Long-Term Comparison of 7 SARS-CoV-2 Antibody Assays in the North Zealand Covid-19 Cohort

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Background: Throughout the coronavirus disease 2019 (Covid-19) pandemic numerous severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody assays have been approved through Emergency Use Authorization and require further evaluation of sensitivity and specificity in clinical laboratory settings prior to implementation.

Methods: We included 1733 samples from 375 PCR-confirmed SARS-CoV-2–positive individuals of the North Zealand Covid-19 Cohort in an 8-month period. We investigated diagnostic sensitivity and specificity against consensus and PCR and interassay agreement over time for 5 SARS-CoV-2 immunoassays [Roche-nucleocapsid (NC)-total, Roche-receptor binding domain (RBD)-total, Siemens-RBD-IgG, Siemens-RBD-total, Thermo Fisher Scientific (TFS)-RBD-IgG] commercially available on automated platforms and 2 ELISA assays (TFS-RBD-total, Wantai-RBD-total).

Results: Early interassay discrepancy in up to 49% of samples decreased steadily during the first 18 days. By day 18, all assays had reached a plateau between 82.3% and 90.5% seropositivity compared to PCR. Assays ranked by closest agreement with the consensus model beyond day 18 (sensitivity/specificity against consensus) were as follows: Roche-RBD-total, 99.8%/100.0%; Wantai-RBD-total, 99.8%/99.7%; Roche-NC-total, 97.8%/100.0%; Siemens-RBD-total, 98.0%/98.7%; TFS-RBD-total, 96.9%/99.7%; TFS-RBD-IgG, 91.5%/100.0%; and Siemens-RBD-IgG, 94.6%/89.9%. We found that 7.8% of PCR-positive patients remained seronegative in all assays throughout the study.

Conclusions: All included assays had sensitivities against consensus >90% past day 18. For the current recommended use of antibody assays to detect former, undocumented Covid-19, our data suggest the use of total antibody assays rather than IgG-specific assays due to higher long-term sensitivity. Finally, a nonresponding subpopulation of 7.8% in our cohort with persistent seronegative results raises concern of a possible substantial number of people with continued low protection following natural SARS-CoV-2 infection.

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IMPACT STATEMENT

This study adds to the current knowledge of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody assay performance, particularly the long-term sensitivity of commercially available assays. The study serves as a decision support for laboratories regarding what assay(s) to implement in routine practice. For the indication of SARS-CoV-2 antibody analysis to identify patients with earlier, undocumented coronavirus disease 2019, the data suggest that total antibody assays are more sensitive long-term than IgG-specific assays. Finally, a subpopulation of 7.8% remained seronegative throughout the study, highlighting the presence of a considerable subgroup with missing immunity following natural SARS-CoV-2 infection, which must be considered when interpreting the results.

INTRODUCTION

Throughout the coronavirus disease 2019 (Covid-19) pandemic, several applications have been suggested for antibody testing against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). PCR testing of viral SARS-CoV-2 RNA is the preferred diagnostic test and has become widely available for diagnostic purposes. Thus, serology testing is almost exclusively limited to epidemiologic surveillance or supporting diagnosis of late complications to an otherwise undocumented SARS-CoV-2 infection (2, 3). Antibody assays cannot currently confirm or disprove immunity as the link to neutralizing activity is not sufficiently established, and it is unclear to what degree the cellular immunity contributes and for what duration (1, 4, 5).

A multitude of SARS-CoV-2 antibody assays have been developed and launched, often very fast using Emergency Use Authorization. Such assays require further evaluation of sensitivity and specificity in a clinical laboratory setting prior to implementation in routine use, preferably covering long-term antibody development.

In this study, we aimed to investigate sensitivity, specificity, and interassay agreement over time of 7 SARS-CoV-2 antibody assays in the North Zealand Covid-19 Cohort.

MATERIALS AND METHODS

The study was performed at the Department of Clinical Biochemistry, Nordsjællands Hospital, Denmark, which handles all blood samples collected in the primary and secondary healthcare institutions from 8 municipalities with approximately 325 000 inhabitants (North Zealand).

Study Population

PCR-positive cohort. From March 21 to November 6, 2020, 1286 individuals in the geographical uptake area of Nordsjællands Hospital were identified SARS-CoV-2–positive by PCR testing at the local Department of Clinical Microbiology. Of these, 39% (503/1286) later had blood drawn for routine biochemical analysis. Residual plasma was systematically collected. Some samples were lost to inclusion due to, for example, shortage of staff, delayed reporting of positive PCR results, etc. We successfully collected 61% (1748/2860) of all possible samples from 75% (375/503) of all relevant PCR-positive patients. Fifteen of 1748 samples were excluded; 4 samples were drawn prior to PCR testing, and 11 samples were lost or yielded no valid results (see Supplemental Fig. 1 in the online Data

Table 1. Sample distribution and patient characteristics of the PCR-positive cohort and PCR-negative control group.

