

Side by side comparison of three fully automated SARS-CoV-2 antibody assays with a focus on specificity

Running head: Specificity of automated Anti-SARS-CoV-2 assays

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Key words:

SARS-CoV-2; serology; specificity; laboratory automation; positive predictive value; seroprevalence;

List of abbreviations:

COVID-19	Coronavirus disease 2019
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
RT-PCR	Reverse transcriptase-polymerase chain reaction
ECLIA	Electrochemiluminescence assay
95% CI	95% confidence interval
CMIA	Chemiluminescent microparticle immunoassay
NPA	Negative percentage agreement
CLIA	Chemiluminescence immunoassay
ROC	Receiver-operating-characteristics
AUC, AUROC	Area under the (ROC-)curve
LOD	Limit of detection
PPV	Positive predictive value
NPV	Negative predictive value
EUA	Emergency Use Authorization

ABSTRACT

Background: In the context of the COVID-19 pandemic, numerous new serological test systems for the detection of anti-SARS-CoV-2 antibodies rapidly have become available. However, the clinical performance of many of these is still insufficiently described. Therefore, we compared three commercial, CE-marked, SARS-CoV-2 antibody assays side by side.

Methods: We included a total of 1,154 specimens from pre-COVID-19 times and 65 samples from COVID-19 patients (≥ 14 days after symptom onset) to evaluate the test performance of SARS-CoV-2 serological assays by Abbott, Roche, and DiaSorin.

Results: All three assays presented with high specificities: 99.2% (98.6-99.7) for Abbott, 99.7% (99.2-100.0) for Roche, and 98.3% (97.3-98.9) for DiaSorin. In contrast to the manufacturers' specifications, sensitivities only ranged from 83.1% to 89.2%. Although the three methods were in good agreement (Cohen's Kappa 0.71-0.87), McNemar tests revealed significant differences between results obtained from Roche and DiaSorin. However, at low seroprevalences, the minor differences in specificity resulted in profound discrepancies of positive predictive values at 1% seroprevalence: 52.3% (36.2-67.9), 77.6% (52.8-91.5), and 32.6% (23.6-43.1) for Abbott, Roche, and DiaSorin, respectively.

Conclusion: We found diagnostically relevant differences in specificities for the anti-SARS-CoV-2 antibody assays by Abbott, Roche, and DiaSorin that have a significant impact on the positive predictive values of these tests.

Introduction

COVID-19 is a new disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which was first described by Chinese scientists in early January 2020 (1). On March 11, the World Health Organization (WHO) officially declared the novel SARS-CoV-2 infections a pandemic, which has now spread rapidly across the entire globe, with more than 13.5 million confirmed cases and over 580,000 confirmed deaths (2). COVID-19 is characterized by a broad spectrum of individual disease courses, ranging from asymptomatic infections to the most severe cases requiring intensive medical care (3).

The reliable detection of infected persons and, subsequently, their isolation is essential for the effort to prevent the rapid spread of the SARS-CoV-2 virus. Therefore, reverse transcriptase-polymerase chain reaction (RT-PCR) testing is required for direct detection of the pathogen. Unfortunately, RT-PCR testing could yield false-negative results, mainly due to preanalytical problems (4,5).

On the other hand, serological testing for SARS-CoV-2 specific antibodies can be used as an additional diagnostic tool in case of suspected false-negative RT-PCR results (6) or for individual determination of antibody levels. Moreover, cross-sectional serological studies provide essential epidemiological information to allow a correct estimation of the spread of the disease within a population (7,8). The first commercially available serological SARS-CoV-2 tests, mostly standard ELISA tests or lateral flow rapid tests, have not always proved to be sufficiently specific and sensitive (9,10). Recently, the first tests for fully automated large-scale laboratory analyzers have been launched. The present evaluation aimed to compare three of these test systems manufactured by

Abbott, Roche, and DiaSorin, with particular emphasis on specificity, which is crucial for an adequate positive predictive value. In view of the currently low seroprevalence worldwide (11), high specificities are important for reliable seroprevalence studies that attempt to close the gap between the number of RT-PCR confirmed COVID-19 cases and the total number of SARS-CoV-2 infections that have occurred (12).

Materials and methods

Study design and patient cohorts

This nonblinded prospective study aims at a detailed comparison of three automated SARS-CoV-2 detection methods with a particular focus on specificity and positive predictive value. A total of 1,154 samples from three cohorts of patients/participants with sampling dates before 01.01.2020 was used to test specificity. The samples derived from three different collections: a cross-section of the Viennese population, LEAD study (13), preselected for samples collected between November and April to enrich seasonal infections (n=494); a collection of healthy voluntary donors (n=302; 269 individuals, 11 donors with a 4-fold repetition of the donation within a median period of 4.5 years [3.6-5.5]); a disease-specific collection of samples from patients with rheumatic diseases (n=358).

For estimation of test sensitivity, samples of 65 COVID-19 donors/patients with a symptom onset to analysis time of ≥ 14 days (median [IQR] time interval of 41 [27-49] days) were evaluated in parallel on all three analysis platforms. In this late phase, we assumed the majority of donors/patients had reached prominent and constant levels of SARS-CoV-2 specific antibodies. 52 of the 65 donors/patients were non-hospitalized convalescent individuals, two-thirds of them with mild symptoms. Of those, 42 donors/patients were RT-PCR confirmed cases, and 10 were close contacts of RT-PCR confirmed cases (similar to(14)).

For asymptomatic donors (n=5), SARS-CoV-2 RT-PCR confirmation to analysis time was used instead. We subjected only a single serum sample per patient to sensitivity analysis to avoid data bias due to uncontrolled multiple measurement points of individual

patients. Symptom onset was determined by a questionnaire in convalescent donors and by reviewing individual health records in patients.

Online **Supplemental Table 1** gives a comprehensive overview of characteristics and cohort-specific inclusion and exclusion criteria; online **Supplemental Tables 2, 3**, and online **Supplemental Fig. 1** provide additional descriptive statistics on donors/patients included in the cohorts.

All included participants gave written informed consent for donating their samples for scientific purposes. From patients, only left-over material from diagnostic procedures was used. The overall evaluation plan conformed with the Declaration of Helsinki as well as with relevant regulatory requirements. It was reviewed and approved by the ethics committee of the Medical University of Vienna (1424/2020).

