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Evaluation of four commercial, fully automated SARS-CoV-2 antibody tests suggests a revision of the Siemens SARS-CoV-2 IgG assay

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Abstract

Objectives: Serological tests detect antibodies against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in the ongoing coronavirus disease-19 (COVID-19) pandemic. Independent external clinical validation of performance characteristics is of paramount importance.

Methods: Four fully automated assays, Roche Elecsys Anti-SARS-CoV-2, Abbott SARS-CoV-2 IgG, Siemens SARS-CoV-2 total (COV2T) and SARS-CoV-2 IgG (COV2G) were evaluated using 350 pre-pandemic samples and 700 samples from 245 COVID-19 patients (158 hospitalized, 87 outpatients).

Results: All tests showed very high diagnostic specificity. Sensitivities in samples collected at least 14 days after disease onset were slightly lower than manufacturers' claims for Roche (93.04%), Abbott (90.83%), and Siemens COV2T (90.26%), and distinctly lower for Siemens COV2G (78.76%). Concordantly negative results were enriched for immunocompromised patients. ROC curve analyses suggest a lowering of

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the cut-off index for the Siemens COV2G assay. Finally, the combination of two anti-SARS-CoV-2 antibody assays is feasible when considering borderline reactive results.

Conclusions: Thorough on-site evaluation of commercially available serologic tests for detection of antibodies against SARS-CoV-2 remains imperative for laboratories. The potentially impaired sensitivity of the Siemens COV2G necessitates a switch to the company's newly filed SARS-CoV-2 IgG assay (sCOVG) for follow-up studies. A combination of tests could be considered in clinical practice.

Abbreviations

CI, confidence interval; CLIA, chemiluminescence immunoassay; COI, cut-off index; COVID-19, Coronavirus disease 2019; ELISA, enzyme-linked immunosorbent assay; LFIA, lateral flow immunoassay; ICU, intensive care unit; Ig, Immunoglobulin; IQR, interquartile range; ROC, receiver operating characteristic; RT-PCR, reverse transcription polymerase chain reaction

Introduction

Coronavirus disease 2019 (COVID-19) is caused by the Severe Acute Respiratory Syndrome Virus 2 (SARS-CoV-2) (1, 2), and was first described in China in December 2019 and declared pandemic by the WHO on March 11, 2020 (3). As of November 24, 2020 more than 59 million confirmed cases and almost 1.4 million deaths have been reported worldwide (for Austria: more than 250,000 confirmed cases and almost 2,500 deaths) (4). The total prevalence is estimated to be higher due to unrecognized infections (5). The gold standard for the primary diagnosis of acute SARS-CoV-2 infection remains the specific detection of viral RNA by molecular methods, including reverse

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transcription polymerase chain reaction (RT-PCR) (6, 7). However, molecular methods have some limitations, in particular the relatively short time frame of detectability post symptom onset and thus possible false negative results in patients who present with low viral load at later stages of the disease course (8-10). In contrast, the presence of SARS-CoV-2 antibodies indicates a recent or prior exposure to the virus (11). Serological tests are therefore important to assess the seroprevalence for monitoring the epidemiology of SARS-CoV-2 infection at the population-level. In view of a still relatively low seroprevalence in many countries, specificities of the assays are crucial for epidemiologic studies and the positive predictive value of a result (12). Furthermore, serologic testing in addition to PCR may aid to increase the accuracy of diagnosis, in particular when seroconversion is documented in consecutive blood samples (8, 13). Moreover, SARS-CoV-2 antibody tests are used to select eligible donors for convalescent plasma (14). Finally, serologic tests are used in SARS-CoV-2 vaccine studies to estimate the immunological response to vaccination (15). The durability of the antibody response as well as the extent and duration of immunity against reinfection with SARS-CoV-2 are still under investigation (16).

Immune response to SARS-CoV-2 includes cell-mediated (17) and antibody-mediated immunity (13). The SARS-CoV-2 genome encodes four major structural proteins, the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins, and several accessory proteins (18, 19). The S protein with its receptor-binding domain (RBD) and the N protein are widely used as antigens to assess the humoral immune responses by detection of specific antibodies (20-22). Serological assays currently used include neutralizing antibody assays, enzyme-linked immunosorbent assays (ELISA), automated chemiluminescence immunoassays (CLIA) and lateral flow immunoassays (LFIA) (21, 23). Distinct serologic assays detect different antibody isotypes: IgG, IgM,

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IgA or all isotypes simultaneously ("total antibodies"). ELISA and CLIA raw data are often calculated as index (in relation to a known sample such as provided control material) and reported as positive or negative depending on a predefined cut-off value.

A rapidly growing number of commercial SARS-CoV-2 antibody assays is available. Whereas the manufacturer of an *in vitro* diagnostic (IVD) device is obliged to declare performance specifications of the test, the need for independent validation of commercial assays in clinical settings has been highlighted in systematic reviews of the literature (24, 25). On the one hand, medical laboratories have to verify that they meet the performance specifications. On the other hand, small sample sizes and a lack of samples from patients with mild to moderate clinical course represent a potential bias in performance studies (26, 27). Our study aimed to evaluate four SARS-CoV-2 antibody assays on three fully automated large-scale laboratory analyzers manufactured by Abbott, Roche, and Siemens, respectively. To or knowledge this is the first published external validation of the Siemens SARS-CoV-2 IgG (COV2G) antibody test.

Materials and methods

Patients and study design

The present study was performed at the Central Institute of Clinical and Chemical Laboratory Diagnostics at the University Hospital of Innsbruck as part of the clinical evaluation of different SARS-CoV-2 serologic assays. All procedures performed in the present study involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments and were approved by the ethics committee of

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the Medical University of Innsbruck (ethics commission numbers: 1103/2020, 1167/2020).

245 patients with RT-PCR confirmed SARS-CoV-2 infection were included: hospitalized COVID-19 patients at the University Hospital of Innsbruck, reconvalescent COVID-19 patients with persistent cardio-pulmonary damage participating in a prospective observational study (CovILD-study, ClinicalTrials.gov number, NCT04416100) and reconvalescent persons volunteering as plasma donors at the Central Institute for Blood Transfusion and Immunology. According to the clinical course, patients were grouped as outpatients, hospitalized patients at the general ward, or hospitalized patients at the intensive care unit (ICU) ward. The patients' characteristics are shown in Table 1. In total, 700 patient samples were assessed. 75 patients had one, 66 patients two, 34 patients three, 23 patients four, 24 patients five and 23 patients six or more blood draws. Disease onset was defined as onset of clinical symptoms compatible with COVID-19 infection. Symptom onset was determined by a questionnaire in convalescent donors and by reviewing individual health records in the other patients. If the patient was asymptomatic or the date of symptom onset was not available (n = 35 patients, 15.2%; corresponding to 81 samples, 11.6%), the date of the first positive SARS-CoV-2 RT-PCR was used instead. The median time span between symptom onset and RT-PCR-based diagnosis was 5 days.

In addition, 350 archived samples drawn in the pre-COVID-19 era were used to validate the specificity of the assays. In detail, 274 unselected samples dated from February 2017 to November 2019, 51 samples from hospitalized patients with bacterial pneumonia and 25 samples from patients with rheumatologic diseases (14 rheumatoid arthritis, six spondylarthritis, three connective tissue disease, two late onset rheuma-

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toid arthritis) drawn before the COVID-19 era were examined. Additionally, an intravenous immunoglobulin formulation (Privigen®, 100 mg/ml, CSL Behring AG, Bern, Switzerland) composed of pre-pandemic pooled immunoglobulins (mainly IgG) of a large number of healthy donors from the US, which should by definition yield negative SARS-CoV-2 antibody results, was used for antibody assay evaluations.

Sample preparation

Blood samples were drawn according to routine clinical procedures. Upon centrifugation, serum specimens for antibody determination were kept at 4°C if analyses were conducted within 7 days or stored at -20°C in the case analyses were conducted at a later time point. Frozen samples were thawed and centrifuged prior to antibody determination.

Anti-SARS-CoV-2 assays

We evaluated the performance of the following fully automated CLIA tests on high throughput random access analyzers widely available in medical laboratories: Roche Elecsys Anti-SARS-CoV-2 assay on the Cobas e602 platform (Roche Diagnostics, Rotkreuz, Switzerland), Abbott SARS-CoV-2 IgG assay on the Architect i2000SR platform (Abbott Laboratories Abbott Park, IL, USA), Siemens SARS-CoV-2 total (COV2T) and SARS-CoV-2 IgG (COV2G) on the Advia Centaur XP platform (Siemens, Munich, Germany). All samples were processed according to the manufacturers' procedures

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with the specified controls and calibrators by trained laboratory staff. Test characteristics given in the manufacturers' product information are summarized in Supplemental Table S1.

Data analysis and statistics

Specificity was analyzed on 350 archived samples drawn in the pre-COVID-19 era and sensitivity on samples from patients with RT-PCR confirmed SARS-CoV-2 infection. Only one sample per patient was subjected to sensitivity analysis to avoid bias due to multiple testing. Patients were included if at least one sample dating between day 14 and day 120 after disease onset was available. In case of multiple samples available per patient, the sample closest to day 28 was included in the sensitivity analysis.

Statistical analyses were performed using MedCalc, version 11.5.1.0 (MedCalc Ltd., Ostend, Belgium) and Excel 2016 (Microsoft, Redmont, USA). Median and interquartile range (IQR) were used as descriptive measures of metric data. Categorical data are given as counts and percentages. 95% confidence intervals (CI) for proportions were calculated according to the Clopper-Pearson exact method. The difference between categorical data was assessed using Chi-square test (McNemar's test for paired data, "N-1" Chi-squared test for unpaired proportions). Receiver operating characteristic (ROC) curve analysis was performed using the DeLong method. The concordance correlation coefficient was calculated according Lin. Statistical significance was defined at a level of 0.05.

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Results

Specificity

Of the 350 pre-pandemic samples included, 341 were analyzed with the Roche Elecsys Anti-SARS-CoV-2 assay, 298 with Abbott SARS-CoV-2 IgG, 288 with Siemens SARS-CoV-2 total (COV2T), and 191 with Siemens SARS-CoV-2 IgG (COV2G). Using the manufacturers' cut-offs, the specificity ranged from 99.33% to 100.00% (Roche 99.71%, Abbott 99.33%, Siemens COV2T 99.65%, Siemens COV2G 100.00%; Table 2, Supplemental Table S2). The small number of false positive samples (Supplemental Table S3) showed no overlap between the tests as none of the samples was tested positive in more than one assay. No false positive result was observed in the subcohorts of pre-pandemic samples from patients with bacterial pneumonia (n=51) or rheumatologic diseases (n=25). Borderline cross reactivity when measuring undiluted intravenous immunoglobulin formulation (Privigen®) was found only for Siemens SARS-CoV-2 IgG (Index: 1.09). However when diluting Privigen® 1:50 with SARS-CoV-2 negative serum or sodium chloride 0.9% (reflecting a more physiological immunoglobulin concentration) all assays shielded negative results (Supplemental Table S4).

Sensitivity

From 230 (93.9%) of the 245 PCR-confirmed COVID-19 patients, a sample dating between day 14 and day 120 after disease onset was available for analysis. The median time between disease onset and blood sampling was 46 days (IQR 24-62, range 14-120; Table 1, Supplemental Figure S1). All single results of the 230 samples are shown in Supplemental Table S5. Using the manufacturers' cut-offs, the observed sensitivity

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of the assays ranged from 78.76% to 93.04% (Roche Elecsys Anti-SARS-CoV-2 93.04%, Abbott SARS-CoV-2 IgG 90.79%, Siemens SARS-CoV-2 total 90.26%, Siemens SARS-CoV-2 IgG 78.76%; Table 2, Supplemental Table S6). Compared to the COV2G assay, the Roche (p<0.0001), Abbott (p=0.0001) and COV2T (p<0.0001) tests were significantly more sensitive.