	All	Females	Males
PCR-positive cohort			
n (%)	375	215 (57)	160 (43)
Age,[a] years, mean (SD)	58.7 (18.8)	56.2 (18.7)	62.1 (18.5)
Samples per patient, median (quartiles)	2 (1–6)	2 (1–5)	3 (1–7)
Days between positive PCR and first sample, median (quartiles)	11 (1–81)	41 (2–99)	3 (1–45)
Admitted in intensive care unit			
n (% of all)	30 (8.0)	13 (6.0)	17 (10.5)
Age,[a] years, median (quartiles)	71.6 (53.9–78.3)	72.5 (56.0–77.9)	70.6 (53.6–78.4)
PCR-positive cohort, patients with more than 1 sample			
n (%)	224	113 (50)	111 (50)
Age,[a] years, mean (SD)	63.0 (18.7)	60.5 (19.7)	65.5 (17.3)
Samples per patient, median (quartiles)	5 (3–8)	5 (3–8)	5 (3–9)
Days between first and last sample, median (quartiles)	60 (11–128)	59 (12–122)	60 (9–131)
PCR-negative control group			
n (%)	269	183 (68)	86 (32)
Age,[a] years, mean (SD)	57.1 (17.8)	55.8 (18.0)	59.9 (17.1)
Days after last negative PCR, median (quartiles)	34 (20–47)	34 (20–47)	34 (20–42)

[a] Age is on the day of the first collected sample.

Supplement). Thus, 1733 samples were included into the study.

Sample distribution and patient characteristics of the cohort are summarized in Table 1. Detailed sample distribution can be found in Supplemental Figs. 2 and 3 and Supplemental Tables 1 and 2.

PCR-negative control group. From March 20 to June 16, 2020, 328 residual plasma samples from 269 SARS-CoV-2–negative patients were collected following a negative PCR test result. Characteristics of the 269 included negative controls are summarized in Table 1. Two patients later tested positive and were included in the positive cohort. A total of 48 samples were collected from a single PCR-negative patient during a longer period of hospital admittances (Fig. 3, F) extending throughout 2020. Four samples from 4 unique patients were seropositive in all assays and were excluded on the clear presumption of previous undiagnosed SARS-CoV-2 infection. Thus, 324 samples were included.

Sample Collection and Storage

Residual heparinized plasma samples from PCR-confirmed SARS-CoV-2-positive patients were collected after routine analysis and stored in one aliquot at −80°C until measurement. Samples were thawed, analyzed and refrozen several times until measurements were performed on all instruments.

Antibody Stability

An independent freeze–thaw experiment was conducted using 2 pools of plasma from a PCR-negative and a PCR-positive donor. Both donors were informed and gave their consent. Eighty-one milliliters of heparinized blood was drawn from each donor, centrifuged, aliquoted in 5 sets of 10

aliquots (1–10) and frozen at −80°C. Aliquots 2 to 10 were thawed at 4°C and refrozen, followed by aliquots 3 to 10 and so on. After 10 freeze–thaw cycles, all aliquots were analyzed on each immunoassay. Aliquot 1 was additionally left at room temperature without cap and reanalyzed after 24 h.

Antibody Assays

The SARS-CoV-2 antibody assays compared in this study are listed in Table 2, including short names used throughout the article. Analysis was performed by experienced medical laboratory technicians following the manufacturer's instructions including internal quality control. A single proficiency test was performed for all assays using sample material from the UKNEQAS quality assurance program (6).The manufacturers' recommended cutoff values were used in interpretation of the results. Borderline results [Thermo Fisher Scientific EliA™ SARS-CoV-2-Sp1 IgG (Thermo Fisher Scientific [TFS]-receptor binding domain [RBD]-IgG) 7–10 EliA U/mL (n = 37) and WANTAI SARS-CoV-2 Ab ELISA (Wantai-RBD-total) 0.9–1.1 absorbance/calculated cutoff (A/CO) (n = 6)] were not interpreted as either positive or negative but included as "no valid result."

The TFS-RBD-IgG assay received CE approval halfway through the study and changed output unit. The results in μg/L were converted to EliA U/mL using a lot-specific correction factor supplied by the manufacturer.

Data Analysis and Statistics

Data were extracted from the respective instruments. Sampling date, requesting unit, patient age, sex, and SARS-CoV-2 PCR test results were retrieved from the local laboratory information management system.

Complete data (valid results from all 7 assays) could be obtained for 57.7% (1000/1733) and 52.5% (170/324) of samples in the cohort and negative control group, respectively. Incomplete data were due to factors such as insufficient volume or clotting of the plasma. Both complete and incomplete data were included in the analysis.

All cohort samples were stratified into time intervals based on days from the date of the initial positive PCR result. The length and number of the created time intervals were based on the inclusion of a minimum of 15 samples per interval. Only the earliest sample was included per time interval from each single patient. Thus, 352 samples (20.3%) were excluded to avoid duplicates, resulting in a total of 1381 samples (79.7%) included into the time intervals.

In the negative control group, only 1 sample from each individual patient was included in the statistical analysis, excluding 55 (17.0%) samples as duplicates. Differences related to sex, age, and requester type were evaluated using Fisher's exact test, the Mann–Whitney U test, and the Kruskal–Wallis test, respectively. The 95% CIs of percentages ($\hat{p}$) were calculated as $\hat{p} \pm 1.96 \cdot \sqrt{(\hat{p} \cdot (1 - \hat{p})/n)}$. Any 2 results were considered significantly different from each other if there was no overlap between the 95% CIs. The outputs of qualitative assays were treated as semiquantitative. For optimal visual presentation the specific cutoffs in each of the different assays were adjusted to 1.0. This enabled plotting of the multiple longitudinal curves into the same logarithmic graph.

All statistical analysis was performed in IBM® SPSS® Statistics version 25 using a P value of ≤ 0.05.

Consensus Model

All assays were evaluated in terms of agreement with a consensus model designed as follows.

Samples with positive results from 3 assays or with 2 positive and no negative results were coded as positive. Any sample with at least 2 negative results and no positive results was coded as negative. In addition, samples with 1 positive

Table 2. Antibody assays included in the study.

