significantly different from the negative groups (RT-PCR-negative, pre-Covid-19 and other HCov) at P<0.0001 (***), as determined by a pairwise t-test comparison with Bonferroni multiplicity adjustment. The ICU patient group is significantly different from the hospitalized patient group (p<0.0001, ###)

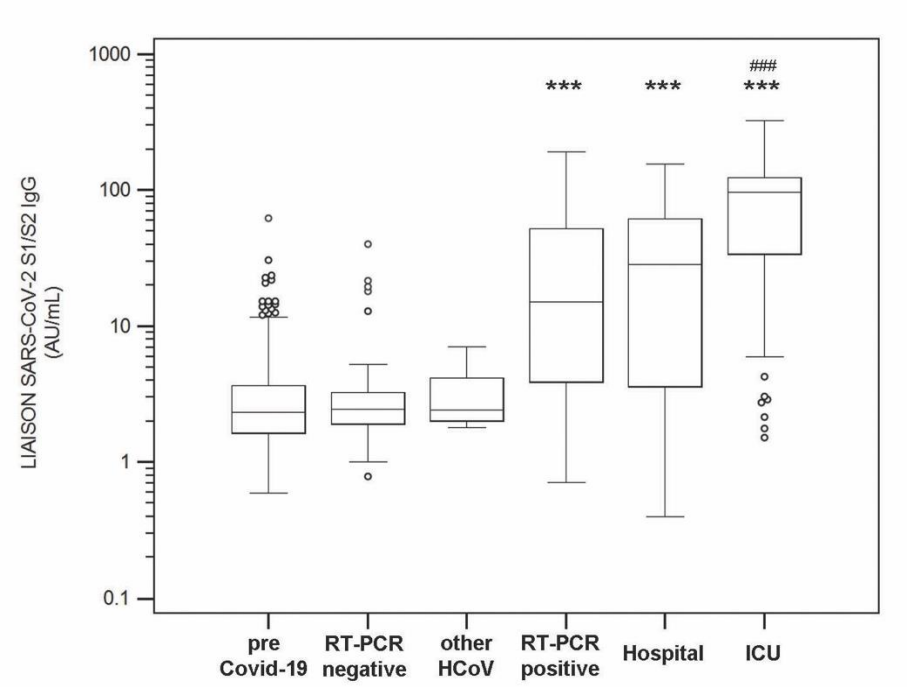
Figure 2: Receiver operating curve for distinguishing samples from patients affected by Covid-19 using the SARS-CoV-2 S1/S2 IgG test in a group of 1568 samples (N=188 positives). Area under the curve, AUC=0.980 (95% CI 0.960-0.990). The Youden Index associated cut-off is 9.4 AU/mL (95% CI >7.1 AU/mL to >12.1 AU/mL).

Figure 3: SARS-CoV-2 S1/S2 IgG measurements for 211 samples collected over the course of time from 84 patients at admission and variably thereafter up to 36 days. The upward trend was modeled using an exponential regression ln(IgG)=A+B* exp(C*Days). The parameter A (4.58) represents the upper limit to which the LIAISON® SARS-CoV-2 S1/S2 IgG trends over time and corresponds to 98 AU/mL. A+B (1.63) is the value at time zero corresponding to 5.1 AU/mL on the original scale. The parameter C (-0.112) is the rate at which the curve moves up to the asymptote and corresponds to 1.1 AU/mL per day. The dotted horizontal line is 15 AU/mL, the positive cut-off of the assay. The

curve cuts this at 5 days, giving an estimate of the delay from diagnosis above which the patient is on average positive.

Figure 4: Distribution of the LIAISON® SARS-CoV-2 S1/S2 IgG assay measurements compared to positive and negative samples by the NT assay.