In an exploratory analysis, we assessed the sensitivity of the assays stratified for gender, age, and disease severity (Supplemental Table S6). When stratifying for gender, sensitivity was higher for males than for females within the Abbott (male: 94.52%, female: 84.15%; p=0.0095) and Siemens COV2G (male: 87.30%, female: 62.69%; p=0.0001) assays (Figure 1A). Stratified for patient age, sensitivity for Siemens COV2G was lower in the age group 18-49 years (63.93%) compared to patients aged 50-69 years (86.32%) and 70-100 years (83.78%) (Figure 1B). Next, we stratified the patients according to intensity of care needed (outpatients, n=85; hospitalized patients in general ward, n=107; and ICU patients, n=39) as a surrogate for disease severity. For outpatients sensitivity ranged from 64.86% to 91.76% (Roche 91.76%, Abbott 85.88%, Siemens COV2T 88.89%, Siemens COV2G 64.86%). For hospitalized patients at the general ward sensitivity ranged from 84.27% to 93.40% (Roche 93.40%, Abbott 93.33%, Siemens COV2T 90.00%, Siemens COV2G 84.27%). For ICU patients sensitivity ranged from 93.94% to 96.67% (Roche 94.87%, Abbott 94.74%, Siemens COV2T 93.94%, Siemens COV2G 96.67%). In particular, the COV2G test had a significantly lower sensitivity in outpatient compared to general ward or ICU patients (Figure 1C).

To address potential reasons for unexpected negative results, clinical records of patients tested negative with the majority of SARS-CoV-2 antibody tests were analyzed in detail. Out of the 230 patient samples included in the primary endpoint analysis, 13

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samples gave negative results in all performed tests and two samples in all but one assay (one was positive only in the Abbott and one only in the Siemens COV2T test). Seven out of those 15 patients were under immunosuppressive treatment not related to COVID-19. Of those, three were under ongoing chemotherapy due to malignancy, two patients had ongoing anti-CD20 therapy with obinutuzumab or rituximab due to lymphoma, one patient received methotrexate due to rheumatoid arthritis and another patient was under treatment with corticosteroids and rituximab because of myasthenia gravis. The other eight patients did not have relevant immunosuppressive conditions or therapies and in all those patients total serum IgG, IgM and IgA immunoglobulins were within the normal range.

Positivity rate across the course of disease

To estimate the time to positivity after disease onset, we analyzed all 700 samples from 245 PCR-confirmed COVID-19 patients and stratified the positivity rate for the time from disease onset (groups days 0-6, 7-13, 14-20, 21-40 and >40). For all tests, the positivity rate increased from week one (range 19.51% to 32.14%) to week two (range 40.62% to 54.64%) and week three (range 80.85% to 95.33%). While the positivity rate after week three slightly decreased for Roche and Abbott, the positivity rate of samples for Siemens COV2T and Siemens COV2G increased from day 14-20 to day 21-40 and dropped again after day 40 (Figure 2, Supplemental Table S7, Supplemental Figure S2). The latter effect might be confounded by the higher number of outpatient samples in the group of samples obtained >40 days from disease onset (Supplemental Figure S1).

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ROC curve analysis

ROC curve analysis revealed area under the curve (AUC) values of 0.984 for Roche Elecsys Anti-SARS-CoV-2, 0.982 for Abbott SARS-CoV-2 IgG, 0.975 for Siemens SARS-CoV-2 total (COV2T) and 0.966 for Siemens SARS-CoV-2 IgG (COV2G) (Figure 3, Supplemental Table S8). When comparing the ROC curves, the AUC of the COV2G test was significantly smaller than the AUC of Roche (p=0.0151) and Abbott (p=0.0174).

In an exploratory analysis, we asked if the assay cut-offs could be modified to improve the sensitivity. The proposed cut-off indexes (COI) based on the maximum sum of sensitivity and specificity in ROC curve analysis were below the manufacturers' COI: >0.15 for Roche, >0.54 for Abbott, >0.42 for Siemens COV2T and >0.32 for Siemens COV2G (Figure 3). Using these optimized COI instead of the manufacturers' COI, sensitivity improved from 93.04% to 95.65% for Roche, from 90.79% to 95.18% for Abbott, from 90.26% to 92.31% for Siemens COV2T and from 78.76% to 89.64% for Siemens COV2G. However, specificity of the single assays diminished from 99.71% to 97.36% for Roche, from 99.33% to 98.32% for Abbott, from 99.65% to 98.61% for Siemens COV2T and from 100.00% to 97.38% for Siemens COV2G when these modified COI were applied (Supplemental Table S9).

Concordance between results of different antibody assays indicates a potential for test combinations

When considering all 1,050 samples the concordance correlation coefficient between the four assays ranged from 77.45% to 92.49% (Supplemental Table S10). Of the 350 pre-pandemic samples included in our study, 235 were tested with all three assays.

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233 of these samples (99.1%) were tested negative with all three assays. In two (0.9%) samples, only the Abbott test yielded a positive result. To compare the sensitivity of the assays in detail, we restricted the analysis to a single sample per patient obtained between day 14 and day 120 after disease onset as described above. 189 samples of PCR-confirmed COVID-19 patients were tested with the four assays Roche, Abbott, and Siemens (COV2T and COV2G). 145 (76.7%) of them were positive with all four assays, and 11 (5.8%) were found negative in all four assays. Of note, 18 samples (9.5%) were negative only with COV2G (Figure 4). Due to the unexpected low sensitivity of the Siemens COV2G test, we restricted further analyses on combinatorial approaches to the three assays Roche Elecsys Anti-SARS-CoV-2, Abbott SARS-CoV-2 IgG and Siemens SARS-CoV-2 total (COV2T).

Finally, we asked whether a combination of two antibody tests could be useful to further improve the clinical performance of serologic SARS-CoV-2 tests. When combining any two out of the Roche, Abbott and Siemens COV2T assays, specificity improves to 100.00%, regardless of the combination used. However, sensitivity dropped to <90% when considering only samples with concordantly positive results in both tests (Table 3). When we used the modified COI instead of the manufacturers' COI, sensitivity of the combinations improved to 95.18% for Roche and Abbott (manufacturers' COI: 89.91%), 92.31% for Roche and Siemens COV2T (manufacturers' COI: 89.23%), and 92.27% for Abbott and Siemens COV2T (manufacturers' COI: 86.60%). In contrast, the specificity of the combined analyses was not affected by the lowering of the COI (Table 3).

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Discussion

In the present study we examined the performance characteristics of four fully automated SARS-CoV-2 antibody CLIA assays focusing on high throughput random access analyzers widely available in many medical laboratories. While the specificity of all assays was well comparable to the performance characteristics provided by the manufacturers, we observed a markedly lower sensitivity in our cohort (78.76% to 93.04% compared to >99%). Similar results have been found in other studies for the Roche, Abbott and the Siemens COV2T assays (28, 29). These findings emphasize the importance of real life data and different clinical scenarios in independent assay validations. While the more subtle differences between observed and expected sensitivity rates for Roche, Abbott and Siemens COV2T were coherent within our study and might be explained by specific characteristics of our patients cohort, the sensitivity of Siemens COV2G was markedly lower in our hands with 78.76% in the total cohort and 64.86% in outpatients. To the best of our knowledge, our study is the first public-available independent evaluation of the COV2G assay and warrants further studies to verify and potentially improve the performance of this test. ROC curve analysis of our results of the Siemens COV2G assay suggests that the cut-off might be too high. Indeed, a number of false negative samples showed borderline reactivity that did not exceed the manufactures' assay cut-off of 1.0. Lowering the cut-off to 0.32 would improve the sensitivity from 78.76% to 89.64% with modest effect on the assay specificity (100.00% to 97.38%). Of course, this post-hoc change of the cut-off would require to repeat the evaluation of sensitivity and specificity for the Siemens COV2G assay in a large cohort. Indeed, the company has just recently filed an FDA-application for approval of a new SARS-CoV-2 IgG assay (sCOVG) with improved sensitivity. Our results indicate that potential differences in the assay performance between the Siemens COV2G and the

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new sCOVG assays need to be considered when evaluating sequential samples of patients.

Interestingly, we found lower sensitivities in female vs. male patients with all four assays, whereby a statistical significant difference was found only in the Abbott and Siemens COV2G assays. In a sub-analysis this trend for a gender difference was found in all three different courses of disease (outpatient, general ward, ICU; data not shown). This is grossly in line with the findings of Korte et al., which also showed that men produce higher amounts of anti SARS-CoV-2 IgG and IgA after SARS-CoV-2 infection (30). This finding should also be included in the interpretation of single antibody test results.

Inconsistent results have been reported regarding the association between antibody titers and disease severity. For example in a serosurvey in health care workers of the Veneto Region of Italy, Plebani et al. found that symptomatic individuals were 100% SARS-CoV-2 antibody positive, whilst only in 58% of asymptomatic carriers antibodies were detectable (31). Phipps et al. could not find an association of IgG (Abbott) and IgM (in-house) antibody response and disease severity, however, patients were segregated in ICU vs. non-ICU care and unlike to the study of Plebani and our study no asymptomatic or outpatient populations, respectively, were described separately (32). The importance of including patients with mild disease course in antibody evaluation studies has already been highlighted (26). We did not observe a major difference between the positivity rates for outpatients, hospitalized patients and ICU patients for the Roche, Abbott and the Siemens COV2T assays. In contrast, the sensitivity rates for the Siemens COV2G were significantly lower in outpatients compared to hospitalized patients. The stringent cut-off of this particular assay might affect the results in patients with low antibody levels. However, none of the assays evaluated was optimized for

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quantification of antibody titers and quantitative analysis was limited by the dynamic range of the tests. Thus, our study was not designed to assess quantitative differences in antibody levels between patients.

Notably, absence of reactivity in serological assays could either reflect antibody test performance or the biological absence of antibodies in individuals as no clear gold standard for the evaluation of SARS-CoV-2 antibody tests exists (33). As samples from 13 patients (5.7%) were constantly negative in all assays, we conclude that the biological absence of antibodies was a relevant factor in our cohort. The biological absence of antibodies in individuals with RT-PCR confirmed SARS-CoV-2 infection might be explained by immediate infection clearance in the naso-pharyngeal space as a consequence of low viral exposure and/or effective immune function which does not induce a systemic immune response but results in detection of viral RNA by RT-PCR. While no data on T-cell mediated immune response were available in our patients, detailed clinical meta-data allowed to correlate the absence of humoral immune response with immunosuppression. Indeed, 5 of those 13 patients with constantly negative antibody tests were found being immunocompromised what likely explains the absence of a detectable systemic humoral immune response. The proportion of immunocompromised patients seems to affect the overall sensitivity rate in our cohort, and our findings warrant for careful interpretation of SARS-CoV-2 antibody results particularly in those.

SARS-CoV-2 seroprevalence is still used as a measure to estimate the true number of affected people during the pandemic. Such studies found seropositivity for SARS-CoV-2 antibodies in 2.4% (n=61,437) of residents in Wuhan in mid-May (34), 0.9% (n=3,186) in German regular blood donors from March to June (35), 1.0% (n=2,500) in Greece university personnel and students during June-July (36), and 4.6% (n=5,933) of health care workers till end of May at the University-Hospitals of Padova and Verona,

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Italy (31). Thus, a very high diagnostic specificity of serologic tests is crucial to minimize false positive results and improve the positive predictive value (PPV). In line with the performance characteristics provided by the manufacturers, we observed a false positive rate <1% for all tests using samples from the pre-pandemic era. However, even a small false positive rate substantially effects the PPV in a low-prevalence situation (12). It has thus been suggested to combine the results of two different SARS-CoV-2 antibody tests to further improve specificity and PPV of serologic testing (37, 38). As a prerequisite for this strategy, it has been shown that the vast majority of false positive results occurred independently with singular anti-SARS-CoV-2 CLIA assays whereas systematic false positive samples affecting multiple assays are very rarely observed (12). This is also in line with our observation. Although the number of false positive samples in our study is rather small, we observed not a single sample being false positive in more than one CLIA assay. In contrast, the concordance of false negative samples between the Roche, Abbott and Siemens COV2T assays was rather high and yielded in a combined sensitivity of >86% for any of these combinations. While singular tests have been optimized for maximal specificity, a combinatorial testing approach could allow to lower the thresholds to recognize borderline reactive samples without impairing specificity (38). With the lower cut-offs according to the ROC analysis, the combined sensitivity improved to >92% for any combination. These results suggest, that borderline reactive results slightly below the threshold of the test could benefit from further analyses with additional CLIA or ELISA assays. However, due to the huge impact of the disease prevalence and pretest probability on predictive values of the assay results, the purpose of the test (detection or exclusion of disease, seroprevalence studies) should be defined before adjustment of thresholds or combining different serologic tests in a certain setting (39).