Manufacturer	Roche Diagnostics, Mannheim, Germany		Siemens Healthineers, Erlangen, Germany		Thermo Fisher Scientific, Waltham, Massachusetts, USA		WANTAI, Beijing, China
Assay name	Elecsys® Anti-SARS-CoV-2	Elecsys® Anti-SARS-CoV-2 S	ADVIA Centaur® SARS-CoV-2 IgG (sCOVG)	ADVIA Centaur® SARS-CoV-2 Total (COV2T)	EliA™ SARS-CoV-2-Sp1 IgG [a]	OmniPATH™ COVID-19 Total Antibody ELISA	WANTAI SARS-CoV-2 Ab Elisa
Assay short name in article	Roche-NC-total	Roche-RBD-total	Siemens-RBD-IgG	Siemens-RBD-total	TFS-RBD-IgG	TFS-RBD-total	Wantai-RBD-total
Platform	COBAS e411	COBAS e411	Centaur XP	Centaur XP	Phadia 250	BEP 2000	BEP 2000
Method	Double antigen sandwich ECLIA	Double antigen sandwich ECLIA	Two-step sandwich CLIA	One-step sandwich CLIA	EliA™ fluoro-enzyme-immunoassay	Two-step sandwich ELISA	Two-step sandwich ELISA
Antigen target	Nucleocapsid	Spike RBD	Spike S1 RBD	Spike S1 RBD	Spike S1 RBD	Spike S1 RBD	Spike RBD
Antibody type	Total	Predominantly IgG, but also IgM and IgA	IgG	Total (IgG, IgM)	IgG	Total (including IgG, IgM, IgA)	Total
Duration: Total (Incubation)	18 min	18 min	57.25 min (36 min)	17.5 min (9 min)	120 min	120 min (70 min)	120 min (75 min)
Sample volume	20 µL	20 µL	40 µL	50 µL	20 µL	50 µL	100 µL
Cutoff and interpretation	Negative: COI <1.0 Positive: COI ≥1.0	Negative: <0.80 U/mL[b] Positive: ≥0.80 U/mL	Negative: <1.00 Index Positive: ≥ 1.00 Index (1.00 Index = 1.00 U/mL)	Negative: <1.00 Index Positive: ≥ 1.00 Index (1.00 Index = 1.00 U/mL)	Negative: <7 EliA U/mL Borderline: 7–10 EliA U/mL Positive: > 10 EliA U/mL	Negative: OD/0.105 < 1 Positive: OD/0.105 ≥ 1	Negative: A/CO ≤ 0.9 Borderline: 0.9 < A/CO < 1.1 Positive: A/CO ≥ 1.1
Result type (Measuring range)	Qualitative result	Quantitative 0.40–250 U/mL[c]	Quantitative 0.5–150.0 U/mL	Quantitative 0.60–75.00 U/mL	Quantitative 0.7–204 EliA U/mL	Qualitative result	Qualitative result

Abbreviations: A, absorbance; CO, calculated cutoff; COI, cutoff index; CLIA, chemiluminescence immunoassay; ECLIA, electrochemiluminescence assay; ELISA, enzyme-linked immunosorbent assay; OD, Optical density; RBD, receptor binding domain.

[a] Two versions of Thermo Fisher's IgG-assay were used on Phadia and results in µg/L were converted to EliA U/mL using a lot-specific correction factor supplied by the manufacturer.

[b] U/mL and Elia U/mL are arbitrary units and should not be confused with enzyme unit (an SI unit) that has the same symbol.

[c] 1 nM Spike-1 RBD antibodies equals 20 U/mL.

result opposed by at least 4 negative results were coded as negative.

Differences in distribution between assays and between assays and the consensus model were evaluated using McNemar's test. Agreement, sensitivity, and specificity were calculated for all assays compared to the consensus model, to the gold standard assays identified, and to the PCR result.

Ethics

The study was performed in accordance to the Helsinki Declaration. The project received approval from the Danish Data Protection Agency (March 20, 2020, "Corona Serologi" #P-2020-279) for the collection of residual plasma following routine measurement.

None of the patients included in the study was registered in the Tissue Utilization Register (Vævsanvendelsesregistret), where citizens may register that the collected blood may solely be used in relation to their own medical treatment.

RESULTS

All assays passed the external control assurance program. The concentration of plasma antibodies was demonstrated to be stable through 10 freeze–thaw cycles and after 24 h at room temperature (Supplemental Fig. 4).

The PCR-Positive Cohort

The distribution of samples from the PCR-positive cohort in time intervals is shown in Fig. 1, A. Initially, following the PCR test, all samples came from hospitalized patients, including patients requiring intensive care. This distribution gradually shifted over time with predominance of outpatient and primary care samples from week 6 and onward (Fig. 1, A).

Overall, in the included samples of the cohort, 58.6% (809/1381) of samples gave uniformly positive results, 17.8% (246/1381) gave uniformly negative results, and 23.6% (326/1381) showed discrepancy between assays. Figure 1, B shows the distribution of uniformity and discrepancy between assay results over time. Discrepancy is further stratified by the result of the consensus model. Initial interassay discrepancy of 49% at day 0 decreased steadily in the first 18 days (Fig. 1, B).

