A rapid test recognizing mucosal SARS-CoV-2-specific antibodies distinguishes prodromal from convalescent COVID-19

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Highlights
A novel rapid antibody test for SARS-CoV-2 N-specific IgG was established
The rapid antibody testing is fast, cost-effective, and reliable
N-specific IgG is detectable in swab specimens during the late phase of infection
Testing of swab specimens discriminates prodromal from declining phases of COVID-19

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SUMMARY

The COVID-19 pandemic poses enormous challenges to global healthcare sectors. To prevent the overburden of medical systems, it is crucial to distinguish individuals approaching the most infectious early phase from those in the declining non-infectious phase. However, a large fraction of transmission events occur during pre- or asymptomatic phases. Especially in the absence of symptoms, it is difficult to distinguish prodromal from late phases of infection just by RT-PCR since both phases are characterized by low viral loads and corresponding high Ct values (>30).

We evaluated a new rapid test detecting IgG antibodies recognizing SARS-CoV-2 nucleocapsid protein using two commercial antibody assays and an in-house neutralization test before determining suitability for testing clinical swab material. Our analyses revealed the combination of the well-known RT-PCR and the new rapid antibody test using one single clinical nasopharyngeal swab specimen as a fast, cost-effective, and reliable way to discriminate prodromal from subsiding phases of COVID-19.

INTRODUCTION

Over 225 million people have acquired a laboratory-confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and more than 4.64 million fatalities were associated with coronavirus infectious disease 19 (COVID-19) (Dong et al., 2020). As indicated by surveillance studies (e.g. (Lau et al., 2020)) and excess mortality calculations (e.g. (Vestergaard et al., 2020)), the true numbers of infections and fatalities are certainly far higher, and the COVID-19 pandemic is far from being over. In December 2019, the SARS-CoV-2 outbreak was first recognized in the Hubei province in China (Zhu et al., 2020). On January 31, 2020, the World Health Organization (WHO) declared the outbreak a Public Health Emergency of International Concern (PHEIC) (Liu et al., 2020) and on March 11, 2020, the spread of SARS-CoV-2 fulfilled all criteria of a global pandemic.

SARS-CoV-2 and SARS-CoV-1 share several virological and clinical similarities, but the easy transmission supported by replication in the upper respiratory is a special feature of SARS-CoV-2 (Liu et al., 2020; Wang et al., 2020). Given the extent, pace, and severity of the COVID-19 pandemic, diagnostics departments around the globe struggle to provide sufficient test capacities, which often limit the possibilities to conduct a series of follow-up tests for individuals with suspected or proven SARS-CoV-2 infections. The most relevant methods for SARS-CoV-2 diagnosis are real-time reverse transcription-PCR (qRT-PCR) assays using nasopharyngeal swab specimens or other upper respiratory tract samples (Tang et al., 2020). Despite some doubts (Woloshin et al., 2020; Arevalo-Rodriguez et al., 2020), we found that the vast majority (>90%) of swab specimens are actually of sufficient quality and contain enough nucleic acids to enable the recognition of viruses such as SARS-CoV-2 (Klingen et al., 2021). An important criterion for the presence and abundance of viral RNAs present in nasopharyngeal swabs is the cycle threshold (Ct) of qRT-PCR. The Ct represents the calculated number of amplification cycles required to reach a defined fluorescence signal intensity above background that reliably documents the presence of the nucleic acid under investigation. Obviously, high viral RNA loads in clinical specimens reach this signal threshold after fewer
antibodies play an important immunological role protecting the host against virus dissemination and viral reinfections. usual targets of immunoglobulin (Ig) M and IgG antibodies are for example the SARS-CoV-2-encoded nucleocapsid protein (N) and the receptor-binding domain (RBD) of the spike protein (S). SARS-CoV-2-specific IgM and IgG responses increase approximately 2 weeks post-infection and during the first week after symptom onset. The amount of IgM positivity peaks by week 5 and then usually becomes undetectable by week 13. SARS-CoV-2-specific IgG peaks at week 4 and stabilizes at intermediate levels during a six-month observation period after a contraction phase between week 6 and 14 (Wu et al., 2020, Wu et al., 2021). Several studies showed that specific IgG antibodies are present in the blood or sera of persons who had been infected with SARS-CoV-2 (see e.g. (Amanat et al., 2020; Zhao et al., 2020; Premkumar et al., 2020)). Interestingly, recent findings suggested that, in addition to IgA, also SARS-CoV-2-specific IgG seems to be present in saliva (Isho et al., 2020). We anticipated that a positive result in a rapid antibody test (rAbT) in conjunction with a very high Ct value might be a simple, inexpensive, and rapid way to discriminate early prodromal and late subsiding phases of the SARS-CoV-2 infection and may be instrumental to support the decision making, e.g., concerning patient discharge and medical staff reemployment following SARS-CoV-2 infections. To this end, we first evaluated a rapid antibody test recognizing IgG molecules binding the nucleocapsid protein of SARS-CoV-2 applying clinical serum specimens. For comparison, two approved and certified antibody binding assays (manufactured by Diasorin and Euroimmun and an in-house in-cell-ELISA-based neutralization test (Scholer et al., 2020) was conducted. To assess the presence of IgG antibodies by the rAbT, we examined nasopharyngeal swab samples obtained from uninfected people, newly infected individuals, and persons being SARS-CoV-2-positive for more than two weeks. Our results demonstrated that it is feasible to differentiate prodromal from convalescent COVID-19 by analyzing one single clinical nasopharyngeal swab specimen.