Figure 5: Relationship and distribution of the LIAISON® SARS-CoV-2 S1/S2 IgG assay levels versus NT dilutions**.** LIAISON® SARS-CoV-2 S1/S2 IgG measurements were separated into 3 groups (<40 AU/mL, 40-80 AU/mL, and >80 AU/mL) and related to NT assay grouped by titer ≥ 1:160 **(A)** or ≥ 1:80 **(B)**. 39% (17/43), 56% (24/43), and 87% (33/38) of samples have a NT assay titer ≥1:160, while 65% (28/43), 79% (34/43), and 92% (35/38) of samples have a NT assay titer ≥1:80. Both titers are considered acceptable by FDA guidelines (5).



469

470 **Figure 1:** Distribution of SARS-CoV-2 S1/S2 IgG levels in various patient groups. All
471 the patient groups categorized as positive (RT-PCR-positive, hospitalized and ICU) are
472 significantly different from the negative groups (RT-PCR-negative, pre-Covid-19 and
473 other HCov) at P<0.0001 (***), as determined by a pairwise t-test comparison with
474 Bonferroni multiplicity adjustment. The ICU patient group is significantly different from
475 the hospitalized patient group (p<0.0001, ###)

476

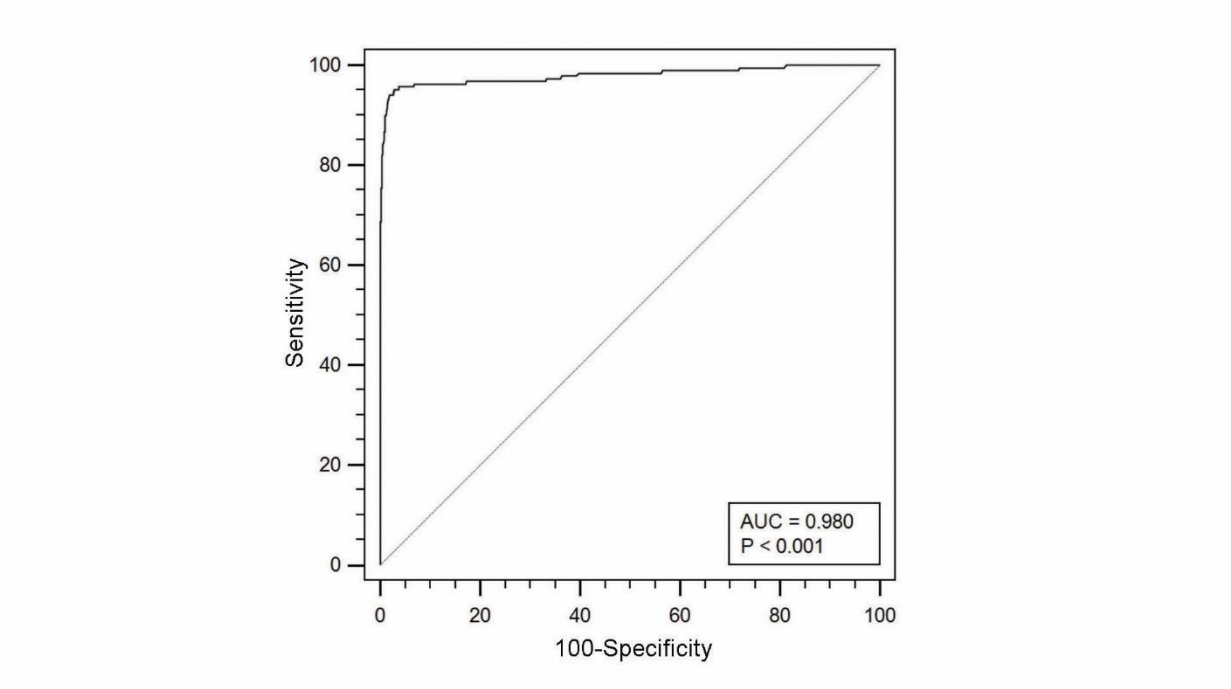


Figure 2: Receiver operating curve for distinguishing samples from patients affected by Covid-19 using the SARS-CoV-2 S1/S2 IgG test in a group of 1568 samples (N=188 positives). Area under the curve, AUC=0.980 (95% CI 0.960-0.990). The Youden Index associated cut-off is 9.4 AU/mL (95% CI >7.1 AU/mL to >12.1 AU/mL).