Biomaterials

Used serum samples were either left-over materials from diagnostic procedures (Department of Laboratory Medicine, Medical University of Vienna) or part of a sample cohort processed and stored at median temperatures $<-70^{\circ}\text{C}$ by the MedUni Wien Biobank. All pre-analytical processes were carried out according to standard operating procedures in an ISO 9001:2008/2015-certified (MedUni Wien Biobank, Department of Laboratory Medicine) and ISO 15189:2012-accredited (Department of Laboratory Medicine) environment. Standard sample protocols were described previously (15).

Antibody testing

SARS-CoV-2 specific antibodies were measured according to the manufacturers' instructions on three different automated platforms at the Department of Laboratory

Medicine of the Medical University of Vienna. Test characteristics are summarized in online **Supplemental Table 4**.

1) IgG antibodies against SARS-CoV-2 nucleocapsid (SARS-CoV-2 IgG) were quantified employing a chemiluminescent microparticle immunoassay (CMIA) on the Abbott ARCHITECT® i2000sr platform (Abbott Laboratories). The manufacturer predefined a cut-off of ≥ 1.4 index (S/C) for the qualitative detection of IgG antibodies to SARS-CoV-2.

2) The Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) was applied on a Cobas e 801 modular analyzer. It detects total antibodies against the SARS-CoV-2 nucleocapsid (N) antigen in a sandwich electrochemiluminescence assay (ECLIA). The cut-off was pre-defined to ≥ 1 COI. According to the manufacturer, the system delivers qualitative results, either being reactive or non-reactive for anti-SARS-CoV-2 antibodies.

3. The LIAISON® SARS-CoV-2 S1/S2 IgG test detects IgG-antibodies against the S1/S2 domains of the virus spike protein in a chemiluminescence immunoassay (CLIA). The test was applied to a LIAISON® XL Analyzer (DiaSorin S.p.A.). The manufacturer suggests a cut-off ≥ 15.0 AU/mL (borderline results 12.0 – <15.0, require a re-test algorithm). Samples that repeatedly tested borderline were classified as positive.

According to the manufacturer, this assay is the only test system in the current comparison to achieve quantitative results (AU/ml). In addition to a two-point calibration, precision measurements at 3 levels and a linearity test according to CLSI EP-6A are cited as proof of this.

Statistical analysis

Unless stated otherwise, continuous data are given as median (quartile 1 – quartile 3). Categorical data are given as counts and percentages. Diagnostic sensitivity and specificity, as well as positive and negative predictive values, were calculated using MedCalc software 19.2.1 (MedCalc Ltd.). 95% confidence intervals (CI) for sensitivity and specificity were calculated according to Clopper and Pearson ("exact" method) with standard logit confidence intervals for the predictive values (16). Receiver-Operating-Characteristic (ROC)-curve analysis was used to evaluate test accuracy and compare the diagnostic performance of the three test systems, according to DeLong et al. (17). Between-test agreements were assessed by interpretation of Cohen's Kappa-statistics and further evaluated with McNemar tests. Statistical significance was assumed at $P < 0.05$. Figures were produced with MedCalc software 19.2.1 and GraphPad Prism 8 (GraphPad Software).

Results

Specificity

To describe assay specificity, we used a total of 1,154 serum samples collected before SARS-CoV-2 circulated in the population and which are, by definition, negative for SARS-CoV-2 specific antibodies. The three different specificity cohorts A-C (described in detail in online **Supplemental Tables 1-3** and **Supplemental Figure 1**) presented with different rates of false-positives (**Table 1**) - cohort C (cohort of rheumatic diseases) showing the highest reactivities. We found in total 9, 3, and 20 false-positive samples for Abbott, Roche, and DiaSorin, leading to an assay specificity of 99.2 (95%CI: 98.6-99.7), 99.7% (95%CI: 99.2-100.0), and 98.3% (95%CI: 97.3-98.9) respectively (**Figure 1A-C**). Median and 90th percentile values of negative samples were 0.025 and 0.115 Index for Abbott, 0.0815 and 0.0927 COI for Roche, and below LOD and 5.52 AU/ml for DiaSorin. False-positive samples yielded median values of 2.21 Index (2.14-2.67) for Abbott SARS-CoV-2 IgG (cut-off: ≥ 1.4 Index), 1.65 COI (1.47-1.72) for Roche Elecsys® Anti-SARS-CoV-2 (cut-off: ≥ 1 COI), and 22.4 AU/ml (17.38-57.35) for DiaSorin LIAISON® SARS-CoV-2 S1/S2 IgG (negative < 12.0 AU/ml, equivocal $12.0 - < 15.0$ AU/ml, positive ≥ 15.0 AU/ml).

Sensitivity

Sensitivity was estimated from 65 samples collected in the median 41 [27-49] days after symptom onset. Surprisingly, we found a relatively high percentage of samples that tested negative for SARS-CoV-2 antibodies: Abbott 10, Roche 7, and DiaSorin 11 false-negatives leading to calculated sensitivities of 84.6% (73.6-92.4), 89.2% (79.1-95.6),

and 83.1% (71.3-91.2), respectively. Five serum samples consistently gave negative test results in all three assays despite being derived from individuals who tested positive for SARS-CoV-2 by RT-PCR. All seven false-negatives in the Roche test overlapped with false-negatives in the Abbott test (both nucleocapsid-antigen based assays), whereas DiaSorin was negative for an additional six serum samples exclusively (S1/S2-domain antigen-based assay).

PPV and NPV

A comparative overview for specificity, sensitivity, as well as positive and negative predictive values at 1%, 5%, and 10% SARS-CoV-2 antibody seroprevalence is shown in online **Supplemental Figures 2A and 2B** and summarized in **Table 2**. While the differences between the test systems for varying seroprevalences did not have a significant impact on NPV (range 98.1%-99.9%), the consequences for PPV were pronounced. At seroprevalence rates of 10%, all three systems showed acceptable PPVs of 92.3%, 97.4%, and 84.2% for Abbott, Roche, and DiaSorin, but at 1% seroprevalence these dropped to unsatisfactory or even unacceptably low values of 52.3% (36.2-67.9), 77.6% (52.8-91.5), and 32.6% (23.6-43.1) for Abbott, Roche, and DiaSorin.