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Limitations of our study include differences in the time between disease onset and serological assessment between patients due to the retrospective design of this assay validation study. To avoid potential biases, we constrained our analysis to samples drawn ≥14 days after onset of COVID-19 specific symptoms in SARS-CoV-2 RT-PCR confirmed patients or two weeks after the first positive RT-PCR result in patients for whom no information on symptom onset was available. The median time of 46 days between disease onset and date of the sample used for the sensitivity analysis fits well to the reported plateau of antibody production against SARS-CoV-2 (40). Thus, our study was designed to assess the maximal sensitivity of antibody tests ≥14 days and did not include the early phase of antibody production within the first days of COVID-19. Potential differences between the performances of the evaluated tests in the symptomatic phase of the disease can thus not be excluded. Likewise, the maximum time between disease onset and sampling was 120 days. The duration of antibody production and immunity after a SARS-CoV-2 infection is a major question. For example, in the study of Long et al. 40% of asymptomatic and 13% of symptomatic patients became seronegative in the early convalescent phase (41). Liu et al. found that SARS-CoV-2 antibodies substantially decreased in about 60 days after symptom onset (42). On the other hand, Isho et al. showed that IgG antibodies to SARS-CoV-2 are maintained in the majority of COVID-19 patients for at least three months post symptom onset (43). However, among others, the observed discrepancies may also be due to differences in the serologic assays used in the different studies. In our study, no substantial decrease of seropositivity was found within 120 days after disease onset. Further studies are needed to assess the performance of different serological tests in longitudinal analysis. In this regard, quantitative measurements of antibody titers and neutralization or pseudo-neutralization assays will be useful to monitor the course of the

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humoral immune response against SARS-CoV-2 in detail. Another limitation of our study was that only adult patients with COVID-19 have been included. For example, Pierce et al. found that serum neutralizing antibody titers were higher in adults compared to pediatric patients with COVID-19 (44). Additional studies are needed to evaluate the performance of the assays tested here for children.

In summary, we independently evaluated the performance of four widely available commercial SARS-CoV-2 antibody assays in an adult COVID-19 cohort including both patients with critical to severe as well as mild courses of the disease. While all assays met the desired specificity criteria, we observed a substantially lower sensitivity compared to the performance reported by the manufacturers. Our study emphasizes the importance of achieving additional performance data in real life including specific populations like immunocompromised patients and asymptomatic carriers. Importantly, our results suggest a limited sensitivity of the Siemens COV2G assay that will be replaced by the newly filed Siemens sCOVG assay. In conclusion, a growing number of fully automated SARS-CoV-2 antibody CLIA assays with sufficient performance characteristics is available for different high throughput analyzers. The selection of specific assays and the interpretation of results should carefully reflect the use case, and a combination of different SARS-CoV-2 antibody assays might be useful.

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Author contributions: CI, AE, WP, LL, MA, AG, and GH performed or analyzed serological tests. SS, TS, WM, HS, JL-R, RB-W, IT, and GW obtained clinical data. CI and AE performed statistical analysis. CI, AE, AG, and GH designed the study and wrote the paper. All authors revised and approved the manuscript.

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Tables

Table 1. Characteristics of COVID-19 patients

		Total		Outpatient		Hospitalized General ward		Hospitalized Intensive care	
		Absolute	**%**	**Absolute**	**%**	**Absolute**	**%**	**Absolute**	**%**
Number	n	245	100%	87	36%	116	47%	42	17%
Age (years)	min	20		20		25		44	
	max	95		83		95		82	
	median (IQR)	56 (44- 69)		39 (29- 56)		64 (54- 77)		59 (54- 67)	
Sex	female	87	36%	34	39%	42	36%	11	26%
	male	158	64%	53	61%	74	64%	31	74%
Symptom onset known	yes	205	84%	61	70%	104	90%	40	95%
	no	40	16%	26	30%	12	10%	2	5%
Time between symptom onset and PCR (days)[a]	median (IQR)	5 (2-7)		2 (1-7)		5 (3-8)		5 (3-7)	
Time between disease onset and blood draw (days)[b]	median (IQR)	46 (24-62)		53 (42-66)		28 (18-59)		28 (26-41)	

[a] Refers to the first positive SARS-CoV-2 PCR of the patient; [b] refers to the representative sample used in the sensitivity analysis. Abbreviations: IQR, interquartile range, PCR, polymerase chain reaction.

Table 2. Sensitivity and specificity of the assays

		Investigated patient cohort	Manufacturers' claims
Roche Elecsys Anti-SARS-CoV-2	Sensitivity (n=230)	93.04% (88.95-95.97)[a]	99.50% (97.00-100.00)[b]
	Specificity (n=341)	99.71% (98.36-99.95)	99.80% (99.69-99.88)
Abbott SARS-CoV-2 IgG	Sensitivity (n=228)	90.79% (86.33-93.90) [a]	100.0% (95.89-100.00)[c]
	Specificity (n=298)	99.33% (97.59-99.82)	99.63% (98.98-99.89)
Siemens SARS-CoV-2 total (COV2T)	Sensitivity (n=195)	90.26% (85.28-93.67) [a]	100.0% (92.45-100.00)[b]
	Specificity (n=288)	99.65% (98.06-99.94)	99.81% (99.41-99.96)
Siemens SARS-CoV-2 IgG (COV2G)	Sensitivity (n=193)	78.76% (72.45-83.94) [a]	100.00% (91.59-100.00)[b]
	Specificity (n=191)	100.00% (98.03-100.00)	99.89% (99.61-99.99)

Sensitivity in the investigated cohort was evaluated using samples of SARS-CoV-2 PCR-confirmed patients dating ≥14 days after disease onset and the sample closest to day 28 after disease onset was chosen (one sample per patient). Specificity was determined on pre-pandemic samples. 95% confidence intervals are shown in brackets. [a]: ≥ day 14 after disease onset; [b]: ≥ day 14 after first PCR-positivity; [c]: ≥ day 14 after symptom onset

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Table 3. Sensitivity and specificity for the combination of two tests

		Manufacturers' COI	Modified COI
Roche + Abbott	Combined sensitivity (n=228)	89.91 (85.32-93.18%)	95.18% (91.57-97.28%)
	Combined specificity (n=291)	100.00% (98.70-100.00%)	100.00% (98.70-100.00%)
Roche + Siemens COV2T	Combined sensitivity (n=195)	89.23% (84.10-92.85%)	92.31% (87.70-95.28%)
	Combined specificity (n=282)	100.00% (98.66-100.00%)	100.00% (98.66-100.00%)
Abbott + Siemens COV2T	Combined sensitivity (n=194)	86.60% (81.09-90.69%)	92.27% (87.64-95.26%)
	Combined specificity (n=241)	100.00% (98.43-100.00%)	100.00% (98.43-100.00%)

Sensitivity and specificity for the combination of assays (double positive samples were regarded as positive, single or double negative samples were regarded as negative) using different cut-off indexes (COI): the COI according to the manufacturer and a modified COI according to ROC curve analysis of the results of our cohort. Sensitivity in the investigated cohort was evaluated using samples of SARS-CoV-2 PCR-confirmed patients dating ≥14 days after disease onset and the sample closest to day 28 after disease onset was chosen (one sample per patient). Specificity was determined on pre-pandemic samples. 95% confidence intervals are shown in brackets. Abbreviations: Roche, Roche Elecsys Anti-SARS-CoV-2; Abbott, Abbott SARS-CoV-2 IgG; Siemens COV2T, Siemens SARS-CoV-2 total.

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Figure legends

Figure 1. Subgroup analyses of sensitivity according to gender, age and severity of disease. Comparison of the sensitivity of the four investigated tests in representative samples of 230 patients with PCR-confirmed COVID-19: Roche (Roche Elecsys Anti-SARS-CoV-2, blue), Abbott (Abbott SARS-CoV-2 IgG, green), Siemens COV2T (Siemens SARS-CoV-2 total, orange) and Siemens COVG (Siemens SARS-CoV-2 IgG, brown). Results were analyzed stratified for gender (A), age (B) and severity of disease (C).

Figure 2. Positivity rate across the course of disease. All 700 samples from 245 PCR-confirmed SARS-CoV-2 infected patients were subjected to antibody determination with Roche (Roche Elecsys Anti-SARS-CoV-2, n=695, blue), Abbott (Abbott SARS-CoV-2 IgG, n=684, green), Siemens COV2T (Siemens SARS-CoV-2 total, n=517, orange) and Siemens COVG (Siemens SARS-CoV-2 IgG, n=498, brown). The positivity rate of the respective tests is shown in dependence of the time between disease onset and blood draw in days (d).

Figure 3. ROC curve analysis and modified cut-off indexes. ROC curves (left) and corresponding raw data indexes (right) for the investigated tests from Roche (Roche Elecsys Anti-SARS-CoV-2, A-B, blue), Abbott (Abbott SARS-CoV-2 IgG, C-D, green), Siemens COV2T (Siemens SARS-CoV-2 total, E-F, orange) and Siemens COV2G (Siemens SARS-CoV-2 IgG, G-H, brown). Analyses are based on pre-pandemic (=negative) samples (samples, Roche: 341, Abbott: 298, Siemens COV2T: 288, Siemens COV2G: 191) and all the samples of SARS-CoV-2 PCR-confirmed patients dating ≥14 days after disease onset (samples, Roche: 537, Abbott: 530, Siemens COV2T: 392, Siemens COV2G: 375). The horizontal lines in the dot plots represent the manufacturers' cut off index (dashed line, Roche and Siemens: 1.0, Abbot: 1.4) and the modified cut off index according to ROC curve analysis (solid line).

Figure 4. Concordance of the SARS-CoV-2 antibody results in PCR-confirmed patients. Overlap of negative (A) and positive (B) antibody test results of different assays in SARS-CoV-2 PCR-confirmed COVID-19 patients (samples dating ≥14 days after disease onset; one representative sample per patient; a total of 189 samples in which data from all four assays were available). Abbreviations: Abbott, Abbott SARS-CoV-2 IgG; Roche, Roche Elecsys Anti-SARS-CoV-2; COV2G, Siemens SARS-CoV-2 IgG; COV2T, Siemens SARS-CoV-2 total.

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References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33.
2. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;382(18):1708-20.
3. World Health Organization. Virtual press conference on COVID-19 – 11 March 2020 [Available from: https://www.who.int/docs/default-source/coronaviruse/transcripts/who-audio-emergencies-coronavirus-press-conference-full-and-final-11mar2020.pdf?sfvrsn=cb432bb3_2.
4. Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. COVID-19 Dashboard [Available from: https://coronavirus.jhu.edu/map.html
5. Nishiura H, Kobayashi T, Yang Y, Hayashi K, Miyama T, Kinoshita R, et al. The Rate of Underascertainment of Novel Coronavirus (2019-nCoV) Infection: Estimation Using Japanese Passengers Data on Evacuation Flights. J Clin Med. 2020;9(2).
6. Ravi N, Cortade DL, Ng E, Wang SX. Diagnostics for SARS-CoV-2 detection: A comprehensive review of the FDA-EUA COVID-19 testing landscape. Biosens Bioelectron. 2020;165:112454.
7. Li C, Zhao C, Bao J, Tang B, Wang Y, Gu B. Laboratory diagnosis of coronavirus disease-2019 (COVID-19). Clin Chim Acta. 2020;510:35-46.
8. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis. 2020.
9. Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, et al. Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. JAMA. 2020.
10. Hu Z, Song C, Xu C, Jin G, Chen Y, Xu X, et al. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. Sci China Life Sci. 2020.
11. Azkur AK, Akdis M, Azkur D, Sokolowska M, van de Veen W, Bruggen MC, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy. 2020;75(7):1564-81.
12. Perkmann T, Perkmann-Nagele N, Breyer MK, Breyer-Kohansal R, Burghuber OC, Hartl S, et al. Side-by-Side Comparison of Three Fully Automated SARS-CoV-2 Antibody Assays with a Focus on Specificity. Clin Chem. 2020;66(11):1405-13.
13. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis. 2020.
14. safety. ECD-gfhaf. An EU programme of COVID-19 convalescent plasma collection and transfusion. Guidance on collection, testing, processing, storage, distribution and monitored use. . June 22, 2020.
15. Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med. 2020.
16. Bohn MK, Lippi G, Horvath A, Sethi S, Koch D, Ferrari M, et al. Molecular, serological, and biochemical diagnosis and monitoring of COVID-19: IFCC taskforce evaluation of the latest evidence. Clin Chem Lab Med. 2020;58(7):1037-52.
17. Braun J, Loyal L, Frentsch M, Wendisch D, Georg P, Kurth F, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature. 2020.
18. Yoshimoto FK. The Proteins of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2 or n-COV19), the Cause of COVID-19. Protein J. 2020;39(3):198-216.
19. Tang YW, Schmitz JE, Persing DH, Stratton CW. The Laboratory Diagnosis of COVID-19 Infection: Current Issues and Challenges. J Clin Microbiol. 2020.
20. Vashist SK. In Vitro Diagnostic Assays for COVID-19: Recent Advances and Emerging Trends. Diagnostics (Basel). 2020;10(4).
21. Espejo AP, Akgun Y, Al Mana AF, Tjendra Y, Millan NC, Gomez-Fernandez C, et al. Review of Current Advances in Serologic Testing for COVID-19. Am J Clin Pathol. 2020;154(3):293-304.