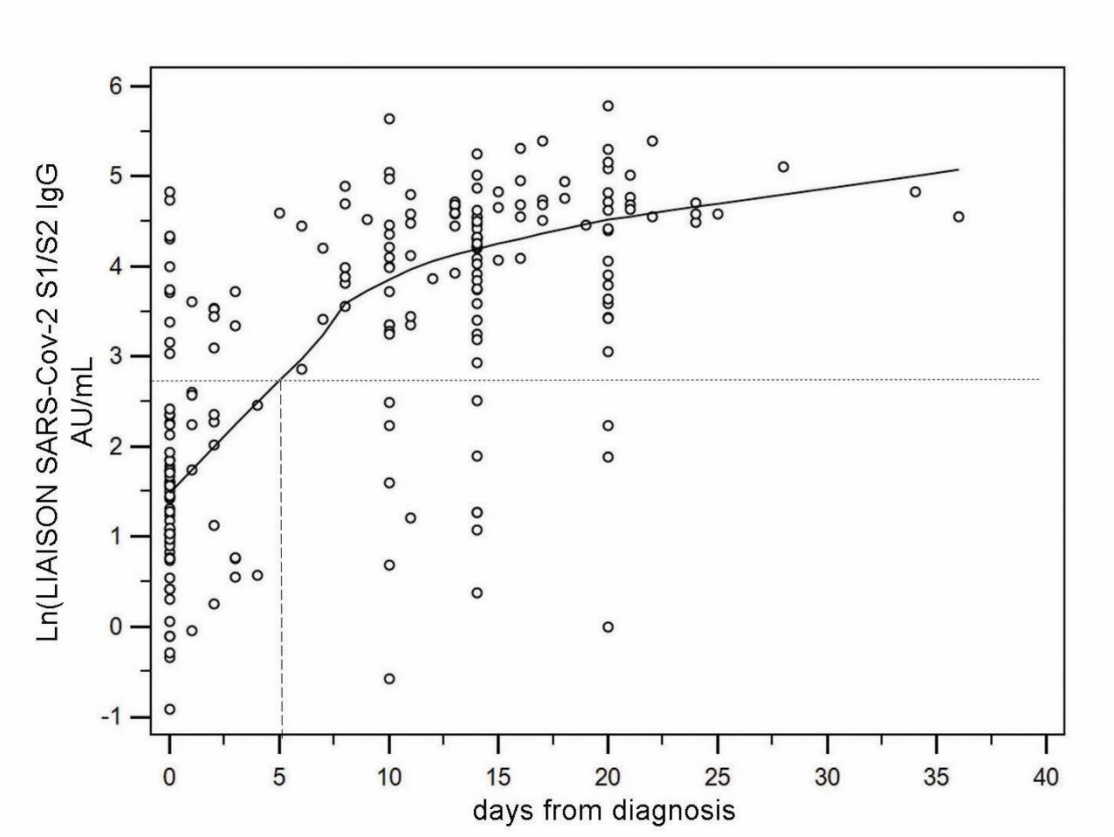


Figure 3: SARS-CoV-2 S1/S2 IgG measurements for 211 samples collected over the course of time from 84 patients at admission and variably thereafter up to 36 days. The upward trend was modeled using an exponential regression ln(IgG)=A+B* exp(C*Days). The parameter A (4.58) represents the upper limit to which the LIAISON® SARS-CoV-2 S1/S2 IgG trends over time and corresponds to 98 AU/mL. A+B (1.63) is the value at time zero corresponding to 5.1 AU/mL on the original scale. The parameter C (-0.112) is the rate at which the curve moves up to the asymptote and corresponds to 1.1 AU/mL per day. The dotted horizontal line is 15 AU/mL, the positive cut-off of the assay. The curve cuts this at 5 days, giving an estimate of the delay from diagnosis above which the patient is on average positive.