RESULTS
Evaluation of the Senova rapid SARS-CoV-2 IgG antibody test using two commercially available antibody binding assays and an in-house neutralization test

We intended to apply a rAbT for the recognition of SARS-CoV-2-specific immune responses in mucosal samples such as clinical nasopharyngeal swab specimens. To this end, we first evaluated the overall performance of the rAbT using clinical serum specimens. For comparative purposes, we used the Euroimmun Anti-SARS-CoV-2 ELISA and the Diasorin Liaison XL SARS-CoV-2 S1/S2 IgG CMIA. According to the manufacturer’s instructions, cut-off ratios of >1.1 and >15 in the Euroimmun ELISA and the Diasorin CMIA, respectively, were considered as clear indications for the presence of SARS-CoV-2-specific IgG recognizing the S protein. Given that the Euroimmun ELISA was more frequently used in our diagnostics department at the time of the study, we applied the ELISA results to stratify and select serum samples for our analysis. We re-tested 22 ELISA-negative clinical serum specimens by CMIA and found 19 serum samples double-negative in both the ELISA and the CMIA test (ELISA ratio <0.8 and CMIA <12). When we performed the Senova rAbT, we obtained negative results for all 22 samples, indicating that the 3 CMIA-positive results were most
likely false positives (Table 1). In light of the discrepancy between ELISA and CMIA, we included an additional antibody assay, the in-cell-ELISA-based neutralization test (icNT) (Scholer et al., 2020), in our analysis to detect the presence of SARS-CoV-2-neutralizing antibodies (nAb). We examined 18 ELISA-positive serum specimens also by CMIA, icNT, and rAbT. In addition, we included two of the three ELISA-negative, but CMIA-positive samples (see Table 1). Fifteen samples were double-positive in both IgG assays (Table 2). The ELISA and the CMIA showed concordant results in 85% (34/40) of cases (Tables 1 and 2). The icNT analysis of the 20 clinical samples that were positive in at least one antibody assay revealed that all samples double-positive in both ELISA and CMIA were also positive in icNT, whereas all samples with discrepant antibody assay results were negative in icNT (Table 2). Notably, an evaluation of the 20 clinical specimens by rAbT yielded high consistency with the icNT results (Table 2). The outcome of rAbT assessment was even more concordant with icNT than the results of ELISA and CMIA, indicating that the Senova rAbT has a very good diagnostic performance.