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22. Poh CM, Carissimo G, Wang B, Amrun SN, Lee CY, Chee RS, et al. Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients. Nat Commun. 2020;11(1):2806.
23. Theel ES, Harring J, Hilgart H, Granger D. Performance Characteristics of Four High-Throughput Immunoassays for Detection of IgG Antibodies against SARS-CoV-2. J Clin Microbiol. 2020;58(8).
24. Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, et al. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ. 2020;370:m2516.
25. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev. 2020;6:CD013652.
26. Patel E, Bloch EM, Clarke W, Hsieh YH, Boon D, Eby YJ, et al. Comparative performance of five commercially available serologic assays to detect antibodies to SARS-CoV-2 and identify individuals with high neutralizing titers. medRxiv. 2020.
27. Plebani M. Antibody responses in mild COVID-19 hospital staff. EBioMedicine. 2020;59:102940.
28. Schnurra C, Reiners N, Biemann R, Kaiser T, Trawinski H, Jassoy C. Comparison of the diagnostic sensitivity of SARS-CoV-2 nucleoprotein and glycoprotein-based antibody tests. J Clin Virol. 2020;129:104544.
29. Manthei DM, Whalen JF, Schroeder LF, Sinay AM, Li SH, Valdez R, et al. Differences in Performance Characteristics Among Four High-Throughput Assays for the Detection of Antibodies Against SARS-CoV-2 Using a Common Set of Patient Samples. Am J Clin Pathol. 2020.
30. Korte W, Buljan M, Rosslein M, Wick P, Golubov V, Jentsch J, et al. SARS-CoV-2 IgG and IgA antibody response is gender dependent; and IgG antibodies rapidly decline early on. J Infect. 2020.
31. Plebani M, Padoan A, Fedeli U, Schievano E, Vecchiato E, Lippi G, et al. SARS-CoV-2 serosurvey in health care workers of the Veneto Region. Clin Chem Lab Med. 2020.
32. Phipps WS, SoRelle JA, Li QZ, Mahimainathan L, Araj E, Markantonis J, et al. SARS-CoV-2 Antibody Responses Do Not Predict COVID-19 Disease Severity. Am J Clin Pathol. 2020.
33. National S-C-SAEG. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. Lancet Infect Dis. 2020.
34. Pan Y, Li X, Yang G, Fan J, Tang Y, Hong X, et al. Seroprevalence of SARS-CoV-2 immunoglobulin antibodies in Wuhan, China: part of the city-wide massive testing campaign. Clin Microbiol Infect. 2020.
35. Fischer B, Knabbe C, Vollmer T. SARS-CoV-2 IgG seroprevalence in blood donors located in three different federal states, Germany, March to June 2020. Euro Surveill. 2020;25(28).
36. Tsitsilonis OE, Paraskevis D, Lianidou E, Pierros V, Akalestos A, Kastritis E, et al. Seroprevalence of Antibodies against SARS-CoV-2 among the Personnel and Students of the National and Kapodistrian University of Athens, Greece: A Preliminary Report. Life (Basel). 2020;10(9).
37. Suhandynata RT, Hoffman MA, Kelner MJ, McLawhon RW, Reed SL, Fitzgerald RL. Multi-platform Comparison of SARS-CoV-2 Serology Assays for the Detection of COVID-19. J Appl Lab Med. 2020.
38. Perkmann T, Oszvar-Kozma M, Koller T, Breyer M-K, Breyer-Kohansal R, et al. Increasing both specificity and sensitivity of SARS-CoV-2 antibody tests by using an adaptive orthogonal testing approach. medRxiv.2020.
39. Plebani M, Padoan A, Sciacovelli L, Basso D. Towards the rational utilization of SARS-CoV-2 serological tests in clinical practice. Clin Chem Lab Med. 2020;58(10):e189-e91.
40. Favresse J, Eucher C, Elsen M, Laffineur K, Dogne JM, Douxfils J. Response of anti-SARS-CoV-2 total antibodies to nucleocapsid antigen in COVID-19 patients: a longitudinal study. Clin Chem Lab Med. 2020;58(10):e193-e6.
41. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020;26(8):1200-4.
42. Liu A, Li Y, Peng J, Huang Y, Xu D. Antibody responses against SARS-CoV-2 in COVID-19 patients. J Med Virol. 2020.

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43. Isho B, Abe KT, Zuo M, Jamal AJ, Rathod B, Wang JH, et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci Immunol. 2020;5(52).

44. Pierce CA, Preston-Hurlburt P, Dai Y, Aschner CB, Cheshenko N, Galen B, et al. Immune responses to SARS-CoV-2 infection in hospitalized pediatric and adult patients. Sci Transl Med. 2020;12(564).

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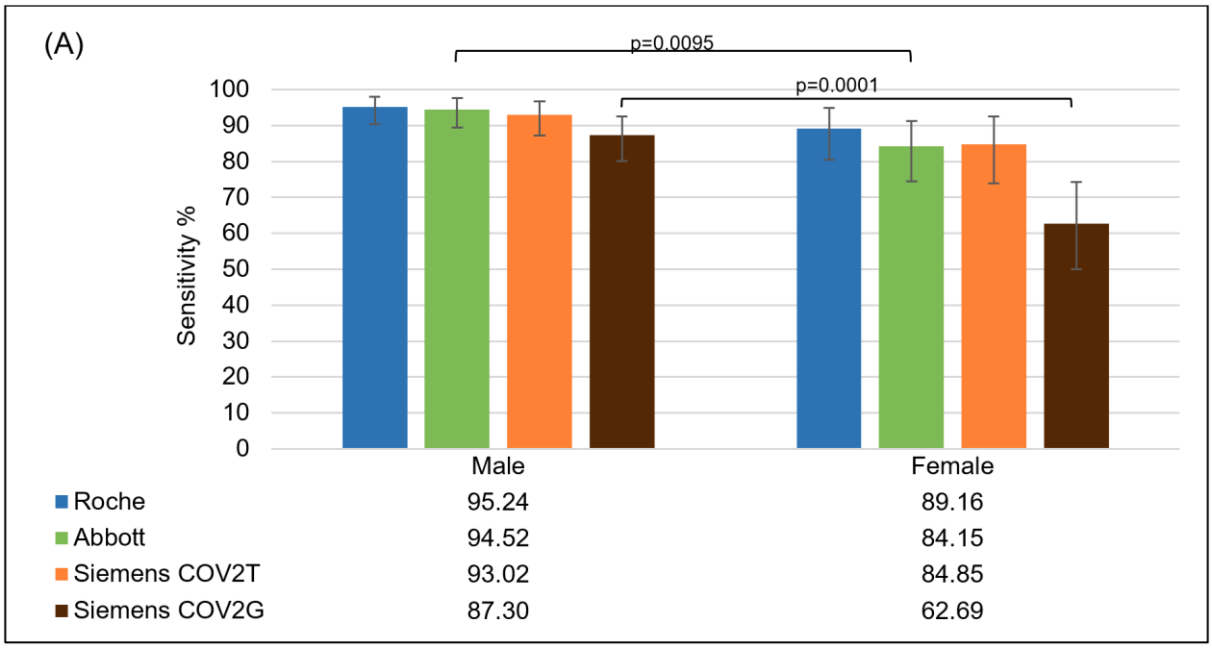
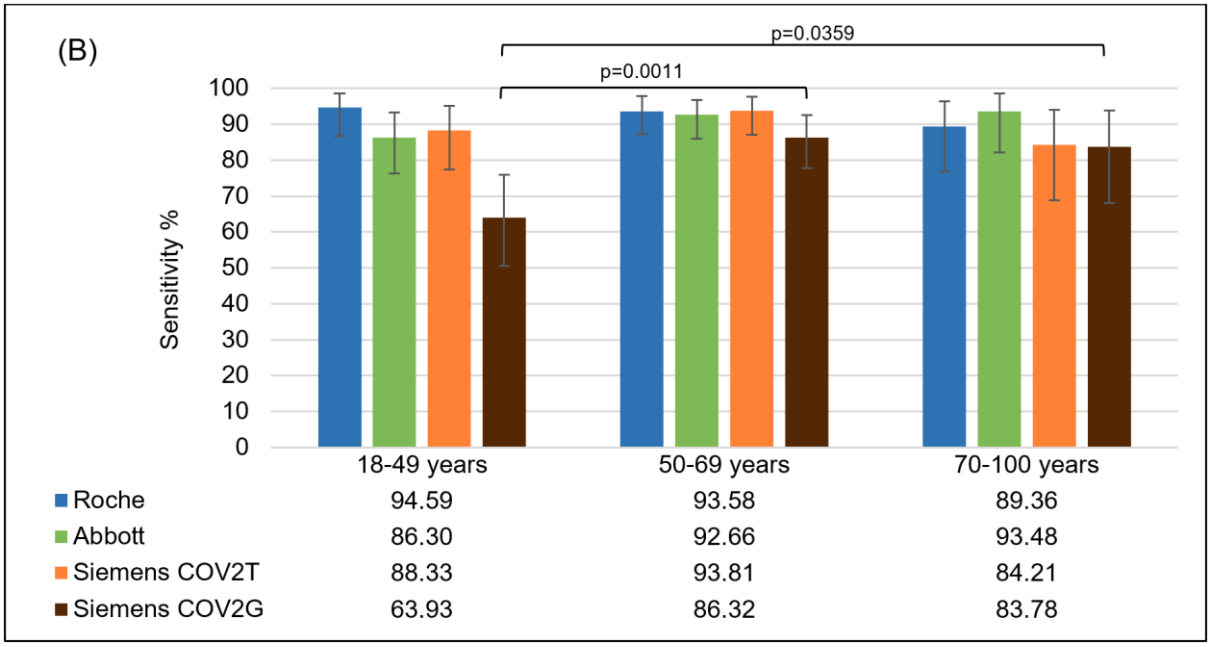
Figure 1 (A-C)





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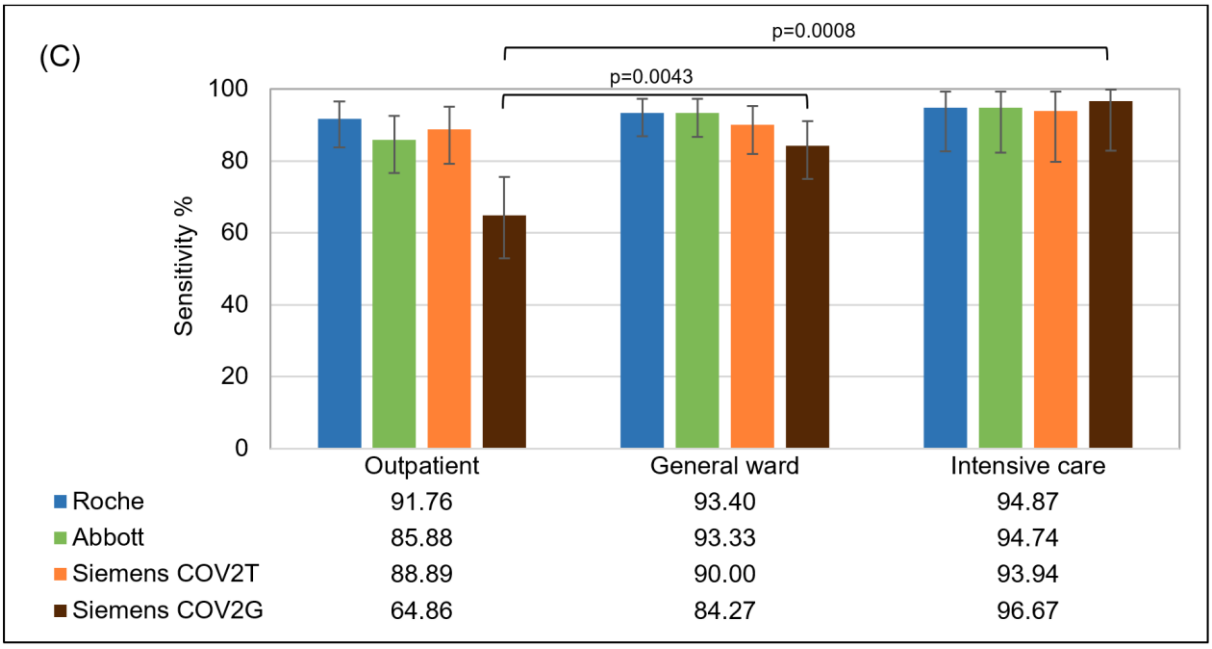