As shown in Fig. 2, A, the seropositivity percentage for all assays rose until day 18, whereafter an assay-wide plateau occurred with a mean seropositive percentage of 82.3% to 90.5%, depending on assay. The cohort samples were split into day 0–17 with 781 samples from 198 individuals and day 18+ with 600 samples from 282 individuals (Supplemental Fig. 1). Limited to day 18+, 79.7% (478/600) of samples were uniformly positive, 8.5% (51/600) were uniformly negative, and 11.8% (71/600) showed discrepant results. The distribution of discrepancy between sexes between day 0 to 17 was similar (33.6% discrepancy in males vs 31.4% in females; $P = 0.537$), while discrepant samples were significantly more frequent in females at day 18+ (16.0% vs 7.4%; $P = 0.002$). The age distribution was similar between the discrepant and uniform results at both day 0–17 ($P = 0.468$) and day 18+ ($P = 0.189$).

Nonresponders of the Cohort

A subgroup of the PCR-positive cohort remained seronegative throughout the study. Thirty-seven samples from 22 unique patients (7.8% of all patients with at least 1 sample beyond day 18) were uniformly negative in all assays. The 22 nonresponding patients collectively contributed 42 samples (5 from day 0–17) which accounted for 0.0%, 1.4%, 5.2%, and 8.8% of samples from intensive care, other hospital wards, outpatient clinics, and primary care centers, respectively. This distribution was significantly different from the remaining samples ($P < 0.001$). On an individual level, these 22 patients were similar to

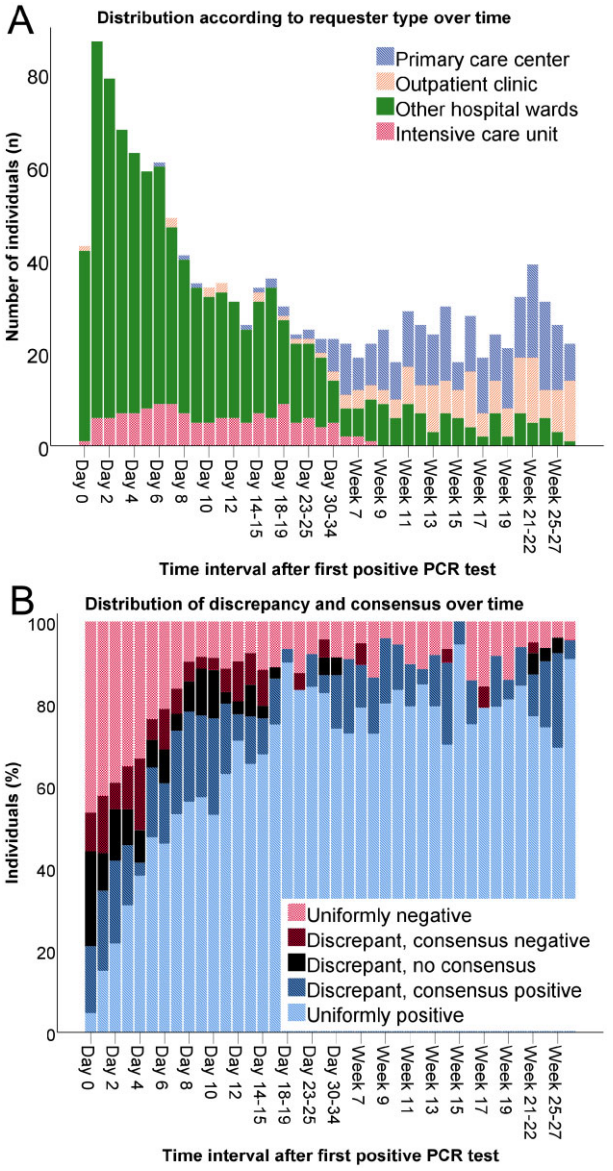

Fig. 1. Distribution of cohort samples in intervals of time since positive PCR test. Each patient is represented maximally once per time interval. (A) Distribution according to requester type. (B) Percentage distribution according to uniformity or discrepancy between all assay results. Discrepancy is further stratified by the result of the consensus model.

the remaining patients according to age ($P = 0.150$) and sex ($P = 0.377$). Although none of the 22 patients had any samples taken at the intensive care unit, this difference was not statistically significant ($P = 0.239$).

The PCR-Negative Control Group

In the negative control group, 90.0% (242/269) of samples were uniformly negative, and 10.0% (27/269 samples) showed discrepancy between assays. The discrepant group was similar in