ROC Curve Analysis

As shown in **Figure 2A-C**, all three ROC curves presented with areas under the curves (AUC) above 0.97 (Abbott: 0.994 [95% CI: 0.987-0.997], Roche: 0.989 [0.981-0.994], DiaSorin: 0.977 [0.967-0.985]). Comparison of ROC-AUCs, according to DeLong et al., did not reveal significant differences (Differences: Abbott/Roche $P=0.487$,

Abbott/DiaSorin $P=0.112$, Roche/DiaSorin $P=0.395$). In the next step, we aimed to assess whether modifying the cut-off values could improve the explanatory power of the ROC-curves (**Figure 2A-C**).

Cut-offs associated with the Youden's index (maximum sum of sensitivity and specificity) of >0.42 , >0.355 , and >8.76 for Abbott, Roche, and DiaSorin lowered the PPV considerably, being as low as 26.4 (20.3-33.6), 62.1 (43.9-77.4), and 24.8 (18.9-31.9) at 1% seroprevalence for Abbott, Roche, and DiaSorin (online **Supplemental Table 5**).

Between-test agreement/disagreement

Correlation analysis of measurement values between the different platforms showed only moderate to weak concordance (online **Supplemental Fig. 3**). The Pearson correlation coefficient was $r=0.66$ ($P<0.001$), for Abbott/DiaSorin (both IgG-assays) and $r=0.63$ ($P<0.001$) for Abbott/Roche (both nucleocapsid-antigen based assays). In contrast, Roche/DiaSorin, with a coefficient of $r=0.23$ did not reach significance ($P=0.067$).

Therefore, the test systems' agreements were studied in a pairwise fashion applying inter-rater agreement statistics (Cohen Kappa). The agreement between Abbott and Roche was very good (0.87 [0.81-0.94]). Agreement between Abbott and DiaSorin, and DiaSorin and Roche was good: 0.71 (0.62-0.80), and 0.76 (0.67-0.84), respectively (**Table 3**). Despite a good overall inter-rater agreement, significant differences could be shown using the McNemar test for DiaSorin and Roche (online **Supplemental Table 6**).

Discussion

SARS-CoV-2 is a new virus closely related to the betacoronaviruses SARS-CoV and MERS. Like SARS-CoV-2, those highly virulent pathogens cause severe respiratory syndromes, often with lethal outcome (18). In contrast, infections with other members of the coronavirus family usually present with mild colds, including 229E, OC43, NL63, and HKU1 (19). Compared to SARS-CoV (which is no longer circulating), cross-reactivity between SARS-CoV-2 and endemic seasonal coronaviruses is low. To date, with few exceptions (20), no accumulation of cross-reactivities between anti-SARS-CoV-2 antibodies and seasonal coronavirus antibodies has been found. We have therefore refrained from screening a coronavirus panel for possible cross-reactivity.

Specificity

To best describe the specificity of a serological test, it is essential to have a reliable reference, i.e., to ensure that the samples used are negative for the target analyte. For SARS-CoV-2, this means using serum/plasma samples obtained before the first appearance of the new virus. Therefore, we have compiled large pre-COVID-19 cohorts, which have the following characteristics: A) samples of an age and sex-controlled population-based cohort of more than 11,000 participants (LEAD-Study) (13), randomly chosen from Vienna and surrounding areas (n=494). B) samples of healthy voluntary donors (n=302), which are typically used at our Department for the evaluation of new assays, and C) samples of a disease-specific collection of patients with rheumatic diseases including rheumatoid arthritis and systemic lupus erythematoses (n=358), known to have a high prevalence of autoantibodies and other atypical immune activities,

enhancing the potential of interference with serological testing. We found several false-positives in the rheumatological cohort (n=13), and to a lesser extent in the other two cohorts (n=9 in the healthy donor cohort and n=10 in the LEAD study). Notably, false-positive samples did not typically overlap in the different systems and only one out of 32 false-positive samples was reactive in more than one assay (Abbott and DiaSorin, a sample from the LEAD study). Since these two test systems use different antigens (nucleocapsid vs. S1/S2 proteins) but the same detection method (IgG), this false-positive reaction is likely associated with interference in the IgG measurement.

Calculated specificities are strongly dependent on the spectrum and the size of a selected specificity cohort. If we calculated the specificities of each cohort separately, we would be able to report variable specificities: cohort A (Abbott 99.2%, Roche 100%, DiaSorin 98.8%), cohort B (Abbott 99%, Roche 99.7%, DiaSorin 98.3%), and cohort C (Abbott 99.4%, Roche 99.4%, DiaSorin 97.5%). Roche would range from ideal 100% down to 99.4%, the same level as the best result for Abbott, and DiaSorin would be nearly as good as the worst Abbott specificity or show a 2.5% difference to the best Roche value. These variable specificities could have an enormous impact on prevalence dependent parameters like PPV. A recent evaluation of the DiaSorin LIAISON® SARS-CoV-2 S1/S2 IgG assay with 1,140 pre-COVID-19 samples reported a specificity of 98.5% (21), nearly perfectly matching the specificity of 98.3% we found when calculating the average of all three cohorts. In contrast, another recent study reported a specificity of 100% for DiaSorin. However, the authors used only n=81 samples for specificity testing (22). Similarly, a further evaluation comparing all three SARS-CoV-2 tests by Abbott, Roche, and DiaSorin found quite different specificities, namely 100%, 98%, and 96.9% for Abbott, Roche, and DiaSorin, respectively. Again, the specificity cohort was

very small (n=100, and n=98 for DiaSorin) (23). This underlines the importance of selecting adequately sized testing cohorts to obtain reliable and comparable results. In summary, the specificities of 99.2%, 99.7%, and 98.3% found in the present study are very close to the values given by the manufacturers of 99.6%, 99.8%, and 98.5% for Abbott, Roche, and Diasorin, which were also established on large collectives.

Sensitivity

The COVID-19 positive cohort used in this study for the estimation of sensitivities is relatively small (n=65). However, it has three distinctive features: only one serum sample per patient/donor was included, blood was sampled median 41 days after the onset of symptoms, and 80% were non-hospitalized COVID-19 patients (two-thirds of them with mild symptoms).