### IgG antibodies to SARS-CoV-2 nucleocapsid protein in the nasopharyngeal swabs of individuals during the late phase of infection are detectable by rAbT

Our aim was to develop a simple method enabling us to distinguish the early prodromal phase from the late declining phase in individuals whose clinical swab specimens exhibited high Ct values in PCR-based SARS-CoV-2 diagnostics. To begin with, we tested if IgA and/or IgG recognizing S or N of SARS-CoV-2 were present in a small number of nasopharyngeal swab specimens of convalescent individuals applying commercially available sandwich ELISAs recognizing IgA and IgG binding to N and S. The sample size was rather small, but the results suggested that N-specific IgG is present rather frequently in nasopharyngeal swabs (data not shown). Given above-described performance of the rAbT and the consistency between rAbT and

### Table 1. Excellent specificity of the Senova rAbT

| ELISA | CMIA | rAb Test |
|-------|------|----------|
| 0.11  | <3.8 | –        |
| 0.12  | <3.8 | –        |
| 0.12  | 4.56 | –        |
| 0.13  | <3.8 | –        |
| 0.13  | <3.8 | –        |
| 0.14  | <3.8 | –        |
| 0.15  | <3.8 | –        |
| 0.15  | <3.8 | –        |
| 0.15  | <3.8 | –        |
| 0.15  | 4.05 | –        |
| 0.16  | <3.8 | –        |
| 0.16  | 18.1 | –        |
| 0.16  | <3.8 | –        |
| 0.17  | <3.8 | –        |
| 0.17  | <3.8 | –        |
| 0.17  | <3.8 | –        |
| 0.18  | <3.8 | –        |
| 0.19  | <3.8 | –        |
| 0.23  | <3.8 | –        |
| 0.25  | 18.3 | –        |
| 0.26  | <3.8 | –        |
| 0.30  | 29.1 | –        |

Twenty-two Euroimmun Anti-SARS-CoV-2 ELISA-negative clinical serum specimens were re-tested concerning the presence of SARS-CoV-2-specific IgG antibodies using the assays Diasorin Liaison XL SARS-CoV-2 S1/S2 IgG CMIA and Senova rAbT. Positivity was defined by ELISA ratios >1.1 and CMIA AU/ml > 15. Negativity was defined by ELISA ratios <0.8 and CMIA AU/ml values < 12. The Senova rAbT was evaluated by absence or presence of the test line.
icNT (Table 2), we tested if we can apply mucosal nasopharyngeal swab material to the rAbT to recognize individuals who had already raised a specific immune response against SARS-CoV-2. To this end, we selected 45 clinical nasopharyngeal swab specimens with positive RT-PCR results based on which the initial SARS-CoV-2 infection was diagnosed, indicating that these samples correspond to early infection events. In addition, we included 45 SARS-CoV-2 RT-PCR-positive swab specimens derived from 34 individuals who had acquired SARS-CoV-2 at least two weeks earlier. Both the group of newly infected and the group of persons infected for at least 2 weeks comprised samples with Ct values exceeding 30, although the mean values of these two groups differ significantly (Figure 1A). This demonstrates that the individual Ct values decline during the course of infection. However, the individual Ct values are insufficient to unambiguously define that a person has progressed to post-infectious period. This also applies to the group of individuals in the late stage of infection: There was no correlation between Ct value and time after the first positive RT-PCR result (Figure 1B).