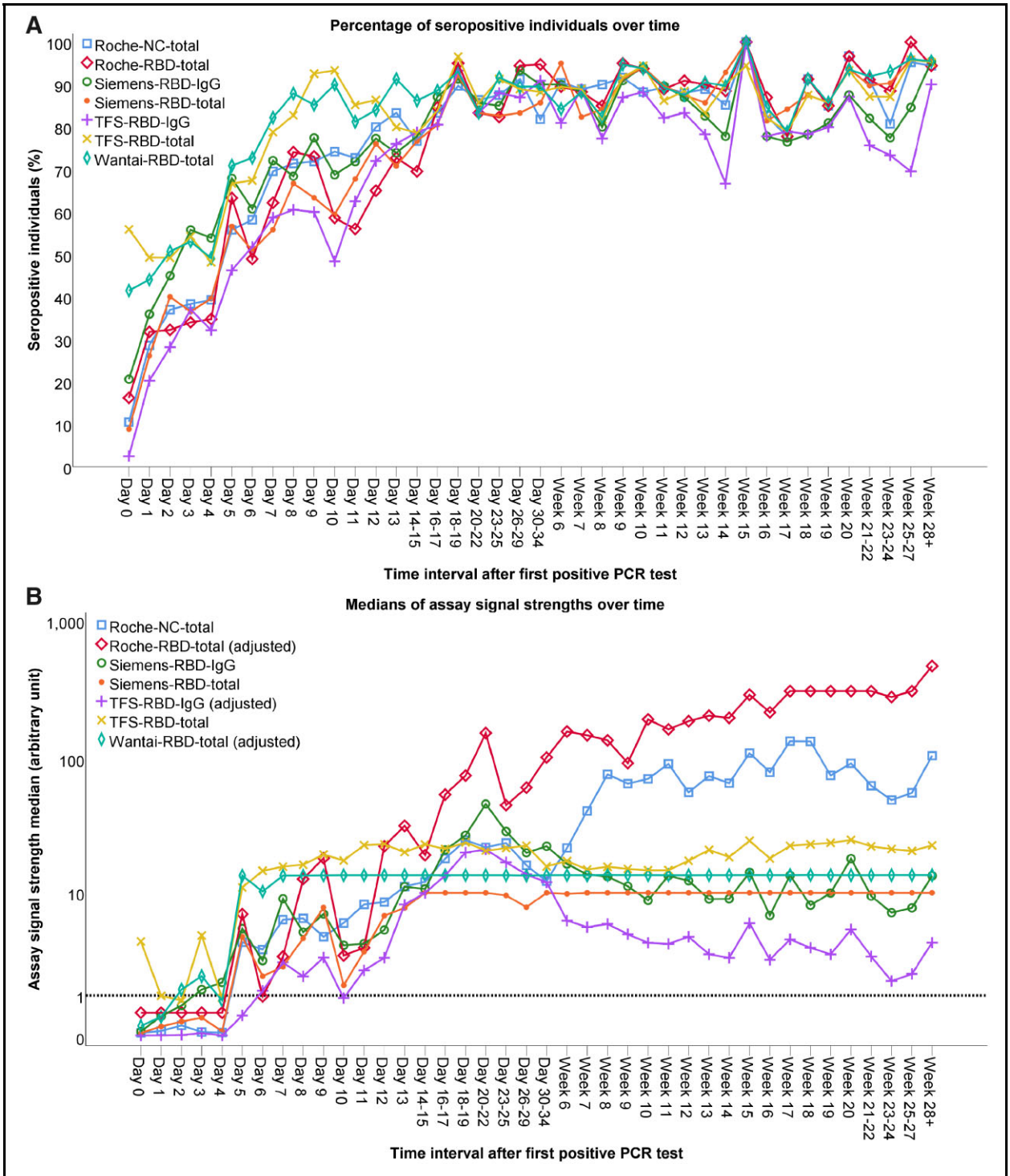

Fig. 2. Longitudinal development of seropositivity and signal strength for 7 SARS-CoV-2 antibody assays. (A) Percentage of seropositive individuals over time. (B) Medians of assay signal strengths over time, presented on a logarithmic scale. Note that all assays have been adjusted to a positive cutoff at 1.0 (dotted line) and that titers are not directly comparable between assays.