Q.-X Long et al. (24) have previously shown that in the majority of COVID-19 patients serum conversion for anti-SARS-CoV-2 IgG and IgM started 13 days after onset of symptoms. In the same publication, serum IgG and IgM levels plateaued within 6 days after seroconversion. This is consistent with the observation that the sensitivity within the first 14 days after symptom onset is highly variable for most SARS-CoV-2 antibody assays but becomes better >14 days (25,26). As our median time between symptom onset and blood sampling was 41 days, we expected high sensitivities for all tested assays. Surprisingly five samples were negative in all three assays: all were RT-PCR confirmed cases, 4/5 non-hospitalized (42-51 days after symptom onset), two with mild and the other two with moderate symptoms, and symptom duration of <1 week for all. None of these patients had a known immune dysfunction or other severe diseases. One patient was an ICU patient with an underlying hematological disease, and the sample was taken at day 15 after symptom onset. For Abbott, there is some evidence available

that the suggested sensitivity of 100% \geq 14 days after symptom onset might not be reached in other cohorts. So, Tang et al. (25) reported a sensitivity of 93.8% \geq 14 days, and Theel et al. (27) of 92.9% \geq 15 days for Abbott. Only Bryan et al. (26) could find higher sensitivities of 96.95% \geq 14 days and 100% \geq 17 days. In our study, we observed a sensitivity of 84.6% for Abbott, which is still far below the reported sensitivities. One possible explanation might be the high proportion of non-hospitalized and mild cases in our sensitivity cohort, as antibody levels could depend on disease severity (28,29). Interestingly, using the same specimens and validation protocol Tang et al. (30) also validated the Roche assay and found a sensitivity of 89.36%, which is very similar to the sensitivity of 89.2% we described in our cohort. Another interesting observation was that in six patients with positive detection of SARS-CoV-2 specific antibodies in the Roche and Abbott test, DiaSorin failed to detect antibodies.

Unfortunately, a clear answer to the question of whether antibody measurements against nucleocapsid- or spike protein-associated antigens are more sensitive and specific is not possible based on the currently available data.

PPV, NPV, ROC-Analysis, test agreement

Specificity and sensitivity alone are not sufficient to judge the performance of a diagnostic test; prevalence-dependent accuracy measures like PPV and NPV are necessary, and especially PPV, in times of low prevalence (31). For most regions affected by the pandemic, the prevalence of SARS-CoV-2 antibody-positive individuals is unknown but can be estimated to be below 5% (32-34). Seroprevalence can change substantially over time and large regional differences have been shown for example in a large nationwide seroprevalence study in Spain (32). In line with this, the FDA compares the performance of all SARS-CoV-2 EUA approved antibody tests based on an assumed

5% seroprevalence (35). At this rate, the results presented here show PPV values of 85.1% (74.7-91.7), 94.8% (85-98), and 71.6% (61.7-79.8) for Abbott, Roche, and DiaSorin, respectively. The PPV values between Roche and DiaSorin differ so clearly that not even the 95% CI intervals overlap. Therefore, we must assume that these two assays differ significantly from each other in terms of positive predictive value. Using these two tests at lower seroprevalences, such as 1%, leads to an even more pronounced difference between Roche and DiaSorin (77.6% vs. 32.6%), with an unacceptable low PPV for DiaSorin.

Although the areas under the curves (0.994, 0.989, and 0.977 for Abbott, Roche, and DiaSorin) did not differ significantly from each other (sensitivity cohort size was too small), modeling of the cut-offs according to Youden's index revealed interesting insights: only Roche could increase the sensitivity without losing specificity dramatically (Sensitivity: 89.2% 98.5%; Specificity: 99.7% 99.4%; cut-off: >0.355 COI, see **Figure 2**). In contrast, DiaSorin at the suggested cut-off of >8.76 AU/ml (similar to(21)) increased the sensitivity from 83.1% to 90.8% but worsened the specificity from 98.3 to 97.2%. In line with this, despite a good overall agreement between Roche and DiaSorin results (Cohen Kappa 0.76 [0.67-0.84]), the McNemar test still showed significant differences, indicating disagreement (in particular in false-positives) more often than expected by chance.

The strength of this study is the side by side evaluation of three assays with a large number of negative samples to give reliable and comparable specificity data (no missing data). Limitations are the moderate numbers of positive samples. Moreover, obtained sensitivities cannot easily be compared to other studies because of the unique feature of our COVID-19 cohort, including 80% non-hospitalized patients with mainly mild

symptoms. The latter is highly relevant for a potential use of antibody tests to assess seroprevalence in large populations.

Conclusion

We find diagnostically relevant differences in specificities for the anti-SARS-CoV-2 antibody assays by Abbott, Roche, and DiaSorin that have a significant impact on the positive predictive value of these tests. We conclude that low seroprevalences require an unusually high specificity for SARS-CoV-2 antibody tests, which pushes some test systems to their limits earlier than others. Therefore, the choice of the test must depend on the respective seroprevalence, and strategies such as confirmation of possible false-positive test results with additional testing must be considered.

Acknowledgments

We sincerely thank Marika Gerdov, Susanne Keim, Karin Mildner, Elisabeth Ponweiser, Manuela Repl, Ilse Steiner, Christine Thun, Martina Trella, for excellent technical assistance and Ass.-Prof. Gerda Leitner for contribution of biomaterial. Finally, we want to thank all the donors of the various study cohorts. Without their voluntary participation in the establishment of the biobanks, this study would not have been possible.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

T. Perkmann, financial support, statistical analysis, administrative support, provision of study material or patients; M.-K. Breyer, provision of study material or patients; R. Breyer-Kohansal, provision of study material or patients; O.C. Burghuber, provision of study material or patients; S. Hartl, provision of study material or patients; D. Aletaha, provision of study material or patients; D. Sieghart, provision of study material or patients; P. Quehenberger, provision of study material or patients; P. Mucher, administrative support, provision of study material or patients; O.F. Wagner, financial support; H. Haslacher, statistical analysis, administrative support.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.