When we determined the suitability of the rAbT for testing clinical nasopharyngeal swab material, we included as control samples swab specimens derived from 42 SARS-CoV-2-uninfected individuals, defined by negative SARS-CoV-2 RT-PCR results and the absence of indications of previous COVID-19 episodes. All of the 42 uninfected individuals showed negative rAbT results (Figure 2A). Furthermore, we applied the rAbT to above-described 45 RT-PCR-positive swab specimens of newly infected individuals. All of these 45 specimens were negative in rAbT (Figure 2A), suggesting that these donors did not have time to develop a SARS-CoV-2-specific IgG response. Conversely, when we tested the 45 SARS-CoV-2 RT-PCR-positive swab specimens derived from individuals infected since 2-8 weeks, 33 specimens clearly showed N-specific antibodies recognizable by the rAbT (Figure 2A). Six samples showed results, which were evaluated as intermediate rAbT-positive because the test line was visible with a weak appearance, and six samples were rAbT-negative (Figure 2A). These findings reveal that most individuals in the late phase of infection

| ELISA | CMIA | icNT | rAb Test |
|-------|------|------|----------|
| 0.25  | 18.3 | nn   | –        |
| 0.30  | 29.1 | nn   | –        |
| 1.13  | <3.8 | nn   | –        |
| 1.13  | 47.8 | neutralizing | +/− |
| 1.18  | 21.6 | neutralizing | +       |
| 1.25  | 9.02 | nn   | –        |
| 1.31  | 15.3 | neutralizing | +       |
| 1.77  | 27.9 | neutralizing | +       |
| 2.19  | 48.7 | neutralizing | +/− |
| 2.43  | 36.4 | neutralizing | +/− |
| 2.93  | 62.7 | neutralizing | +       |
| 3.04  | 26.1 | neutralizing | +       |
| 3.50  | 42.4 | neutralizing | +       |
| 4.06  | <3.8 | nn   | –        |
| 4.34  | 57.6 | neutralizing | +/− |
| 4.90  | 146  | neutralizing | +       |
| 4.91  | 66.1 | neutralizing | +       |
| 5.33  | 39   | neutralizing | +       |
| 6.41  | 139  | neutralizing | +       |
| 9.92  | 20.8 | neutralizing | +       |

Twenty clinical serum specimens were tested concerning the presence of SARS-CoV-2-specific IgG antibodies using the approved and certified antibody binding assays Euroimmun Anti-SARS-CoV-2 ELISA and Diasorin Liaison XL SARS-CoV-2 S1/S2 IgG CMIA. Additionally, icNT and the Senova rAbT were performed. Positivity was defined by ELISA ratios >1.1 and CMIA AU/ml values > 15. Negativity was defined by ELISA ratios <0.8 and CMIA AU/ml values < 12. The rAbT was evaluated as negative (‘−’), positive (‘+’), and intermediate positive (‘+/−’), based on the presence or absence of the test line. Abbreviations: nn, non-neutralizing.
exhibited IgG antibodies to SARS-CoV-2 N which can be detected in clinical nasopharyngeal swab specimens by rAbT. Accordingly, classification into infectious and non-infectious individuals based on a combination of high Ct value (e.g., >30) and positive rAbT appear reliable, whereas assessment based only on Ct values has the potential to be misleading.

The 9 newly infected individuals who had a Ct value above 30 were most likely in their prodromal phase, as indicated by the absence of N-specific antibodies, and might have proceeded to higher viral loads briefly after. In the group of individuals infected for 2-8 weeks, 14 swab specimens had a Ct value under 30. Here, the rAbT was positive in 71.4% of cases (10/14), in 14.3% (2/14) the rAbT was negative or intermediate positive (Figure 2B). When we grouped the samples according to the rAbT result, there was no significant difference between the groups in terms of post-infection time (as indicated by days since the first positive RT-PCR), although a tendency to a shorter post-infection period was observed for the rAbT-negatives (Figure 2C). Of these 6 rAbT-negative specimens, 3 samples belonged to individuals who were tested more than once. In these 3 cases, additional clinical swab samples were obtained 2-7 days after the rAbT-negative sampling, all of which tested positive in rAbT (Table 3), suggesting that the rAbT-negative sample collection was conducted just before antibodies developed. Of the 3 remaining rAbT-negative specimens, 2 individuals were known to have underlying conditions (Table 3). One patient was treated with corticosteroids, the other suffered from a B cell lymphoma and received chemotherapy. Remarkably, the B cell lymphoma patient exhibited high virus burden (Ct 15.7) even after 22 days of infection. Similar cases have been described previously (Avanzato et al., 2020). For the last rAbT-negative specimen (Ct 38.31, day 38 after first positive RT-PCR), the absence of virus-controlling antibodies may account for a very long period of RT-PCR positivity and could be indicative for an undiagnosed underlying condition. These data (Table 3) corroborate the validity of the rAbT results and provide evidence for the suitability of the rAbT for diagnostics.