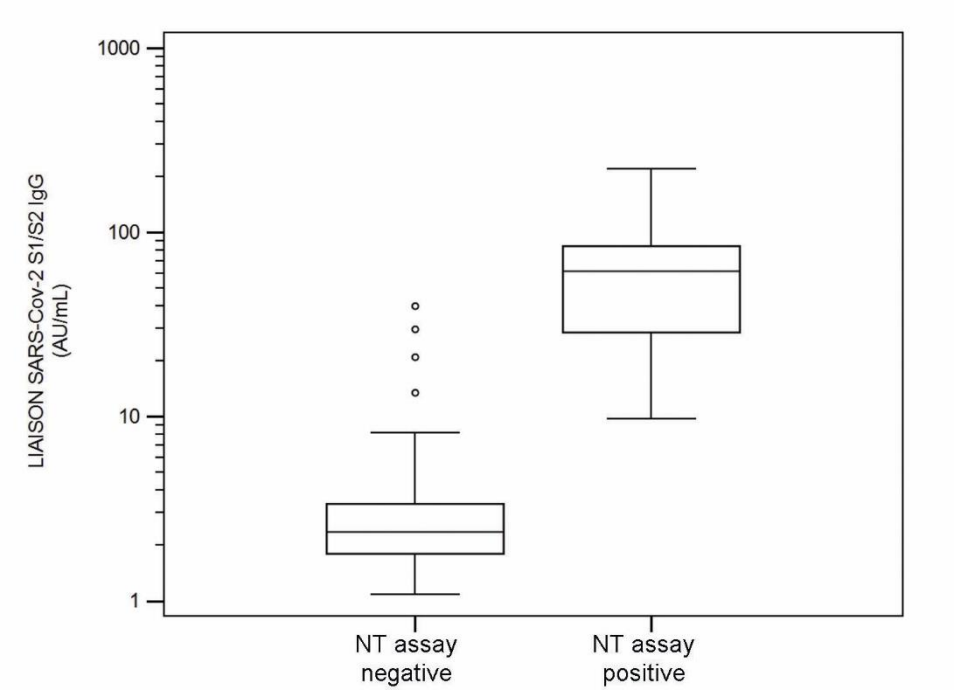


Figure 4: Distribution of the LIAISON® SARS-CoV-2 S1/S2 IgG assay measurements compared to positive and negative samples by the NT assay.