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Figure 2

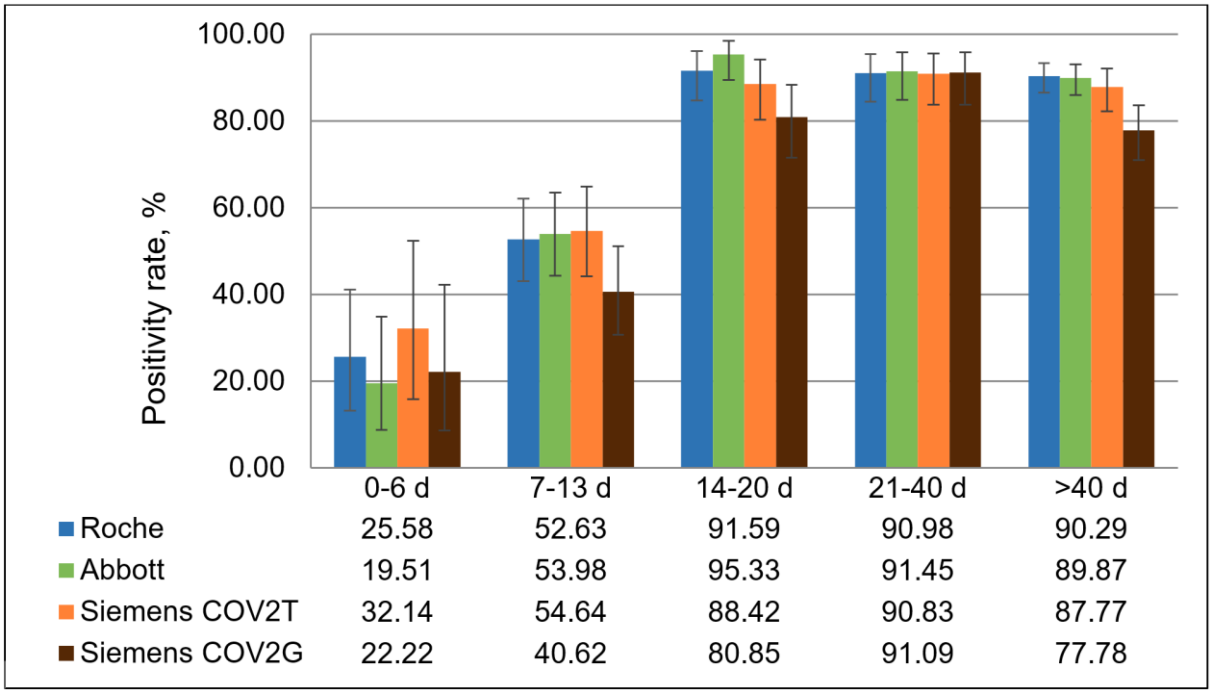

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Figure 3 (A-H)

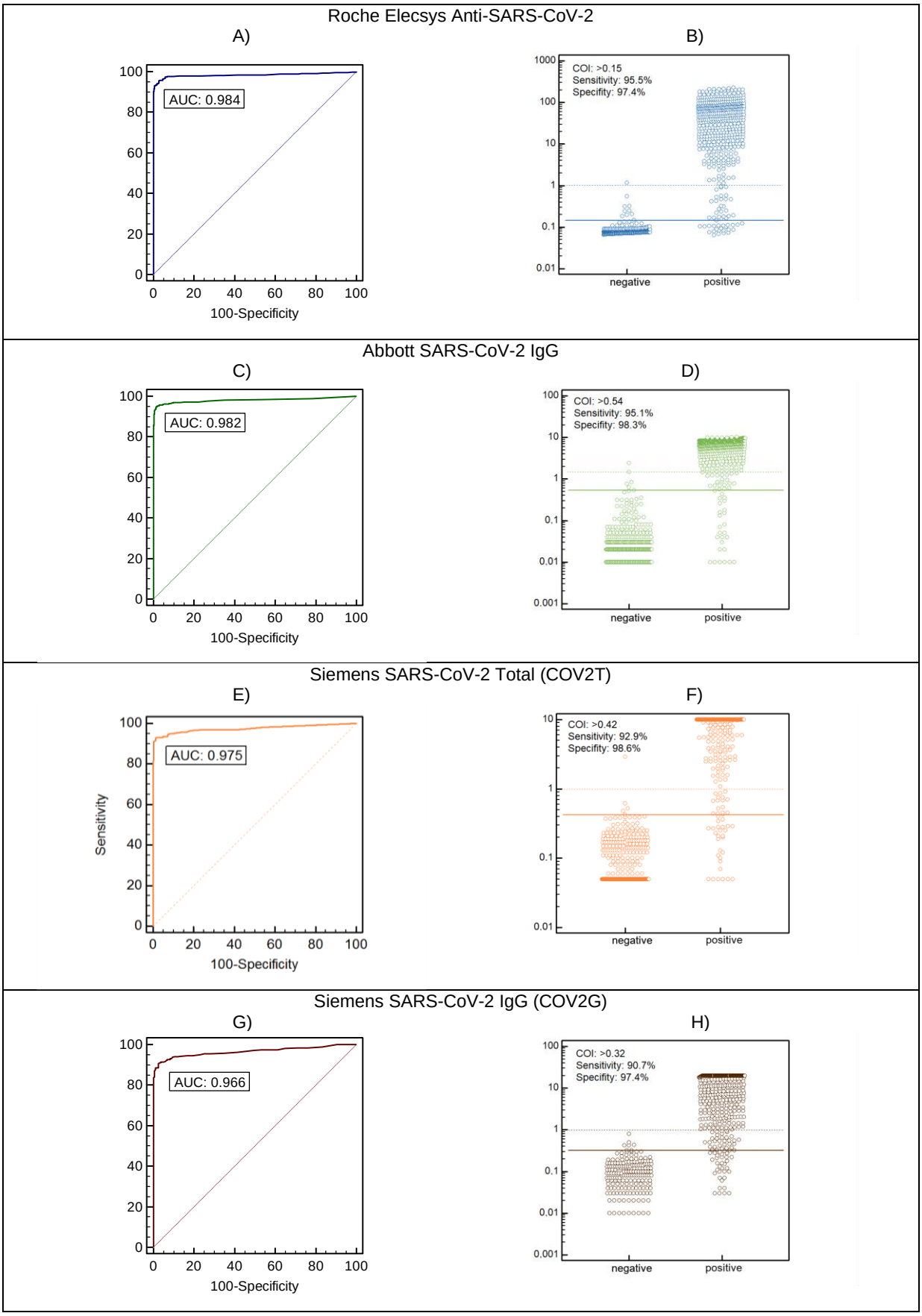

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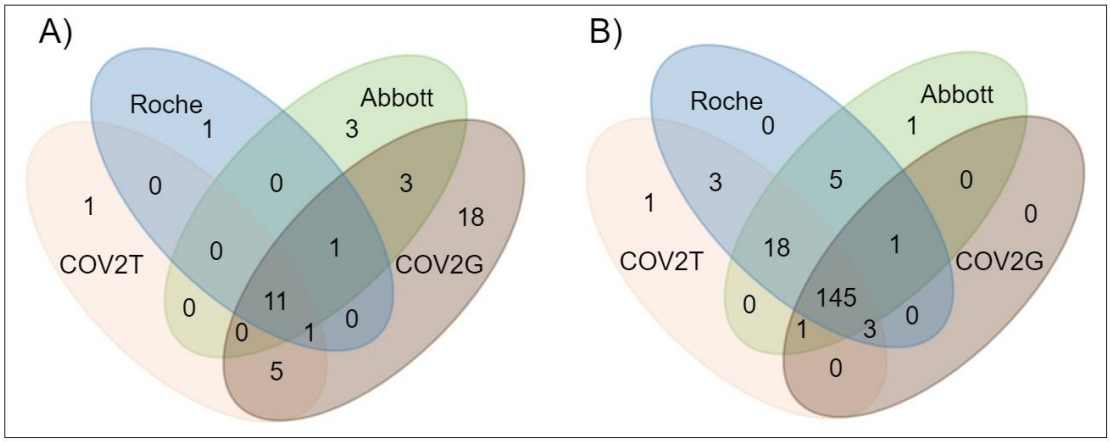
Figure 4 (A-B)



SUPPLEMENTAL MATERIAL

Supplemental Tables

Supplemental Table S1. Attributes for the evaluated serologic assays as given by the manufacturers' package inserts.

Attribute	Elecsys Anti-SARS-CoV-2	SARS-CoV-2 IgG	SARS-CoV-2 total (COV2T)	SARS-CoV-2 IgG (COV2G)
Manufacturer	**Roche**	**Abbott**	**Siemens**	**Siemens**
Version of the package insert	July 2020	April 2020	Mai 2020	June 2020
CE-IVD status	Conform	Conform	Conform	Conform
EUA status	Granted	Granted	Granted	Granted
Method	ECLIA	CMIA	CLIA	CLIA
Platform	Cobas e411, e601, e602[a], e801	ARCHITECT i2000SR [a], i1000SR	ADVIA Centaur XP [a], XPT	ADVIA Centaur XP [a], XPT
Specimen type	Serum, plasma (Li-heparin, K-EDTA)	Serum, plasma (EDTA)	Serum, plasma (Li-heparin, K-EDTA)	Serum, plasma (Li-heparin, K-EDTA)
Sample volume (without dead volume)	20 µl	25 µl	50 µl	10 µl
Detected antibodies	Total antibodies, including IgG	IgG	Total antibodies, including IgG and IgM	IgG
Solid-phase antigen target	N protein	N protein	RBD of the S1 protein	RBD of the S1 protein
Result calculation	Cut-off index	Index (S/Co)	Index	Index
Interpretation	<1,0: negative ≥1,0: positive	<1,4: negative ≥1,4: positive	<1,0: negative ≥1,0: positive	<1,0: negative ≥1,0: positive
Diagnostic specificity*	99.80% (99.69-99.88%). n=10,432	99.63% (98.98-99.89%). n=1,066	99.81% (99.41-99.96%). n=1,589	99.89% (99.61-99.99%). n=1,831)
Diagnostic sensitivity*	*Days after first PCR-positivity*	*Days after symptom onset*	*Days after first PCR-positivity*	*Days after first PCR-positivity*
	0-6: 60.20% (52.30-67.80%). n=161	<3: 0.00% (0.00-60.24%). n=4	0-6: 61.05% (50.50-70.89%). n=95	0-6: 53.49% (42.41-64.23%). n=86
	7-13: 85.30% (78.60-90.60%). n=150	3-7: 25.00% (3.19-65.09%). n=8	7-13: 97.50% (92.87-99.48%). n=120	7-13: 93.44% (84.05-98.18%). n=61
	≥14: 99.50% (97.00-100.00%). n=185	8-13: 86.36% (65.09-97.09%). n=22	≥14:100.00% (92.45-100.00%). n=47	≥14:100.00% (91.59-100.00%). n=42
		≥14:100.00% (95.89-100.00%). n=88		
Accuracy	Within-day: 1.3-2.8% Between-day: 2.2-7.2%	Within-day: 1.1-5.9% Between-day: 1.2-5.9%	Within-day: 2.6-10.8% Between-day: 5.2-12.4%	Within-day: 2.0-4.6% Between-day: 2.8-6.1%

In brackets 95% confidence intervals are shown. Abbreviations: CE-IVD, European Conformity - In Vitro Diagnostic Medical Device; CLIA, chemiluminescence immunoassay; CMIA, chemiluminescent microparticle immunoassay; ECLIA, electrochemiluminiscence immunoassay; ELISA, enzyme-linked immunosorbent assay; EUA: Emergency Use Authorization; K, potassium; Li, Lithium; n: number of samples tested; RBD: receptor-binding domain; S/Co, signal-to-cutoff ratio. [a] indicates the platform used in our study.

Supplemental Table S2. Diagnostic specificity of the four assays.

Assay	Total	Negative	Positive	Specificity
Roche Elecsys Anti-SARS-CoV-2	341	340	1	99.71% (98.38-99.99%)
Abbott SARS-CoV-2 IgG	298	296	2	99.33% (97.60-99.92%)
Siemens SARS-CoV-2 total (COV2T)	288	287	1	99.65% (98.08-99.99%)
Siemens SARS-CoV-2 IgG (COV2G)	191	191	0	100.00% (98.09-100.00%)

Specificity of the assays was determined on 350 pre-pandemic samples; 95% confidence intervals are given in brackets.