Taken together, our analysis revealed the combination of positive rAbT in conjunction with low but positive RT-PCR (e.g., Ct > 30) conducted on the basis of a single clinical nasopharyngeal swab specimen as fast, cost-effective, and reliable way to discriminate prodromal from subsiding phases of COVID-19 and to distinguish increasing from decreasing trends of SARS-CoV-2 virus burdens.

**DISCUSSION**

We established and validated a rAbT to assess the presence of SARS-CoV-2 N-specific IgG antibodies in clinical serum samples and nasopharyngeal swab specimens. Using SARS-CoV-2-positive and -negative serum samples, we first demonstrated that the Senova rAbT correlates very well with the approved and
certified antibody binding assays Euroimmun Anti-SARS-CoV-2 ELISA and Diasorin Liaison XL SARS-CoV-2 S1/S2 IgG CMIA (Amanat et al., 2020; Zhao et al., 2020; Premkumar et al., 2020). In 100% of cases, the rAbT showed consistent results with the ELISA and the CMIA double-positive serum samples and the icNT analyses. All samples only single-positive in the ELISA or the CMIA were rAbT- and icNT-negative. Thus, the result of the rAbT assessment matched even better with the icNT than the results of ELISA and CMIA. Therefore, we concluded that the IgG antibody levels required for a positive rAbT result seem to correlate

Figure 2. IgG antibodies to SARS-CoV-2 N are detectable by rAbT in the nasopharyngeal swabs of individuals during the late phase of infection

For a Figure360 author presentation of this figure, see https://doi.org/10.1016/j.isci.2021.103194.

(A) In total, 132 nasopharyngeal swab specimens (derived from 42 uninfected, 45 newly infected and 45 individuals infected since 2-8 weeks) were tested concerning the presence of IgG antibodies to SARS-CoV-2 N using the Senova rAbT. Negative results are marked as ‘rAbT-‘, intermediate positives as ‘rAbT+/-‘, and rAbT positives as ‘rAbT+‘. The relative numbers of test outcomes are depicted as bars complemented by the absolute numbers. The ‘rAbT+‘, ‘rAbT+/-‘, and ‘rAbT-‘ samples of the group of individuals infected for 2-8 weeks were subgrouped and analyzed regarding the Ct value (B) and time post first positive RT-PCR (C).

Table 3. Details to the rAbT-negative specimens of individuals infected since 2-8 weeks

| Sample | Ct    | rAbT | Days post 1st pos. RT-PCR | Comment                      |
|--------|-------|------|---------------------------|------------------------------|
| 1      | 30.38 | –    | 14                        | rAbT+ at day 16 pos. RT-PCR  |
| 2      | 25.57 | –    | 20                        | rAbT+/- at day 22 pos. RT-PCR |
| 3      | 39.10 | –    | 21                        | rAbT+/- at day 28 pos. RT-PCR |
| 4      | 36.07 | –    | 16                        | Corticosteroids              |
| 5      | 15.70 | –    | 22                        | B cell lymphoma, CTx         |
| 6      | 38.31 | –    | 38                        | No known underlying conditions|

The 6 rAbT-negative specimens of the group of individuals infected since 2-8 weeks were analyzed in terms of possible causes for the negative rAbT outcome. Available data are listed.
very well with the neutralization capacities and that the Senova rAbT has a very good diagnostic performance.