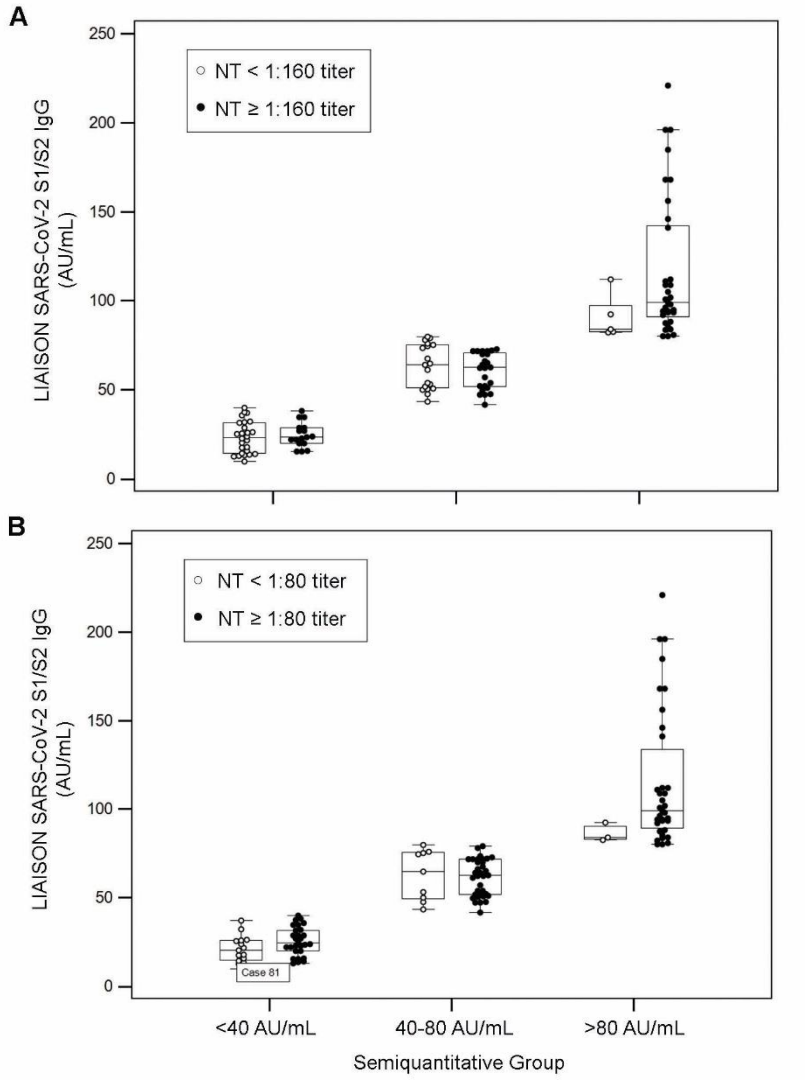


Figure 5: Relationship and distribution of the LIAISON® SARS-CoV-2 S1/S2 IgG assay levels versus NT dilutions**.** LIAISON® SARS-CoV-2 S1/S2 IgG measurements were separated into 3 groups (<40 AU/mL, 40-80 AU/mL, and >80 AU/mL) and related to NT assay grouped by titer ≥ 1:160 **(A)** or ≥ 1:80 **(B)**. 39% (17/43), 56% (24/43), and 87% (33/38) of samples have a NT assay titer ≥1:160, while 65% (28/43), 79% (34/43), and 92% (35/38) of samples have a NT assay titer ≥1:80. Both titers are considered acceptable by FDA guidelines (5).

Table 1: Longitudinal Assessment of Positive Predictive Agreement (PPA) to RT-PCR Diagnosis in Covid-19 Patients. Serial samples from 104 Covid-19 patients positive by RT-PCR admitted to the hospital or ICU were tested with the LIAISON® SARS-CoV-2 S1/S2 IgG assay. A value of 9 AU/mL was used as the cut-off for positivity.

Days from Diagnosis	**First Serial Measurement**		**Second Serial Measurement**		**Third Serial Measurement**		**Cumulative**	
	Total N	S1/S2 IgG^+	Total N	S1/S2 IgG^+	Total N	S1/S2 IgG^+	S1/S2 IgG^+/Total	**PPA (95%CI)**
≤ 5 days	84	28					28/84	33.3% (23.4% to 44.5%)
6-14 days	7	7	71	62	1	1	70/79	88.6% (79.5% to 94.7%)
≥15 days	13	13	12	12	22	20	45/47	95.7% (85.5% to 99.5%)
Total Subjects	104		83		23			

512 **Table 2:** Clinical Performance of the LIAISON® SARS-CoV-2 S1/S2 IgG Assay Using 9

513 and 15 AU/mL as Cut-off Based on RT-PCR Diagnoses.

514

			LIAISON® SARS-CoV-2 S1/S2 IgG Assay		
				95% CI	N
Cut-off 9 AU/mL	Sensitivity	≤ 5 days	33.3%	23.4% to 44.5%	84
		> 5 days	91.3%	85.0% - 95.6%	127
	Specificity	Pre-Covid-19	97.1%	96.0% - 98.0%	1140
		All negatives	97.0%	95.9% - 97.8%	1380
Cut-off 15 AU/mL	Sensitivity	≤ 5 days	22.6%	14.2% to 33.0%	84
		> 5 days	88.2%	81.3% - 93.2%	127
	Specificity	Pre-Covid-19	98.5%	97.6% - 99.1%	1140
		All negatives	98.1%	97.2% - 98.8%	1380

515

516 **Table 3:** Positive And Negative Predictive Values at Various Disease Prevalence