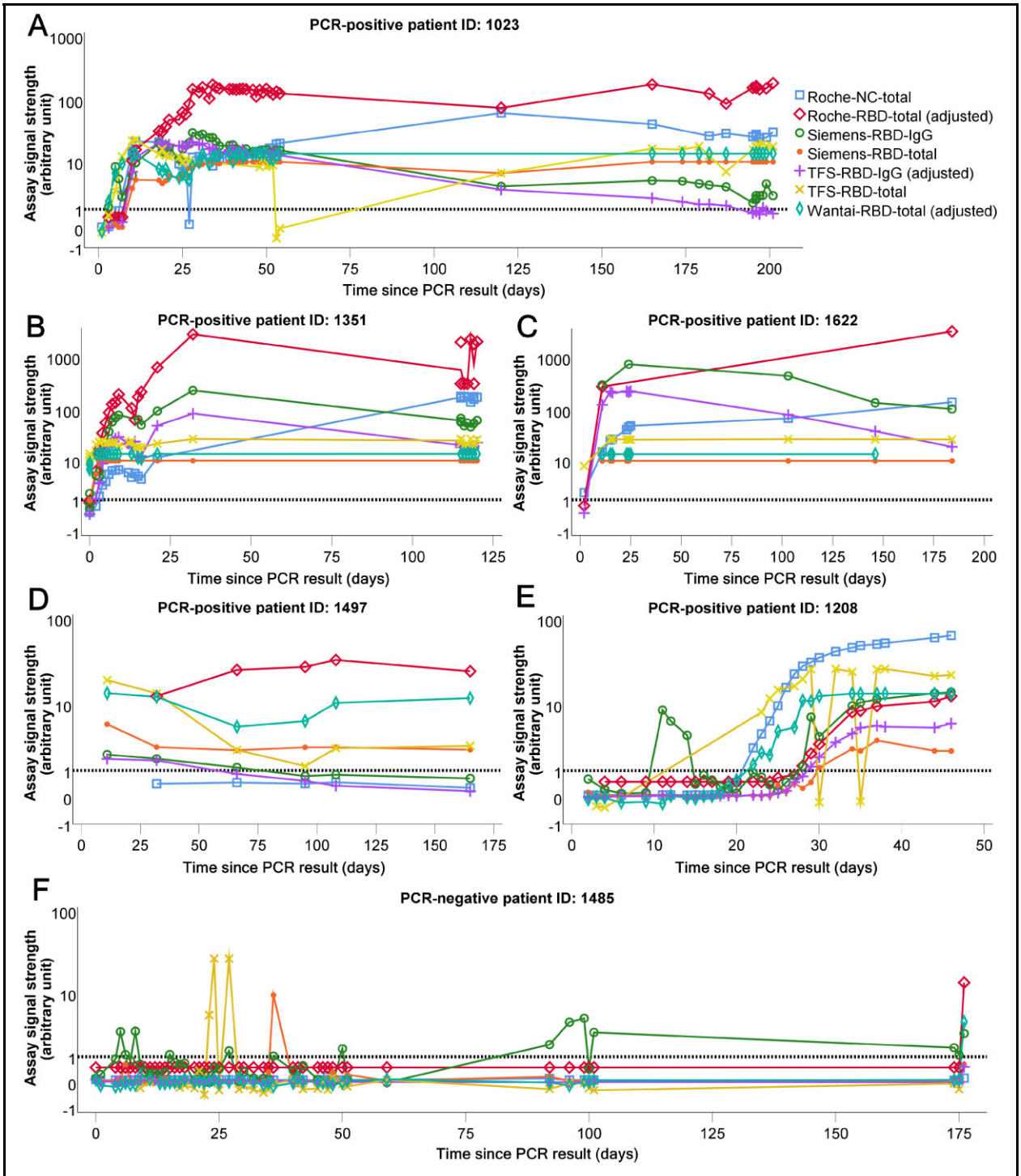

Fig. 3. **Illustrative longitudinal patient courses by 7 SARS-CoV-2 antibody assays. All assays are adjusted to a positive cutoff of 1.0. (A–C) Patient courses with strong and persistent antibody levels. (D) Weak and declining response. (E) Delayed response. Note incidences of false negatives in (A + E), clustered false positives in (E + F) and weak, declining signal strength of IgG assays in (A + D).**

distribution of age ($P = 0.312$), sex ($P = 0.384$), and sampling location ($P = 0.781$) compared to the uniform group.

The discrepancy between assays was in 81.5% (22/27) of the discrepant samples (8.2% of all included control group samples) solely due to positive results of the Siemens ADVIA Centaur® SARS-CoV-2 IgG (Siemens-RBD-IgG) assay, interpreted as false positives. As illustration, the entire patient antibody course of patient 1485 (PCR-negative) with 48 consecutive samples is presented in Fig. 3, F.

Results of the Consensus Model

The consensus model was applied on the cohort samples and controls as described in the Materials and Methods section. In the cohort samples, the model resulted in 71.8% (991/1381) consensus positive samples, 22.7% (314/1381) consensus negative samples, and 5.5% (76/1381) undetermined samples. Limited to samples from day 18+, 89.7% (538/600) of samples were coded consensus positive, 9.0% (54/600) consensus negative, and 1.3% (8/600) were undetermined. Further limited to 435 samples of day 18+ with complete data from all 7 assays, 90.3% (393/435) of samples were consensus positive, 9.4% (41/435) consensus negative, and 0.2% (1/435) undetermined.

In the unique negative controls, 98.1% (264/269) of samples were consensus negative, 0.4% (1/269) consensus positive, and 1.5% (4/269) inconclusive.

Assay Agreement with Consensus Model

Roche Elecsys® Anti-SARS-CoV-2 S (Roche-RBD-total) and Wantai-RBD-total had the highest percentage agreement with the consensus model of 99.9 (95% CI 99.6–100) and 99.8 (95% CI 99.4–100), respectively (Table 3). Neither was significantly different from the consensus model ($P_{\text{Roche-RBD-total}} = 1.000$ and $P_{\text{Wantai-RBD-total}} = 1.000$) or each other ($P = 0.125$). The remaining total assays had high agreement: Roche Elecsys® Anti-SARS-CoV-2 (nucleocapsid) (Roche-NC-total; 98.6% agreement, 95% CI 97.7–99.4), Siemens ADVIA Centaur® SARS-CoV-2 total (Siemens-RBD-total; 98.2% agreement, 95% CI 97.3–99.2), and Thermo Fisher Scientific Omnipath™ COVID-19 total antibody ELISA (TFS-RBD-total); 97.9% agreement, 95% CI 97.0–98.9). The 2 selective IgG assays, TFS-RBD-IgG (94.6% agreement, 95% CI 93.1–96.2) and Siemens-RBD-IgG (93.1% agreement, 95% CI 91.2–94.9), showed lower agreement. Agreement, sensitivity, and specificity of all assays against the consensus model, the Roche-RBD-total and Wantai-RBD-total assays as well as the PCR test result are presented in Table 3.

Illustrative patient antibody courses are presented in Fig. 3 to visualize different types and observations of antibody development over time.

DISCUSSION

The Cohort

We have introduced the North Zealand Covid-19 Cohort, a unique collection of serial plasma samples representing 75% of all PCR-confirmed SARS-CoV-2-positive individuals from North Zealand having blood samples taken during the initial (wild-type) Covid-19 wave in Denmark in 2020. The cohort's diversity is a direct reflection of the patient composition of our clinical laboratory's sample flow, including samples from primary care centers. This is to our knowledge one of the largest comparative studies of SARS-CoV-2 antibody assays in respect to the large number of patients with several serial samples (Supplemental Table 2).

Early Serology

This stage was defined as day 0-17 after the positive PCR test and was characterized by an initial low seropositivity and a high degree of

discrepancy between assays, which supports current recommendations not to rely on antibody measurements in the first 3 weeks following infection (2).