Based on recent findings suggesting the presence of SARS-CoV-2-specific IgG at the nasopharyngeal mucosa (e.g., Isho et al., 2020), we suspected that clinical swab specimens used for RT-PCR-based SARS-CoV-2 diagnostics might also contain sufficient N-specific IgG to be suitable for antibody tests. Application of the rAbT faithfully discriminated samples derived from individuals who were in the prodromal early phase of COVID-19 from samples derived from late phases of SARS-CoV-2 infections when humoral immune response already raised N-specific IgG recognizable by the rAbT. Indeed, we found that a high frequency of nasopharyngeal swab samples derived from donors who were shedding low levels of SARS-CoV-2 genomes at least two weeks post primary infection contain rAbT-reactive N-specific IgG, whereas the specimens either derived from uninfected individuals or individuals who were infected very recently remained rAbT-negative. Thus, rAbT positivity can provide additional information and add a layer of safety concerning the decision if individuals with low virus doses (Ct > 30) are currently in the increasing or the decreasing phase concerning viral burden. Obviously, this discrimination is particularly relevant during pre-symptomatic phases of infection and in the case of asymptomatic courses when symptoms cannot be used as a reference. The latter is in fact also important given that a considerable fraction of people acquire a SARS-CoV-2 infection without experiencing symptoms (Buitrago-Garcia et al., 2020; Wu et al., 2021). Additionally, modeling studies suggest that approximately half of all infections originate from individuals without symptoms (Johansson et al., 2021). Thus, pre- and asymptomatic infections are an urgent issue which needs to be correctly diagnosed.

Following the evaluation of the rAbT, we demonstrated that of 45 SARS-CoV-2 RT-PCR-positive samples from individuals, who had acquired the virus 2-8 weeks earlier, 86.6% had a positive or intermediate positive result in rAbT. In comparison, most studies agree that IgG antibodies to SARS-CoV-2 spike and RBD antigens are detected in the blood in more than 90% of cases by 10-11 days post-symptom onset (e.g., Amanat et al., 2020; Long et al., 2020). Thus, rather similar results were evident here. The small difference may be explained by the assumption that IgG antibody responses may be detected a bit earlier or may be more abundant in the blood compared to the nasopharyngeal mucosa.

In conclusion, our study provides evidence that the Senova rapid SARS-CoV-2 IgG antibody test is an easy and cost-effective way to diagnose IgG antibodies in the nasopharyngeal swab specimens of individuals during the late phase of infection. The entire rAbT can be processed in less than 20 min. The fact that the identical clinical nasopharyngeal swab specimens can be used for the RT-PCR as well as the rAbT is clearly an advantage. It saves time for clinicians, laboratory staff and patients, because no further sampling is necessary. In contrast to automated diagnostic IgG tests or ELISA that require plate readers, the rapid antibody test does not require equipment and can be processed under a laminar airflow workbench. Thus, undiluted and non-inactivated specimens can be examined safely. In addition, the test is inexpensive (in our case, approx. 5 € per test), ready-to-use and easy to handle. Taken together, the rAbT may have a diagnostic value and may support clinical decision-making.

**Limitations of the study**

In our study, we analyzed 132 nasopharyngeal swab specimens derived from 42 uninfected, 45 newly infected, and 45 individuals infected since 2-8 weeks. Although our cohort showed clear differences among the groups, the number of samples is rather limited for a clinical cohort.

Because neutralizing antibodies are not determined by the rAbT, we would like to emphasize that we do not claim that rAbT-positive individuals are immune to SARS-CoV-2. Instead, our test discriminates prodromal from late phases of infection. We are convinced that a very high CT value in combination with a positive rapid antibody test reduces the likelihood that the person sheds infectious SARS-CoV-2.