Consultant or Advisory Role: S. Hartl, GSK, Boehringer, Menarini, Chiesi, Astra Zeneca, MSD, Novartis, Roche, Abbvie, TEVA, Takeda.

Stock Ownership: None declared.

Honoraria: D. Aletaha, Abbvie, Amgen, Merck, Celltrion, Gilead, Galappagos, Lilly, Merck, Novartis, Pfizer, Roche, Sanofi, Sandoz.

Research Funding: None declared.

Expert Testimony: None declared.

Patents: None declared.

Other Remuneration: T. Perkmann, travel support from Menarini, travel support from Werfen, lecture fees from ThermoFisher-Phadia; N. Perkmann-Nagele, DiaSorin Austria GmbH, travel grant for DiaSorin; O.F. Wagner, Roche, Abbott, DiaSorin.

Role of Sponsor: No sponsor was declared.

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;382:727–33.
2. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis* 2020;20:533–4.
3. Chen Q, Zheng Z, Zhang C, Zhang X, Wu H, Wang J, et al. Clinical characteristics of 145 patients with corona virus disease 2019 (COVID-19) in Taizhou, Zhejiang, China. *Infection* 2020;92:401–9.
4. Hase R, Kurita T, Muranaka E, Sasazawa H, Mito H, Yano Y. A case of imported COVID-19 diagnosed by PCR-positive lower respiratory specimen but with PCR-negative throat swabs. *Infect Dis (Lond)* 2020;52:423–6.
5. Liu R, Han H, Liu F, Lv Z, Wu K, Liu Y, et al. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. *Clin Chim Acta* 2020;505:172–5.
6. Yong G, Yi Y, Tuantuan L, Xiaowu W, Xiuyong L, Ang L, et al. Evaluation of the auxiliary diagnostic value of antibody assays for the detection of novel coronavirus (SARS-CoV-2). [Epub ahead of print] *J Med Virol* 2020 Apr 22 as DOI: 10.1002/jmv.25919.
7. Farnsworth CW, Anderson NW. SARS-CoV-2 Serology: Much Hype, Little Data. *Clin Chem* 2020;66:875-7.

8. Theel ES, Slev P, Wheeler S, Couturier MR, Wong SJ, Kadkhoda K. The Role of Antibody Testing for SARS-CoV-2: Is There One? *J Clin Microbiol* 2020;58:e00797-20.
9. Infantino M, Grossi V, Lari B, Bambi R, Perri A, Manneschi M, et al. Diagnostic accuracy of an automated chemiluminescent immunoassay for anti-SARS-CoV-2 IgM and IgG antibodies: an Italian experience. *J Med Virol* 2020 Apr 24 as DOI: 10.1002/jmv.25932.
10. Whitman JD, Hiatt J, Mowery CT, Shy BR, Yu R, Yamamoto TN, et al. Test performance evaluation of SARS-CoV-2 serological assays. *medRxiv*. Cold Spring Harbor Laboratory Press; 2020;:2020.04.25.20074856.
11. Eckerle I, Meyer B. SARS-CoV-2 seroprevalence in COVID-19 hotspots. [Epub ahead of print] *Lancet* 2020 Jul 3 as DOI: 10.1016/S0140-6736(20)31482-3.
12. 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.
13. Breyer-Kohansal R, Hartl S, Burghuber OC, Urban M, Schrott A, Agusti A, et al. The LEAD (Lung, Heart, Social, Body) Study: Objectives, Methodology, and External Validity of the Population-Based Cohort Study. *J Epidemiol* 2019;29:315–24.

14. Robbiani DF, Gaebler C, Muecksch F, Lorenzi JCC, Wang Z, Cho A, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. [Epub ahead of print] *Nature* 2020 Jun 18 as DOI: 10.1038/s41586-020-2456-9.
15. Haslacher H, Gerner M, Hofer P, Jurkowitsch A, Hainfellner J, Kain R, et al. Usage Data and Scientific Impact of the Prospectively Established Fluid Bioresources at the Hospital-Based MedUni Wien Biobank. *Biopreserv Biobank* 2018;16:477-82.
16. Mercaldo ND, Lau KF, Zhou XH. Confidence intervals for predictive values with an emphasis to case–control studies. *Statistics in Medicine* 2007;26:2170–83.
17. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the Areas under Two or More Correlated Receiver Operating Characteristic Curves: A Nonparametric Approach. *Biometrics* 1988;44:837.
18. Rockx B, Kuiken T, Herfst S, Bestebroer T, Lamers MM, Oude Munnink BB, et al. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. *Science* 2020;368:1012-5
19. Weiss SR. Forty years with coronaviruses. *J Exp Med* 2020;217:e20200537.
20. Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients. *Emerging Infect Dis* 2020;26:1478-88.
21. Bonelli F, Sarasini A, Zierold C, Calleri M, Bonetti A, Vismara C, et al. Clinical And Analytical Performance Of An Automated Serological Test That Identifies S1/S2

Neutralizing IgG In Covid-19 Patients Semiquantitatively. [Epub ahead of print] J Clin Microbiol 2020 Jun 24 as DOI: 10.1128/JCM.01224-20.

22. Tré-Hardy M, Wilmet A, Beukinga I, Dogné J-M, Douxfils J, Blairon L. Validation of a chemiluminescent assay for specific SARS-CoV-2 antibody. Clin Chem Lab Med 2020;58:1357-64.
23. Ekelund O, Ekblom K, Somajo S, Pattison-Granberg J, Olsson K, Petersson A. High-throughput immunoassays for SARS-CoV-2, considerable differences in performance when comparing three methods. medRxiv. Cold Spring Harbor Laboratory Press; 2020;:2020.05.22.20106294.
24. Long Q-X, Liu B-Z, Deng H-J, Wu G-C, Deng K, Chen Y-K, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020;26:845–8.
25. Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, et al. Clinical Performance of Two SARS-CoV-2 Serologic Assays. Clin Chem 2020;66:1055-62.
26. Bryan A, Pepper G, Wener MH, Fink SL, Morishima C, Chaudhary A, et al. Performance Characteristics of the Abbott Architect SARS-CoV-2 IgG Assay and Seroprevalence in Boise, Idaho. J Clin Microbiol. 2020; 58:e00941-20.
27. 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:e01243-20.