In the first 2 days after the positive PCR test, the 2 total antibody ELISA assays (Wantai-RBD-total and TFS-RBD-total) showed significantly higher rates of seroconversion compared to the 5 automated immunoassays (Fig. 2, A and example in Fig. 3, B). The subsequent drop in seropositive percentage reflects a shift in patient composition in the following time intervals. The ELISA assays also reached approximately 90% seropositivity (the plateau level) by day 10, whereas the assay-wide plateau was not reached until day 18. These results contradict early reports of an overall lower sensitivity of ELISA assays compared to chemiluminescence immunoassays (7), but are in line with later reports on specific ELISA assays, including Wantai-RBD-total (8, 9).

Late Serology

Past day 18, where the plateau had been reached for all assays, the median signal strength of all total antibody assays was steady, capped at maximum value, or slightly increasing throughout the study period (Fig. 2, B). In contrast, the IgG assays had a decreasing signal strength after 4 weeks, most attenuated in the TFS-RBD-IgG assay (Fig. 2, B and examples in Fig. 3, A, D). A similar decrease in signal strength of the TFS-RBD-IgG assay has been described by Favresse et al. in a 10-month follow-up study (10). Yet, the same study included 2 additional IgG-specific assays (Ortho and DiaSorin), which did not decrease to the same extent. This could indicate that the decreasing signal strength observed for TFS-RBD-IgG is an assay-specific phenomenon rather than a general trait of IgG-specific assays. With regards to the TFS-RBD-IgG assay, we suspect an inappropriately high cutoff for the TFS-RBD-IgG assay, which results in a sensitivity below its potential. Based on our data, a positive cutoff at 3.4 (currently at 10 and borderline results at 7–10) would result in 96.6% sensitivity and 99.7% specificity against consensus.

A declining signal strength of nucleocapsid (NC) antibodies over time was reported by Masiá et al. (11). In their 12-month followup period, 56% of patients with moderate/severe disease reverted to seronegativity according to the Euroimmun-NC-IgG assay. In our 8-month study and in the 10-month study of Favresse et al. (10), the signal strength of Roche-NC-total was very persistent over time. Thus, the NC signal loss observed by Masiá et al. seems likely to be assay-specific rather than a general trait of NC antibody development.

We found interassay discrepancy to be significantly more frequent in females than in males at day 18+ (16.0% vs 7.3%; $P = 0.002$). This is likely a result of differences between sexes in the background demographics of our cohort. We observed an overrepresentation of women having their first blood sample drawn months after diagnosis (i.e., following mild or asymptomatic Covid-19 without need of health care services) (Supplemental Fig. 3). Such mild cases more often yielded long-term samples with discrepancy due to low signal strength of TFS-RBD-IgG.

Comparison of Assay Performance

We introduced a consensus model and excluded samples of day 0–17 to achieve the optimal comparability between assays despite differences in IgG targets—IgG or total. The consensus model was successfully applied to 98.7% of samples with comparable distribution of results between complete and incomplete data. Of the 7 assays tested (Table 2), Roche-RBD-total and Wantai-RBD-total had the closest agreement with the consensus model of 99.9% and 99.8%, respectively. The Wantai-RBD-total ELISA assay has proved superior to competing assays in earlier studies (8, 9, 12–14). The Roche-RBD-total assay is

Table 3. Agreement, sensitivity, and specificity of all 7 assays against the consensus model, the Roche-RBD-total assay, the Wantai-RBD-total assay, and the PCR test result.

Assay	Compared to the consensus model			Compared to Roche-RBD-total as gold standard			Compared to Wantai-RBD-total as gold standard			Compared to PCR test result		
	Agreement % (95% CI)	Sensitivity %	Specificity %	Agreement % (95% CI)	Sensitivity %	Specificity %	Agreement % (95% CI)	Sensitivity %	Specificity %	Agreement % (95% CI)	Sensitivity %	Specificity %
Roche-NC-total	98.6 (97.7–99.4)	97.8	100.0	98.4 (97.5–99.3)	97.8	99.6	97.9 (96.9–99.0)	97.0	99.6	91.9 (90.0–93.9)	88.8	99.6
Roche-RBD-total	99.9 (99.6–100)	99.8	100.0	–	–	–	99.4 (98.8–100)	99.1	100.0	93.1 (91.2–95.0)	90.5	99.5
Siemens-RBD-IgG	93.1 (91.2–94.9)	94.6	89.9	93.1 (91.2–95.1)	94.8	89.6	92.4 (90.4–94.4)	94.0	89.0	85.8 (83.3–88.3)	85.5	86.7
Siemens-RBD-total	98.2 (97.3–99.2)	98.0	98.7	98.1 (97.1–99.2)	98.0	98.5	97.5 (96.3–98.6)	97.3	97.8	91.4 (89.4–93.4)	89.1	98.9
TFS-RBD-IgG	94.6 (93.1–96.2)	91.5	100.0	94.5 (92.8–96.2)	91.6	100.0	94.1 (92.4–95.8)	90.6	99.7	87.6 (85.4–89.9)	82.3	100.0
TFS-RBD-total	97.9 (97.0–98.9)	96.9	99.7	97.4 (96.2–98.6)	96.6	98.8	97.8 (96.8–98.8)	96.9	99.3	92.3 (90.5–94.1)	88.8	100.0
Wantai-RBD-total	99.8 (99.4–100)	99.8	99.7	99.4 (98.8–100)	100.0	98.4	–	–	–	92.9 (91.1–94.7)	90.1	98.9

Calculations are based on all cohort samples included in time intervals past day 18 and all unique negative controls.

newer but has emerging reports of a very acceptable performance (10, 15). The remaining total assays performed acceptably in comparison to consensus (97.9–98.6% agreement) (Table 3). The 2 selective IgG assays displayed lower agreement with consensus; the TFS-RBD-IgG (94.6% agreement) primarily due to low sensitivity, especially long-term (91.5% sensitivity compared to consensus) and Siemens-RBD-IgG (93.1% agreement) primarily due to false-positive results and a resulting low specificity (89.9% specificity compared to consensus). Earlier reports on the Siemens-RBD-IgG assay did not report false-positive results, in contrast they reported a specificity of 99.4% (16). We observed several incidences with timely clustering of false-positive results for Siemens-RBD-IgG (Fig. 3, E and F), which raises the suspicion that a specific condition or treatment might interfere with this specific assay.