Supplemental Table S3. False positive results in pre-pandemic samples.

Sample number	Roche Elecsys Anti-SARS-CoV-2		Abbott SARS-CoV-2 IgG		Siemens SARS-CoV-2 total (COV2T)		Siemens SARS-CoV-2 IgG (COV2G)	
1	n	0.08	p	1.46	n	0.13		
2	p	1.18	n	0.04				
3	n	0.09	p	2.42	n	0.21	n	0.17
4	n	0.09			p	2.91	n	0.04

Individual results (assay index) of all samples tested positive in at least one assay of 350 pre-pandemic samples are shown. Positive results (p) are shown in red, negative results (n) are shown in green.

Supplemental Table S4. Measurements of the immunoglobulin formulation Privigen®, undiluted and 1:50 diluted.

Privigen	Roche Elecsys Anti-SARS-CoV-2	Abbott SARS-CoV-2 IgG	Siemens SARS-CoV-2 total (COV2T)	Siemens SARS-CoV-2 IgG (COV2G)
Undiluted	0.14	0.65	<0.05	1.09
1:50, negative serum	0.08	0.01	<0.05	0.01
1:50, sodium chloride	0.15	0.05	0.26	0.23

Data indicate the respective results of the raw data indexes of the tests. Privigen® was diluted with negative pre-pandemic serum or sodium chloride, respectively.

Supplemental Table S5. Individual results of all 230 sensitivity samples.

#	#	Disease course	Days	Roche Elecsys Anti-SARS-CoV-2		Abbott SARS-CoV-2 IgG		Siemens SARS-CoV-2 total (COV2T)		Siemens SARS-CoV-2 IgG (COV2G)	
1	1	outpatient	16	p	4.42	p	4.81				
2	2	outpatient	20	p	4.46	p	4.24				
3	3	outpatient	21	p	19.93	p	6.92	p	6.50	p	3.45
4	4	outpatient	24	p	24.56	p	6.72	p	>10.00	p	3.20
5	5	outpatient	25	p	17.66	p	1.49				
6	6	outpatient	25	p	5.32	p	3.85				
7	7	outpatient	27	p	26.00	p	4.80	p	>10.00	p	1.96
8	8	outpatient	30	p	3.82	p	3.05	p	1.58	n	0.32
9	9	outpatient	30	p	46.16	p	9.15				
10	10	outpatient	32	p	2.86	n	0.71	p	4.07	p	1.28
11	11	outpatient	32	p	3.00	p	1.78	p	6.14	p	2.59
12	12	outpatient	33	p	30.01	p	4.68	p	3.63	n	0.36
13	13	outpatient	34	n	0.24	n	0.03	n	0.29	n	0.00
14	14	outpatient	36	p	28.47	p	4.96	p	4.84	p	2.03
15	15	outpatient	38	p	8.58	p	3.10	p	2.55	p	1.14
16	16	outpatient	38	n	0.11	n	0.01			n	0.06
17	17	outpatient	39	p	38.64	p	7.68	p	>10.00	p	11.35
18	18	outpatient	39	p	43.54	p	5.66	p	3.86	p	1.97
19	19	outpatient	40	p	13.36	p	2.45	p	>10.00	p	14.28
20	20	outpatient	40	p	84.46	p	7.99	p	>10.00	p	12.05
21	21	outpatient	42	p	4.58	p	2.52				

22	22	outpatient	42	p	32.47	p	4.04				
23	23	outpatient	43	p	88.73	p	8.67	p	>10.00	p	6.75
24	24	outpatient	43	n	0.11	n	0.18	n	<0.05	n	0.04
25	25	outpatient	45	p	92.52	p	6.79	p	1.98	p	1.86
26	26	outpatient	45	p	39.48	p	9.90	p	7.36	p	6.76
27	27	outpatient	46	p	108.40	p	7.56	p	9.68	p	1.11
28	28	outpatient	47	p	14.35	p	2.29	p	2.48	n	0.15
29	29	outpatient	47	p	144.40	p	3.94	p	9.11	p	1.80
30	30	outpatient	48	p	58.86	p	6.18	p	>10.00	p	9.86
31	31	outpatient	48	p	9.34	p	2.64	p	3.12	n	0.39
32	32	outpatient	48	p	31.36	p	5.13	p	3.38	n	0.50
33	33	outpatient	48	p	68.75	p	6.04	p	1.35	p	1.08
34	34	outpatient	49	p	29.76	p	2.31	p	>10.00	p	1.86
35	35	outpatient	49	p	33.96	p	4.70	n	0.87	n	0.45
36	36	outpatient	49	n	0.11	n	0.30	n	0.28	n	0.10
37	37	outpatient	50	p	47.36	p	6.32	p	7.65	p	1.86
38	38	outpatient	50	p	27.04	p	3.93	p	7.76	n	0.62
39	39	outpatient	51	p	67.02	p	6.94	p	>10.00	p	5.97
40	40	outpatient	51	p	69.06	p	9.11	p	9.79	p	2.53
41	41	outpatient	52	p	37.19	p	6.63	p	5.94	n	0.88
42	42	outpatient	52	p	12.66	p	3.18	p	2.33	n	0.55
43	43	outpatient	53	p	45.49	p	4.17	p	3.50	p	1.45
44	44	outpatient	55	n	0.09	n	0.07	n	0.40	n	0.12
45	45	outpatient	55	p	198.00	p	5.73	p	8.16	p	2.84
46	46	outpatient	56	p	20.74	n	1.23			n	0.37
47	47	outpatient	57	p	85.05	p	9.21	p	>10.00	p	16.47
48	48	outpatient	58	p	69.65	p	5.16	p	1.08	n	0.22
49	49	outpatient	58	p	88.64	p	9.55	p	>10.00	p	1.37
50	50	outpatient	58	p	49.01	p	5.58	p	3.62	n	0.64
51	51	outpatient	59	p	50.49	p	4.64	p	>10.00	p	1.34
52	52	outpatient	59	p	37.06	p	4.50	p	4.45	p	1.67
53	53	outpatient	59	p	68.29	p	5.98	p	>10.00	p	3.51
54	54	outpatient	60	p	7.79	p	2.66	p	2.86	n	0.53
55	55	outpatient	61	p	13.53	p	1.53	p	2.59	p	1.34
56	56	outpatient	61	p	45.69	p	4.23	n	0.27	n	0.28
57	57	outpatient	61	p	77.00	p	8.41	p	>10.00	p	>20.00
58	58	outpatient	62	p	229.00	p	9.10	p	>10.00	p	5.83
59	59	outpatient	64	p	23.78	p	7.16	p	1.95	p	3.73
60	60	outpatient	64	p	24.67	p	4.25	p	5.82	n	0.44
61	61	outpatient	65	p	122.10	p	6.40	p	9.17	p	1.03
62	62	outpatient	65	p	51.98	p	5.67	p	5.97	n	0.57
63	63	outpatient	65	p	3.15	n	0.70	p	1.54	n	0.21
64	64	outpatient	66	p	15.00	p	2.79	p	>10.00	p	1.12
65	65	outpatient	68	n	0.09	n	0.02	n	0.13	n	0.07
66	66	outpatient	69	p	28.61	p	2.16	p	7.83	p	2.17
67	67	outpatient	70	p	1.52	n	0.84	p	1.27	n	0.22
68	68	outpatient	71	p	209.50	p	6.20	p	9.04	p	2.00

69	69	outpatient	71	p	97.84	p	3.59	p	9.45	p	2.53
70	70	outpatient	72	p	53.74	p	3.54	p	>10.00	p	2.09
71	71	outpatient	72	p	13.01	n	0.87	p	5.62	p	1.15
72	72	outpatient	75	n	0.11	n	0.02	n	0.11	n	0.03
73	73	outpatient	77	p	18.45	p	3.46	p	6.68	p	1.14
74	74	outpatient	77	p	149.40	p	9.26	p	>10.00	p	12.18
75	75	outpatient	82	p	215.30	p	3.58	p	>10.00	p	1.12
76	76	outpatient	82	p	163.90	p	7.97				
77	77	outpatient	85	p	159.60	p	6.16	p	>10.00	p	2.38
78	78	outpatient	87	p	126.50	p	3.04	p	5.54	n	0.89
79	79	outpatient	89	p	159.00	p	2.73	p	>10.00	p	1.12
80	80	outpatient	90	p	153.00	p	6.37	p	6.93	p	3.95
81	81	outpatient	90	p	212.20	p	7.67	p	4.28	p	1.31
82	82	outpatient	97	p	194.20	p	4.53	p	>10.00	p	6.43
83	83	outpatient	104	p	51.14	p	2.06				
84	84	outpatient	111	p	72.73	p	3.70				
85	85	outpatient	120	p	93.09	p	3.61				
86	1	general ward	14	p	15.79	p	4.55	p	>10.00	p	7.58
87	2	general ward	14	p	8.29	p	5.80	p	8.52	p	1.74
88	3	general ward	14	p	8.53	p	6.61	p	4.99	p	17.86
89	4	general ward	14	n	0.47	n	0.94	n	0.34	n	0.12
90	5	general ward	14	p	3.56	p	3.14	p	>10.00	n	0.45
91	6	general ward	14	p	3.25	p	6.66				
92	7	general ward	14	p	5.27	p	4.02				
93	8	general ward	15	p	10.91	p	3.63	p	>10.00	p	5.67
94	9	general ward	15	p	27.00	p	4.74	p	9.13	p	2.47
95	10	general ward	15	p	15.63	p	4.70	p	3.42	n	0.24
96	11	general ward	15	p	24.61	p	8.39	p	>10.00	p	10.50
97	12	general ward	15	p	45.55	p	8.27	p	>10.00	p	>20.00
98	13	general ward	15	p	1.65	p	3.93				
99	14	general ward	15	p	13.66	p	1.87				
100	15	general ward	16	p	14.70	p	8.47	p	>10.00	p	14.47
101	16	general ward	16	p	11.99	p	6.45	p	>10.00	p	9.65
102	17	general ward	16	p	18.97	n	0.78	p	>10.00	p	>20.00
103	18	general ward	17	p	3.50	p	5.96	p	>10.00	p	1.85
104	19	general ward	17	p	3.71	p	5.69	p	>10.00	p	7.32
105	20	general ward	17	p	14.37	p	8.71	p	2.55	p	3.42
106	21	general ward	17	p	52.97	p	8.96	p	>10.00	p	>20.00
107	22	general ward	17	p	25.00	p	7.05	p	7.06	p	11.61
108	23	general ward	17	p	15.84	p	4.97				
109	24	general ward	17	p	1.43						
110	25	general ward	18	p	19.79	p	2.08	p	>10.00	p	2.66
111	26	general ward	18	p	11.51	p	7.59	p	>10.00	p	16.18
112	27	general ward	18	p	21.65	p	8.25	p	>10.00	p	14.52
113	28	general ward	19	p	19.30	p	8.08	p	>10.00	p	15.71
114	29	general ward	19	n	0.25	p	8.30	n	0.67	n	0.03
115	30	general ward	19	n	0.18	n	0.08	p	1.75	n	0.53