28. Long Q-X, Tang X-J, Shi Q-L, Li Q, Deng H-J, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. [Epub ahead of print] *Nat Med* 2020 Jun 18 as DOI: 10.1038/s41591-020-0965-6
29. Liu Z-L, Liu Y, Wan L-G, Xiang T-X, Le A-P, Liu P, et al. Antibody profiles in mild and severe cases of COVID-19. *Clin Chem* 2020;66:1102-3.
30. Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, et al. Clinical Performance of the Roche SARS-CoV-2 Serologic Assay. *Clin Chem*. 2020;66:1107-8.
31. Šimundić A-M. Measures of Diagnostic Accuracy: Basic Definitions. *EJIFCC* 2009;19:203–11.
32. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, Oteo J, Hernán MA, Pérez-Olmeda M, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. [Epub ahead of print] *Lancet*. 2020 Jul 3 as DOI: 10.1016/S0140-6736(20)31483-5.
33. Stringhini S, Wisniak A, Piumatti G, Azman AS, Lauer SA, Baysson H, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. [Epub ahead of print] *Lancet* 2020 Jun 11 as DOI: 10.1016/S0140-6736(20)31304-0.
34. Xu X, Sun J, Nie S, Li H, Kong Y, Liang M, et al. Seroprevalence of immunoglobulin M and G antibodies against SARS-CoV-2 in China. [Epub ahead of print] *Nat Med* 2020 Jun 5 as DOI: 10.1038/s41591-020-0949-6.

35. US Food Drug Administration. EUA Authorized Serology Test Performance [Internet]. fda.gov. 2020. Available from: <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance> [accessed 2020 Jun 2]

Tables

Table 1. Numbers and percentages of false positive SARS-CoV-2 antibody reactivities in three different specificity cohorts: Cohort A (LEAD-Study), Cohort B (Healthy donor collective), and Cohort C (Rheumatic diseases cohort). χ^2 -tests for differences of proportions: *... Roche vs. DiaSorin $P=0.015$, \$... Roche vs. DiaSorin $P=0.040$, &... Abbott vs. Diasorin $P<0.040$, %... Roche vs. DiaSorin $P<0.001$

	COHORT A	COHORT B	COHORT C	TOTAL
	<i>n=494</i>	<i>n=302</i>	<i>n=358</i>	<i>n=1,154</i>
Abbott SARS-CoV-2 IgG	4 (0.8%)	3 (1.0%)	2 (0.6%) ^{&}	9 (0.8%)
Roche Elecsys® Anti-SARS-CoV-2	0 (0.0%)*	1 (0.3%)	2 (0.6%) ^{\$}	3 (0.3%)[%]
DiaSorin LIAISON® SARS-CoV-2 S1/S2 IgG	6 (1.2%)*	5 (1.7%)	9 (2.5%) ^{\$&}	20 (1.7%)[%]

Table 2. Values for Specificity, Sensitivity, Positive-Predictive-Value (PPV) and Negative-Predictive-Value (NPV) at 1%, 5% and 10% SARS-CoV-2 seroprevalence (SP) with 95% confidence intervals (95% CI).

	Abbott SARS-CoV-2 IgG		Roche Elecsys® Anti-SARS-CoV-2		DiaSorin LIAISON® SARS-CoV-2 S1/S2 IgG	
Statistic	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	84.6%	73.6-92.4	89.2%	79.1-95.6	83.1%	71.3-91.2
Specificity	99.2%	98.6-99.7	99.7%	99.2-100	98.3%	97.3-98.9
1% Seroprevalence						
<i>PPV</i>	52.3%	36.2-67.9	77.6%	52.8-91.5	32.6%	23.6-43.1
<i>NPV</i>	99.9%	99.7-99.9	99.9%	99.8-100	99.8%	99.7-99.9
5% Seroprevalence						
<i>PPV</i>	85.1%	74.7-91.7	94.8%	85.3-98.3	71.6%	61.7-79.8
<i>NPV</i>	99.2%	98.6-99.5	99.4%	98.9-99.7	99.1%	98.5-99.5
10 % Seroprevalence						
<i>PPV</i>	92.3%	86.2-95.9	97.4%	92.5-99.2	84.2%	77.3-89.3
<i>NPV</i>	98.3%	97.0-99.0	98.8%	97.6-99.4	98.1%	96.8-98.9

Table 3. Inter-rater agreement (Cohen kappa) with linear weights. Value of Kappa <0.20 poor agreement, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 good agreement, and 0.81-1.00 very good agreement.

	Abbott			Kappa (95% CI)
Roche	NEG	POS		0.87 (0.81-0.94)
NEG	1149	9	1158 (95.0%)	Standard Error
POS	6	55	61 (5.0%)	0.032
	1155 (94.7%)	64 (5.3%)	1219	

	Abbott			Kappa (95% CI)
DiaSorin	NEG	POS		0.71 (0.62-0.79)
NEG	1131	14	1145 (93.9%)	Standard Error
POS	24	50	74 (6.1%)	0.045
	1155 (94.7%)	64 (5.3%)	1219	

	DiaSorin			Kappa (95% CI)
Roche	NEG	POS		0.76 (0.67-0.84)
NEG	1136	22	1158 (95.0%)	Standard Error
POS	9	52	61 (5.0%)	0.042
	1145 (93.9%)	74 (6.1%)		

Figure Captions

Figure 1: Specificity was determined using 1,154 serum samples taken before the circulation of SARS-CoV-2. For SARS-CoV-2 antibody tests Abbott SARS-CoV-2 IgG (A), Roche Elecsys® Anti-SARS-CoV-2 (B), and DiaSorin LIAISON® SARS-CoV-2 S1/S2 IgG (C), values of specificity samples are shown in rank order. Horizontal dotted lines mark the respective cut-offs recommended by the manufacturer and, in the case of DiaSorin, a gray zone for equivocal results. Vertical dotted lines indicate the median (*) and the 90th percentile values (**). Median and 90th percentile values of negative samples were 0.025 and 0.115 for Abbott, 0.0815 and 0.0927 for Roche, below LOD and 5.52 for DiaSorin.