517 Thresholds for the LIAISON® SARS-CoV-2 S1/S2 IgG Assay at Cut-offs of 9 and 15

518 AU/mL

		LIAISON® SARS-CoV-2 S1/S2 IgG Assay			
Disease Prevalence	**Cut-off**	**PPV**	**95% CI**	**NPV**	**95% CI**
5%	9 AU/mL	62.2%	55.0 – 69.0%	99.7%	99.5 – 99.9%
	15 AU/mL	80.3%	71.4% - 86.9%	99.5%	99.2% - 99.7%
20%	9 AU/mL	88.7%	85.3% - 91.3%	98.8%	97.7% - 99.3%
	15 AU/mL	95.1%	92.2% - 96.9%	97.5%	96.2% - 98.3%
90%	9 AU/mL	99.6%	99.5% - 99.7%	69.2%	54.3% - 81.0%
	15 AU/mL	99.9%	99.8% - 99.9%	52.1%	41.5% - 62.5%

519

520 **Table 4:** Comparison of the LIAISON® SARS-CoV-2 S1/S2 IgG Assay Measurement

521 Using 9 and 15 AU/mL as Cut-offs from Samples with Known Neutralizing Titers

522 Defined as Negative (< 1:40) or Positive for Neutralizing Antibodies (≥ 1:40).

		LIAISON® SARS-CoV-2 S1/S2 IgG Assay	
Cut-off			**95% CI**
9 AU/mL	Sensitivity	100%	97.1 – 100%
	Specificity	97.8%	94.4% - 99.4%
15 AU/mL	Sensitivity	94.4%	80.7% - 97.7%
	Specificity	98.3%	95.2% - 99.7%

523

524 **Table 5**: Imprecision Data of the LIAISON® SARS-CoV-2 S1/S2 Assay from a 5 Day
525 Precision Study Conducted According to CLSI EP5-A3 Guidelines. The Panel Samples
526 Were Tested in 6 Replicates per Run, 3 Runs per Day for 5 Operating Days.

527

			Intra		Total	
Sample	**N**	**Mean (AU/mL)**	**SD**	**CV %**	**SD**	**CV %**
Negative 1	90	5.45	0.14	2.5	0.15	2.7
Negative 2	90	6.72	0.23	3.4	0.26	3.9
Low 1	90	11.1	0.34	3.0	0.38	3.4
Low 2	90	20.2	0.54	2.7	0.67	3.3
Moderate 1	90	40.1	1.14	2.8	1.17	2.9
Moderate 2	90	64.1	1.68	2.6	1.86	2.9

528

529 **Table 6:** Cross-reactivity with Other Coronaviruses Tested in Patient Samples Positive

530 by their Respective RT-PCR Tests.

Non-SARS Human Coronavirus	N	S1/S2 IgG+
HCoV-OC43	4	0
HCoV-HKU1	1	0
HCoV-229E	1	0
HCoV-untyped strain	4	0
Total	**10**	**0**

531

532 **Table 7:** Cross-reactivity with Other Conditions Caused by Other Viruses, Other Organ-

533 isms, or with Atypical Immune System Activity with Symptoms Similar to Covid-19.

534

Condition	N	S1/S2 IgG$^+$
Nuclear autoantibodies (ANA)	10	0
HBV	10	1
HCV	10	0
Influenza A	10	1
Influenza B	10	0
Respiratory syncytial virus	10	0
Borrelia burgdorferi	10	0
Mycoplasma pneumoniae	10	0
EBV	10	0
CMV	10	0
HSV-1/2	10	0
HAMA	10	0
Parvovirus B19	10	0
Rheumatoid factor	10	1
Rubella	10	0
VZV	10	0
Total	**160**	**3**

535
536

537

ABBREVIATIONS

ICU: intensive care unit

NT assay: neutralization assay

PPA: positive predictive agreement

PPV: positive predictive value

NPV: negative predictive value

S1: Spike protein fragment 1

S2: Spike protein fragment 2

IgG: immunoglobulin G