116	31	general ward	19	p	9.95	p	5.98	p	>10.00	p	6.80
117	32	general ward	19	p	37.50	p	6.12	p	>10.00	p	>20.00
118	33	general ward	20	p	4.02	p	3.38	p	>10.00	p	8.24
119	34	general ward	20	p	22.21	p	7.05	p	>10.00	p	>20.00
120	35	general ward	20	p	5.84	p	1.84	n	0.68	n	0.29
121	36	general ward	20	p	13.03	p	6.69			p	3.80
122	37	general ward	20	p	9.58	p	4.86				
123	38	general ward	21	p	1.15	p	5.28	n	0.29	n	0.50
124	39	general ward	21	p	36.54	p	7.18	p	>10.00	p	16.86
125	40	general ward	21	p	21.26	p	6.13	p	>10.00	p	>20.00
126	41	general ward	21	p	13.90	p	5.50	p	>10.00	p	11.43
127	42	general ward	22	p	39.78	p	8.90	p	>10.00	p	5.74
128	43	general ward	23	p	33.15	p	8.25	p	>10.00	p	>20.00
129	44	general ward	23	p	21.92	p	8.16	p	>10.00	p	>20.00
130	45	general ward	24	p	44.42	p	2.25	p	5.83	p	1.68
131	46	general ward	24	n	0.80	p	6.47	p	>10.00	p	10.70
132	47	general ward	24	p	14.02	p	7.35	p	>10.00	p	14.59
133	48	general ward	24	p	39.26	p	9.46	p	>10.00	p	6.44
134	49	general ward	24	p	41.21	p	8.50	p	>10.00	p	>20.00
135	50	general ward	25	p	35.27	p	8.70	p	>10.00	p	>20.00
136	51	general ward	26	p	9.41	p	6.88	p	>10.00	p	>20.00
137	52	general ward	27	p	12.94	p	8.00	p	>10.00	p	9.12
138	53	general ward	28	p	15.53	p	8.83	p	>10.00	p	6.44
139	54	general ward	28	p	3.23	p	5.91	p	>10.00	p	>20.00
140	55	general ward	28	p	35.48	p	8.77	p	>10.00	p	8.24
141	56	general ward	33	p	60.67	p	6.60			p	14.07
142	57	general ward	34	p	84.65	p	8.00	p	>10.00		
143	58	general ward	39	p	70.82	p	9.50				
144	59	general ward	44	p	86.18	p	8.53	p	>10.00	p	4.46
145	60	general ward	46	p	87.75	p	6.27	p	>10.00	p	5.15
146	61	general ward	46	p	53.67	p	7.24	p	>10.00	p	4.82
147	62	general ward	46	p	95.25	p	9.12				
148	63	general ward	47	p	75.33	p	6.05	p	>10.00	p	5.16
149	64	general ward	48	p	64.57	p	7.68	p	>10.00	p	16.59
150	65	general ward	49	p	14.45	p	4.60	p	>10.00	p	2.60
151	66	general ward	50	n	0.09	n	0.01	n	0.19	n	0.03
152	67	general ward	50	p	63.92	p	6.15	p	>10.00	p	>20.00
153	68	general ward	50	n	0.32	n	0.69	n	0.10	n	0.93
154	69	general ward	51	p	51.54	p	8.63	p	>10.00	p	6.90
155	70	general ward	51	p	47.70	p	6.30	p	2.73	p	1.01
156	71	general ward	53	p	15.11	p	7.84	n	0.75	p	1.43
157	72	general ward	54	p	19.18	p	8.44	p	>10.00	p	7.18
158	73	general ward	54	p	66.68	p	8.18	p	>10.00	p	4.95
159	74	general ward	57	p	76.02	p	7.48	p	>10.00	p	4.90
160	75	general ward	58	p	70.55	p	7.95	p	>10.00	p	>20.00
161	76	general ward	58	p	3.94	n	1.35	p	2.78	n	0.54
162	77	general ward	58	p	36.85	p	3.64				

163	78	general ward	59	p	50.29	p	2.53	n	0.36	n	0.55
164	79	general ward	59	p	98.70	p	9.02	p	>10.00	p	3.13
165	80	general ward	59	p	42.36	p	7.14	p	>10.00	p	>20.00
166	81	general ward	60	p	53.45	p	7.65				
167	82	general ward	61	p	49.65	p	6.58	p	>10.00	p	7.04
168	83	general ward	61	p	210.10	p	7.34	p	9.51	p	1.97
169	84	general ward	61	p	64.63	p	3.42	p	>10.00		
170	85	general ward	62	p	92.64	p	7.43	p	>10.00	p	8.49
171	86	general ward	62	p	16.23	p	2.50	p	7.66	p	2.05
172	87	general ward	63	n	0.10	n	0.04	n	<0.05	n	0.07
173	88	general ward	63	p	84.20	p	9.44	p	>10.00	p	7.20
174	89	general ward	63	p	60.58	p	9.03	p	>10.00	p	6.47
175	90	general ward	63	p	94.06	p	8.45	p	>10.00	p	>20.00
176	91	general ward	65	p	161.80	p	8.39	p	5.91	p	2.84
177	92	general ward	65	p	139.40	p	7.02	p	>10.00	p	4.39
178	93	general ward	67	p	70.86	p	5.13	p	>10.00	p	4.91
179	94	general ward	68	p	36.67	p	3.42	p	2.87	n	0.87
180	95	general ward	69	p	71.77	p	9.45	p	>10.00	p	4.72
181	96	general ward	70	p	80.46	p	5.26	p	1.87	n	0.33
182	97	general ward	71	p	81.51	p	4.53	p	>10.00	p	>20.00
183	98	general ward	71	p	92.71	p	9.59	p	5.57	p	2.79
184	99	general ward	71	p	131.20	p	7.04	p	>10.00	p	10.69
185	100	general ward	71	p	141.40	p	8.91	p	>10.00	p	11.69
186	101	general ward	72	p	178.50	p	7.33	p	>10.00	p	19.26
187	102	general ward	73	p	85.78	p	9.28	p	>10.00		
188	103	general ward	74	p	71.67	p	8.98	p	>10.00	p	18.08
189	104	general ward	96	p	104.10	p	7.02				
190	105	general ward	107	p	106.40	p	7.50				
191	106	general ward	108	p	17.44	p	1.56				
192	1	intensive care	18	p	17.25	p	7.01	p	>10.00	p	16.86
193	2	intensive care	19	p	68.42	p	8.86	p	>10.00	p	7.03
194	3	intensive care	21	p	26.31	p	8.42	p	>10.00	p	>20.00
195	4	intensive care	21	p	9.31	p	6.68	p	>10.00	p	10.95
196	5	intensive care	21	p	43.80	p	8.02	p	>10.00	p	3.41
197	6	intensive care	21	p	47.57	p	4.40				
198	7	intensive care	23	p	1.35	p	7.65	p	>10.00	p	1.86
199	8	intensive care	24	p	52.95	p	9.54	p	>10.00	p	>20.00
200	9	intensive care	24	p	47.73	p	8.03	p	>10.00	p	>20.00
201	10	intensive care	25	p	75.09	p	8.76	p	>10.00	p	7.59
202	11	intensive care	26	p	28.22	p	8.70	p	>10.00	p	>20.00
203	12	intensive care	26	p	77.10	p	8.63	p	>10.00	p	>20.00
204	13	intensive care	27	p	11.43	p	7.59	p	>10.00	p	8.59
205	14	intensive care	27	p	38.86	p	8.60	p	>10.00	p	14.35
206	15	intensive care	28	p	5.54	p	7.70	p	>10.00	p	5.21
207	16	intensive care	28	p	10.13	p	6.24	p	>10.00	p	7.25
208	17	intensive care	28	p	39.27	p	7.61	p	>10.00	p	>20.00
209	18	intensive care	28	p	7.43	n	1.36	p	2.74		

210	19	intensive care	28	p	6.42	p	4.99	p	>10.00	p	>20.00
211	20	intensive care	28	p	52.77	p	8.17	p	>10.00	p	13.39
212	21	intensive care	28	p	23.44	p	8.40	p	>10.00	p	19.09
213	22	intensive care	29	p	75.52	p	9.26	p	>10.00	p	>20.00
214	23	intensive care	31	p	16.55	p	8.30	p	>10.00	p	6.73
215	24	intensive care	31	p	70.01	p	8.76	p	>10.00	p	>20.00
216	25	intensive care	31	p	72.69	p	8.99	p	>10.00	p	>20.00
217	26	intensive care	31	p	77.61	p	8.34	p	>10.00	p	>20.00
218	27	intensive care	34	n	0.08	n	0.01	n	0.20	n	0.13
219	28	intensive care	35	n	0.11			n	<0.05		
220	29	intensive care	40	p	77.42	p	9.29				
221	30	intensive care	41	p	58.18	p	7.68	p	>10.00	p	7.46
222	31	intensive care	65	p	44.20	p	4.77				
223	32	intensive care	66	p	117.50	p	8.01	p	>10.00	p	19.63
224	33	intensive care	67	p	191.60	p	8.63	p	>10.00	p	9.48
225	34	intensive care	78	p	94.79	p	4.24	p	>10.00	p	>20.00
226	35	intensive care	80	p	84.76	p	6.06	p	>10.00		
227	36	intensive care	84	p	88.00	p	8.24	p	>10.00	p	>20.00
228	37	intensive care	87	p		p	8.76				
229	38	intensive care	98	p	105.70	p	8.90				
230	39	intensive care	99	p	73.59	p	8.74				

Individual results (raw data assay index) of the samples of SARS-CoV-2 PCR-confirmed patients are shown (one sample per patient; the sample ≥14 days and closest to day 28 after symptom onset was used). Positive results (p) are shown in red, negative results (n) are shown in green. Days indicate the time between disease onset and blood sampling. # indicates the patient number.

Supplemental Table S6. Diagnostic sensitivity of the four assays stratified for disease course, age and gender.

		Roche Elecsys Anti-SARS-CoV-2	Abbott SARS-CoV-2 IgG	Siemens SARS-CoV-2 total (COV2T)	Siemens SARS-CoV-2 IgG (COV2G)
Sensitivity according to PI		**99.50% (97.00-100.00%)**	**100.00% (95.89-100.00%)**	**100.00% (92.45-100.00%)**	**100.00% (91.59-100.00%)**
All patients	Total	230	229	195	193
	Positive	214	208	176	152
	Negative	16	21	19	41
	Sensitivity	93.04% (88.95-95.97%)	90.83% (86.32-94.23%)	90.26% (85.20-94.03%)	78.76% (72.30-84.30%)
Outpatient	Total	85	85	72	74
	Positive	78	73	64	48
	Negative	7	12	8	26
	Sensitivity	91.76% (83.77-96.62%)	85.88% (76.64-92.49%)	88.89% (79.28-95.08%)	64.86% (52.89-75.61%)
Gen. ward	Total	106	105	90	89
	Positive	99	98	81	75
	Negative	7	7	9	14
	Sensitivity	93.40% (86.87-97.30%)	93.33% (86.75-97.28%)	90.00% (81.86-95.32%)	84.27% (75.02-91.12%)
ICU	Total	39	38	33	30
	Positive	37	36	31	29
	Negative	2	2	2	1
	Sensitivity	94.87% (82.68-99.37%)	94.74% (82.25-99.36%)	93.94% (79.77-99.26%)	96.67% (82.78-99.92%)
18-49	Total	74	73	60	61
	Positive	70	63	53	39
	Negative	4	10	7	22
	Sensitivity	94.59% (86.73-98.51%)	86.30% (76.25-93.23%)	88.33% (77.43-95.18%)	63.93% (50.63-75.84%)
50-69	Total	109	109	97	95
	Positive	102	101	91	82
	Negative	7	8	6	13
	Sensitivity	93.58% (87.22-97.83%)	92.66% (86.05-96.78%)	93.81% (87.02-97.70%)	86.32% (77.74-92.51%)
70-100	Total	47	46	38	37
	Positive	42	43	32	31
	Negative	5	3	6	6
	Sensitivity	89.36% (76.90-96.45%)	93.48% (82.10-98.63%)	84.21% (68.75-93.98%)	83.78% (67.99-93.81%)
Male	Total	147	146	129	126
	Positive	140	138	120	110
	Negative	7	8	9	16
	Sensitivity	95.24% (90.43-98.06%)	94.52% (89.49-97.60%)	93.02% (87.17-96.76%)	87.30% (80.20-92.56%)
Female	Total	83	82	66	67
	Positive	74	69	56	42
	Negative	9	13	10	25
	Sensitivity	89.16% (80.41-94.92%)	84.15% (74.42-91.28%)	84.85% (73.90-92.49%)	62.69% (50.01-74.20%)

Sensitivity of the evaluated test-systems including all SARS-CoV-2 PCR-confirmed patients (one sample per patient; the sample ≥14 days and closest to day 28 after symptom onset was used) and stratified for severity of the disease (outpatient, hospitalised at the general ward, hospitalized at the intensive care unit) as well as age (18-49, 50-69, 70-100 years), respectively. 95% confidence intervals are given in brackets. Abbreviations: PI: package insert.

Supplemental Table S7. Positivity rate across the time course of the disease.