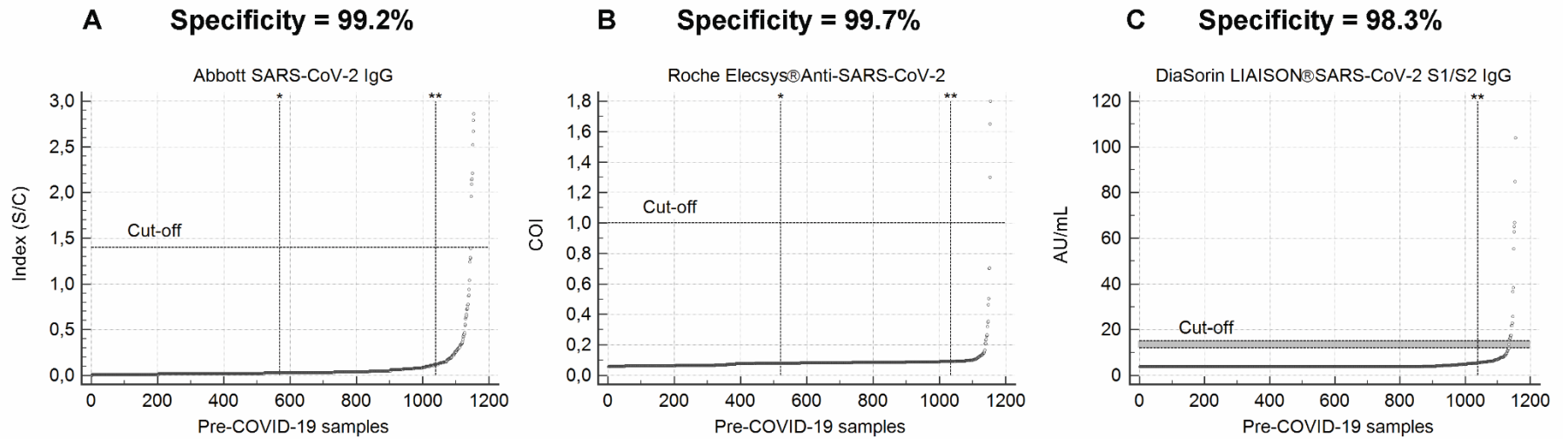
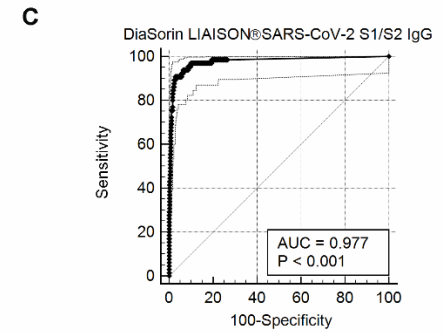
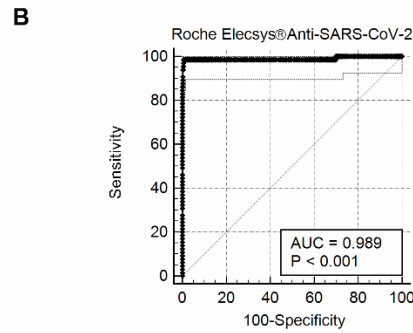
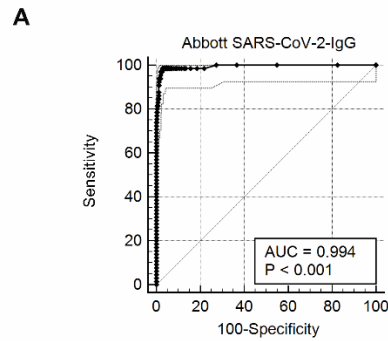


Figure 2: Receiver Operating Characteristic (ROC) curves for Abbott (A), Roche (B), and DiaSorin (C) are shown. The Area Under the Curve (AUC) indicates the test accuracy, 95% confidence intervals are represented by gray dotted lines. The tables below the ROC curves give sensitivities, specificities, and PPVs at different criterion values surrounding the cut-offs suggested by the manufacturers.



Criterion	Sensitivity	Specificity	PPV (1%)	PPV (5%)	PPV (10%)
>1.00	92.3 (83.0 - 97.5)	98.8 (98.0 - 99.3)	43.5 (31.2 - 56.5)	80.0 (70.3 - 87.1)	89.4 (83.3 - 93.5)
>1.02	90.8 (81.0 - 96.5)	98.8 (98.0 - 99.3)	43.0 (30.9 - 56.1)	79.7 (69.9 - 87.0)	89.3 (83.1 - 93.4)
>1.04	90.8 (81.0 - 96.5)	98.9 (98.1 - 99.4)	44.9 (32.0 - 58.4)	80.9 (71.1 - 88.0)	90.0 (83.8 - 93.9)
>1.12	87.7 (77.2 - 94.5)	98.9 (98.1 - 99.4)	44.0 (31.2 - 57.6)	80.4 (70.3 - 87.6)	89.6 (83.3 - 93.7)
>1.14	86.2 (75.3 - 93.5)	98.9 (98.1 - 99.4)	43.6 (30.8 - 57.2)	80.1 (69.9 - 87.5)	89.5 (83.1 - 93.6)
>1.17	84.6 (73.5 - 92.4)	98.9 (98.1 - 99.4)	43.1 (30.4 - 56.8)	79.8 (69.5 - 87.3)	89.3 (82.8 - 93.5)
>1.24	84.6 (73.5 - 92.4)	99.0 (98.2 - 99.5)	45.1 (31.7 - 59.3)	81.1 (70.7 - 88.4)	90.0 (83.6 - 94.1)
>1.28	84.6 (73.5 - 92.4)	99.1 (98.3 - 99.5)	47.3 (33.0 - 62.0)	82.4 (72.0 - 89.5)	90.8 (84.4 - 94.7)
>1.29	84.6 (73.5 - 92.4)	99.1 (98.4 - 99.6)	49.7 (34.5 - 64.8)	83.7 (73.3 - 90.6)	91.6 (85.3 - 95.3)
>1.39	84.6 (73.5 - 92.4)	99.2 (98.5 - 99.6)	52.3 (36.2 - 67.9)	85.1 (74.7 - 91.7)	92.3 (86.2 - 95.9)
>1.79	83.1 (71.7 - 91.2)	99.2 (98.5 - 99.6)	51.8 (35.7 - 67.6)	84.9 (74.3 - 91.6)	92.2 (86.0 - 95.8)
>1.89	81.5 (70.0 - 90.1)	99.2 (98.5 - 99.6)	51.4 (35.3 - 67.2)	84.6 (74.0 - 91.4)	92.1 (85.7 - 95.7)
>1.96	81.5 (70.0 - 90.1)	99.3 (98.6 - 99.7)	54.3 (37.1 - 70.5)	86.1 (75.5 - 92.6)	92.9 (86.6 - 96.3)