For the current use of antibody assays to detect former, undocumented Covid-19, our data suggest the use of total antibody assays rather than IgG-specific assays due to a more persistent long-term sensitivity. This is further supported by reports of more homologous results across total antibody assays compared to IgG-specific antibody assays (17).

Low Overall Seroconversion Rate

We observed a low overall seropositivity past day 18 of 82% to 91% (depending on assay). Interestingly, for 7.8% of all PCR-positive patients in our cohort with samples beyond day 18, we saw no seroconversion at all; that is, all their samples were uniformly negative. This group (nonresponders) was not significantly different from the remaining patients regarding age or sex. Yet, on a sample level, the nonresponders had a significantly different distribution of requester type with no intensive care samples and an overrepresentation of samples from primary care, which may be reflective of a subpopulation with lower need for hospital care (i.e., with lower disease severity). This would be in line with previous observations of a stronger antibody response in patients with severe illness (8, 11, 18, 19).

Our findings of low assay sensitivities compared to PCR of Wantai-RBD-total, Roche-NC-total, and Siemens-RBD-total between 89% and 90% are comparable to reports of Herroelen et al. (20) (Wantai-RBD-total 92.1%, Roche-NC-total 88.2%, day 20+ after symptom onset, 52.6% severe illness) and Oved et al. (21) (Siemens-RBD-total 85.9%, Roche-NC-total 89.0%, day 14+ after PCR, 4.9% severe illness and 22.6% unknown severity). We initially hypothesized this shared limit at approximately 90% seropositivity compared to PCR to be due to similar inclusion of mild and asymptomatic cases, unlike many earlier studies primarily concerning hospitalized patients (7). Yet, there are conflicting reports of significantly higher sensitivity between 95% and 100% for Wantai-RBD-total (9), Roche-RBD-total (10), Roche-NC-total (9, 10, 22, 23), and Siemens-RBD-total (22, 23) in populations with hospitalization percentages ranging from 0.0% to 21.8%. The freeze–thaw experiment and consistency of seronegativity in several patient courses suggests our sample collection practice is unlikely to explain low seroconversion. We hypothesize that the low seropositivity reflects the cohort composition and by extension our laboratory sample flow and background population.

Implications of Missing Seroconversion

Of all PCR-positive patients in our cohort with samples beyond day 18, 7.8% remained seronegative throughout the study (nonresponders). If interpreted as convalescent Covid-19 patients with impaired or missing immunity, this subpopulation is of concern. Immunocompromised patients without any measurable antibody response have been observed (24). Yet, cellular

immunity may be present in the absence of circulating antibodies (25). Meanwhile, correlation between virus neutralization tests and protective immunity is increasingly documented (26, 27). But the correlation between virus neutralization tests and nonneutralizing antibody assays is ongoingly debated with conflicting reports (23, 28–31). Such correlations and the immunity status of the nonresponders requires further clarification, especially if antibody titers in the future will guide booster vaccination strategies as earlier suggested (1).

Compared to PCR, the assay sensitivities ranged from 82.3% to 90.5%. Thus, depending on assay choice, up to 18% of patients suffering from long-term sequelae following undocumented Covid-19 may remain undiagnosed in the absence of detectable antibodies. If using any of the many lateral flow assays available, false-negative rates may be even higher (7, 32). Such risk of false negatives should be considered when choosing an assay and when interpreting antibody measurements.

Limitations

The focus of this study was to compare the qualitative assay results alone (positive vs negative). Although early in the pandemic, inclusion of the negative control group through negative PCR results alone introduces a small risk of false negatives. Finally, since there is no agreed upon international gold standard assay, we evaluated all assays against consensus, which was our closest approximation of an objective truth.

CONCLUSIONS

In summary, all assays had sensitivities against consensus of >90% past day 18. For the currently recommended use of antibody assays to detect former Covid-19, our data suggest the use of total antibody assays rather than IgG-specific assays due to higher long-term sensitivities. Finally, a nonresponding subpopulation of 7.8% in our cohort with persistent seronegative results raises concern of a possible substantial number of people with continued low protection following natural SARS-CoV-2 infection.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

Nonstandard Abbreviations: Covid-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RBD, receptor binding domain (of the spike glycoprotein); TFS-RBD-IgG, Thermo Fisher Scientific EliA™ SARS-CoV-2-Sp1 IgG; Wantai-RBD-total: WANTAI SARS-CoV-2 Ab ELISA; A/CO, absorbance/calculated cutoff (Wantai-RBD-total output); Roche-RBD-total, Roche Elecsys® Anti-SARS-CoV-2 S; Roche-NC-total, Roche Elecsys® Anti-SARS-CoV-2 (nucleocapsid); TFS-RBD-total: Thermo Fisher Scientific Omnipath™ COVID-19 total antibody ELISA; Siemens-RBD-IgG, Siemens ADVIA Centaur® SARS-CoV-2 IgG; Siemens-RBD-total: Siemens ADVIA Centaur® SARS-CoV-2 total.

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