	Roche Elecsys Anti-SARS-CoV-2		Abbott SARS-CoV-2 IgG		Siemens SARS-CoV-2 total (COV2T)		Siemens SARS-CoV-2 IgG (COV2G)	
days	**n**	**Positivity rate**	**n**	**Positivity rate**	**n**	**Positivity rate**	**n**	**Positivity rate**
0-6	43	**25,58%**	41	**19,51%**	28	**32,14%**	27	**22,22%**
		(13.25-41.17%)		(8.82-34.87%)		(15.88-52.35%)		(8.62-42.26%)
7-13	114	**52,63%**	113	**53,98%**	97	**54,64%**	96	**40,62%**
		(43.06-62.06%)		(44.35-63.40%)		(44.21-64.78%)		(30.71-51.13%)
14-20	107	**91,59%**	107	**95,33%**	95	**88,42%**	94	**80,85%**
		(84.63-96.08%)		(89.43-98.47%)		(80.23-94.08%)		(71.44-88.24%)
21-40	122	**90,98%**	117	**91,45%**	109	**90,83%**	101	**91,09%**
		(84.44-95.41%)		(84.84-95.83%)		(83.77-95.51%)		(83.76-95.84%)
>40	309	**90,29%**	306	**89,87%**	188	**87,77%**	180	**77,78%**
		(86.43-93.35%)		(85.93-93.01%)		(82.21-92.08%)		(70.99-83.62%)

A total of 700 samples of 245 SARS-CoV-2 PCR-confirmed patients were measured. The table shows the positivity rate of the four investigated assays depending on the time course of the disease. Days indicate the time between disease onset and blood sampling. N indicates the total number of samples tested. 95% confidence intervals are given in brackets.

Supplemental Table S8. ROC curve analysis of the four assays.

Assay	AUC
Roche Elecsys Anti-SARS-CoV-2	0.984 (0.974-0.991)
Abbott SARS-CoV-2 IgG	0.982 (0.970-0.990)
Siemens SARS-CoV-2 total (COV2T)	0.975 (0.961-9.986)
Siemens SARS-CoV-2 IgG (COV2G)	0.966 (0.948-0.980)

ROC curve analysis was based on 350 pre-pandemic (=negative) samples (samples, Roche: 341, Abbott: 298, Siemens COV2T: 288, Siemens COV2G: 191) and the samples of SARS-CoV-2 PCR-confirmed (=positive) patients (samples, Roche: 537, Abbott: 530, Siemens COV2T: 392, Siemens COV2G: 375). Samples of SARS-CoV-2 positive patients were only considered if time-span between disease onset and blood draw was ≥14 days. 95% confidence intervals are given in brackets. Abbreviations: AUC, area under the curve; ROC, receiver operating characteristic.

Supplemental Table S9. Sensitivity and specificity of the assays using the modified cut-off index.

		Manufacturer's COI	Modified COI
	COI	**≥1.0: positive**	**>0.15: positive**
Roche Elecsys Anti-SARS-CoV-2	Sensitivity (n=230)	93.04% (89.00-95.67%)	95.65% (92.18-97.62%)
	Specificity (n=341)	99.71% (98.36-99.95%)	97.36% (95.06-98.61%)
	COI	**≥1.4: positive**	**>0.54: positive**
Abbott SARS-CoV-2 IgG	Sensitivity (n=228)	90.79% (86.33-93.90%)	95.18% (91.57-97.28%)
	Specificity (n=298)	99.33% (97.59-99.82%)	98.32% (96.13-99.28%)
	COI	**≥1.0: positive**	**>0.42: positive**
Siemens SARS-CoV-2 total (COV2T)	Sensitivity (n=195)	90.26% (85.28-93.67%)	92.31% (87.70-95.28%)
	Specificity (n=288)	99.65% (98.06-99.94%)	98.61% (96.48-99.46%)
	COI	**≥1.0: positive**	**>0.32: positive**
Siemens SARS-CoV-2 IgG (COV2G)	Sensitivity (n=193)	78.76% (72.45-83.94%)	89.64% (84.54-93.19%)
	Specificity (n=191)	100.00% (98.03-100.00%)	97.38% (94.02-98.88%)

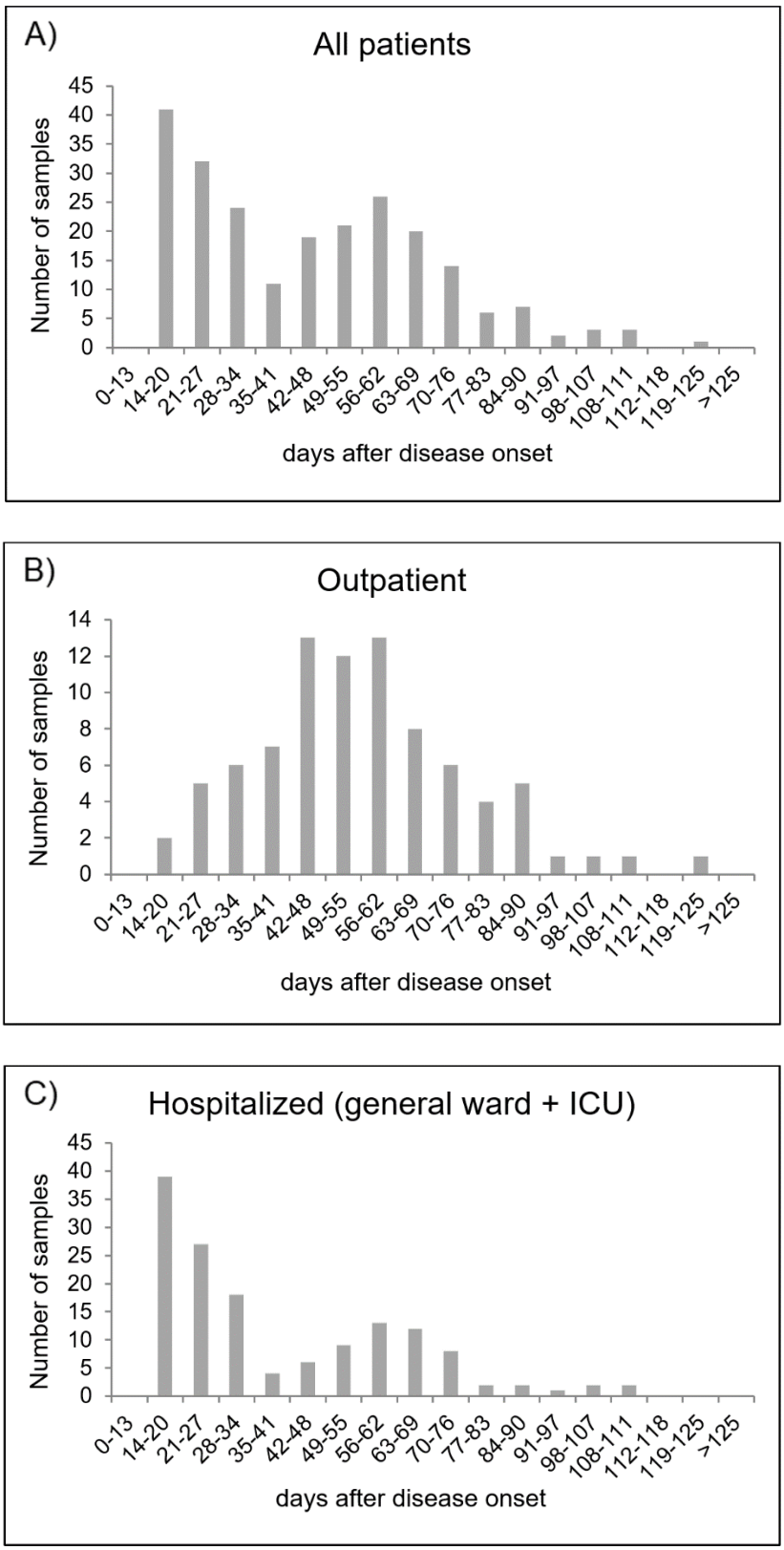
Sensitivity and specificity of the assays using different cut-off indexes (COI): the COI according to the manufacturer and a modified COI according to our ROC curve analysis. Sensitivity was evaluated on samples of SARS-CoV-2 PCR-confirmed patients (one sample per patient; the sample ≥14 days and closest to day 28 after symptom onset was used). Specificity was determined on 350 pre-pandemic samples. 95% confidence intervals are shown in brackets.

Supplemental Table S10. Concordance correlation coefficient between the four assays.

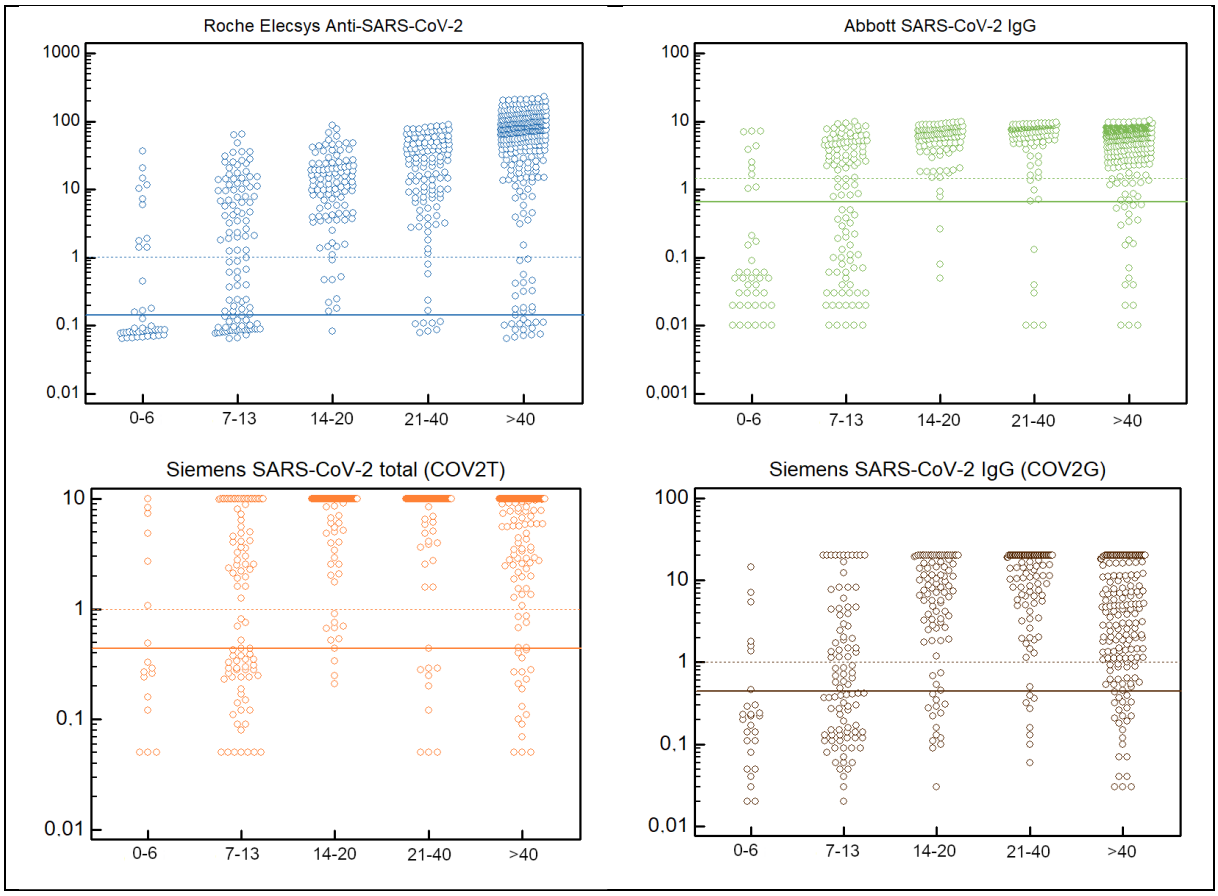
Test	Roche	Abbott	Siemens COV2T	Siemens COV2G
Roche	x	90.13 (n=972)	92.49 (n=800)	81.93 (n=689)
Abbott		x	85.47 (n=750)	77.45 (n=645)
Siemens COV2T			x	84.93 (n=680)

Concordance correlation coefficient of the qualitative results of the four assays (350 pre-pandemic samples and all 700 samples of SARS-CoV-2 PCR-confirmed patients). Abbreviations: n, total number of samples measured with both assays. Abbott = Abbott SARS-CoV-2 IgG; Roche = Roche Elecsys Anti SARS-CoV-2; Siemens COV2G = Siemens SARS-CoV-2 IgG; Siemens COV2T = Siemens SARS-CoV-2 Total.

Supplemental Figures



Supplemental Figure S1. Distribution of sample timing. Horizontal axis: time span between disease onset and timing of the blood draw of the sample used for the sensitivity analysis. Vertical axis: number of samples. A) all patients; B) outpatient; C) hospitalized patients (general ward and intensive care unit, ICU).



Supplemental Figure S2. Raw data index results of all 700 samples of SARS-CoV-2 PCR-confirmed patients, stratified for the time course of the disease. Horizontal axis: days after symptom onset. Vertical axis: raw data index of the respective test. Dashed horizontal line: manufacturer's cut-off index. Solid horizontal line: modified cut-off index.