Criterion	Sensitivity	Specificity	PPV (1%)	PPV (5%)	PPV (10%)
>0.448	95.4 (87.1 - 99.0)	99.4 (98.8 - 99.8)	61.4 (43.1 - 76.9)	89.2 (79.8 - 94.6)	94.6 (89.3 - 97.3)
>0.464	95.4 (87.1 - 99.0)	99.5 (98.9 - 99.8)	65.0 (45.4 - 80.5)	90.6 (81.3 - 95.6)	95.3 (90.2 - 97.8)
>0.504	95.4 (87.1 - 99.0)	99.6 (99.0 - 99.9)	69.0 (48.1 - 84.2)	92.1 (82.8 - 96.5)	96.1 (91.1 - 98.3)
>0.575	93.9 (85.0 - 98.3)	99.6 (99.0 - 99.9)	68.6 (47.7 - 84.0)	91.9 (82.6 - 96.5)	96.0 (90.9 - 98.3)
>0.701	93.9 (85.0 - 98.3)	99.7 (99.1 - 99.9)	73.2 (50.6 - 87.9)	93.4 (84.2 - 97.4)	96.8 (91.9 - 98.8)
>0.705	93.9 (85.0 - 98.3)	99.7 (99.2 - 99.9)	78.5 (54.0 - 91.9)	95.0 (86.0 - 98.3)	97.6 (92.8 - 99.2)
>0.812	90.8 (81.0 - 96.5)	99.7 (99.2 - 99.9)	77.9 (53.2 - 91.6)	94.8 (85.5 - 98.3)	97.5 (92.6 - 99.2)
>0.893	89.2 (79.1 - 95.6)	99.7 (99.2 - 99.9)	77.6 (52.7 - 91.5)	94.8 (85.3 - 98.2)	97.4 (92.5 - 99.2)
>1.120	87.7 (77.2 - 94.5)	99.7 (99.2 - 99.9)	77.3 (52.3 - 91.4)	94.7 (85.1 - 98.2)	97.4 (92.3 - 99.1)
>1.190	86.2 (75.3 - 93.5)	99.7 (99.2 - 99.9)	77.0 (51.8 - 91.2)	94.6 (84.9 - 98.2)	97.4 (92.2 - 99.1)
>1.300	86.2 (75.3 - 93.5)	99.8 (99.4 - 100.0)	83.4 (55.6 - 95.3)	96.3 (86.7 - 99.1)	98.2 (93.2 - 99.6)
>1.650	86.2 (75.3 - 93.5)	99.9 (99.5 - 100.0)	90.9 (58.5 - 98.6)	98.1 (88.0 - 99.7)	99.1 (94.0 - 99.9)
>1.800	86.2 (75.3 - 93.5)	100.0 (99.7 - 100.0)	100.0	100.0	100.0

Criterion	Sensitivity	Specificity	PPV (1%)	PPV (5%)	PPV (10%)
>10.0	86.2 (75.3 - 93.5)	97.8 (96.7 - 98.5)	27.9 (20.7 - 36.4)	66.8 (57.6 - 74.9)	80.9 (74.2 - 86.3)
>10.6	86.2 (75.3 - 93.5)	97.8 (96.8 - 98.6)	28.7 (21.2 - 37.5)	67.7 (58.4 - 75.7)	81.5 (74.8 - 86.8)
>10.7	86.2 (75.3 - 93.5)	97.9 (96.9 - 98.7)	29.5 (21.8 - 38.6)	68.6 (59.2 - 76.6)	82.2 (75.4 - 87.4)
>10.8	86.2 (75.3 - 93.5)	98.0 (97.0 - 98.7)	30.4 (22.4 - 39.8)	69.5 (60.0 - 77.5)	82.8 (76.0 - 87.9)
>10.9	84.6 (73.5 - 92.4)	98.0 (97.0 - 98.7)	30.0 (22.0 - 39.4)	69.1 (59.5 - 77.2)	82.5 (75.6 - 87.7)
>11.5	84.6 (73.5 - 92.4)	98.1 (97.1 - 98.8)	31.0 (22.6 - 40.7)	70.0 (60.4 - 78.2)	83.1 (76.3 - 88.3)
>11.6	84.6 (73.5 - 92.4)	98.2 (97.2 - 98.9)	32.0 (23.3 - 42.1)	71.0 (61.3 - 79.1)	83.8 (77.0 - 88.9)
>11.8	84.6 (73.5 - 92.4)	98.3 (97.3 - 98.9)	33.0 (24.0 - 43.5)	72.0 (62.2 - 80.1)	84.4 (77.6 - 89.5)
>11.9	83.1 (71.7 - 91.2)	98.3 (97.3 - 98.9)	32.6 (23.6 - 43.1)	71.6 (61.7 - 79.8)	84.2 (77.3 - 89.3)
>13.5	83.1 (71.7 - 91.2)	98.4 (97.4 - 99.0)	33.8 (24.4 - 44.7)	72.6 (62.7 - 80.8)	84.9 (78.0 - 89.9)
>13.8	81.5 (70.0 - 90.1)	98.4 (97.4 - 99.0)	33.3 (24.0 - 44.2)	72.3 (62.2 - 80.5)	84.6 (77.6 - 89.7)
>14.2	80.0 (68.2 - 88.9)	98.4 (97.4 - 99.0)	32.9 (23.6 - 43.8)	71.9 (61.7 - 80.2)	84.4 (77.3 - 89.6)
>15.3	76.9 (64.8 - 86.5)	98.4 (97.4 - 99.0)	32.1 (22.9 - 42.9)	71.1 (60.7 - 79.7)	83.8 (76.5 - 89.2)