**STAR METHODS**

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- RealStar SARS-CoV-2 RT-PCR
- SARS-CoV-2 in-cell-ELISA-based neutralization test (icNT)
- Senova rapid SARS-CoV-2 IgG antibody test

QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103194.

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AUTHOR CONTRIBUTIONS

F. K., L. S., B. K., and V. T. K. L.-T did research. A. H., O. E. A., C. E., R. S. R., U. D. collected, provided, and curated clinical specimens and corresponding information. F. S. and P. M. developed the Senova rAbT. F. K., L. S., B. K., V. T. K. L.-T., and M. T. analyzed data. F. K., V. T. K. L.-T., and M. T. wrote the manuscript. All authors read, edited, and approved the manuscript.

DECLARATION OF INTERESTS

F. K., L. S., B. K., A. H., O. E. A., C. E., R. S. R., U. D., P. M., V. T. K. L.-T. declare no competing interests. F. S. is an employee of Senova, the distributor of the rAbT used in this study. M. T. received research donations not related to the current project and preceding herein described experiments from fzmib.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| SARS-CoV-2 Nucleocapsid antibody (SARS-CoV-2 N) | Bioss | Cat# ABIN6952435; RRID:AB_2890255 |
| Bacterial and virus strains |        |            |
| SARS-CoV-2 isolate | clinical sample | N/A |
| Biological samples  |        |            |
| serum and swab specimens | diagnostic procedure | N/A |
| Critical commercial assays |        |            |
| Euroimmun Anti-SARS-CoV-2 ELISA (S) | Euroimmun | Cat# EI2606-9601G |
| Diasorin Liaison® XL SARS-CoV-2 S1/S2 IgG CMIA | Diasorin | Cat# 311451 |
| RealStar® SARS-CoV-2 RT-PCR kit | Altona | Cat# 821015 |
| Rapid SARS-CoV-2 IgG antibody test (Corona Pro-IgG Kassetten) | Senova | Cat# 2.13.1.541 |
| Experimental models: Cell lines |        |            |
| African green monkey kidney Vero E6 cells | ATCC | CRL-1586 |
| Software and algorithms |        |            |
| MicroWin (Mithras2 LB 943) | Berthold Technologies | N/A |
| GraphPad Prism | GraphPad Software Inc. | https://www.graphpad.com |

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Mirko Trilling (mirko.trilling@uk-essen.de).

Materials availability
No new materials have been generated. All reagents are commercially available and all protocols are either presented here or are publicly available e.g., in previously published open-access papers. Technical questions should be directed and will be answered by the Lead Contact, Mirko Trilling (mirko.trilling@uk-essen.de).

Data and code availability
The published article includes all data sets generated or analyzed in this study. This study did not generate new algorithms.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cells, viruses, and infection
Vero E6 cells (ATCC CRL-1586) were cultivated in high glucose Dulbecco’s minimal essential medium (DMEM [Gibco 41966-029]), supplemented with 10% (v/v) FCS, penicillin, and streptomycin at 37°C in an atmosphere of 5% CO2. SARS-CoV-2 was isolated from a patient sample using Vero E6 and confirmed by SARS-CoV-2 diagnostic qRT-PCR. Viral titers were determined by TCID50 titration.

Swab and serum specimens
The collection of serum and swab specimens was part of the routine diagnostic procedure.
Ethics
The assessment of test samples for the improvement of diagnostic procedures and the virus isolation have been approved by the ethics committee of the medical faculty of the University of Duisburg-Essen (20-9208-BO, 20-9511-BO, and 20-9512-BO).

METHOD DETAILS

**Euroimmun Anti-SARS-CoV-2 ELISA**
The Euroimmun Anti-SARS-CoV-2 ELISA was conducted in accordance to the recommendations of the manufacturer (Euroimmun, Lübeck, Germany). If the ratio exceeded 1.1, the test was considered to be positive. Results below 0.8 were judged to be negative. Borderline samples (ELISA ratio between 0.8 and 1.1) were excluded.

**Diasorin Liaison® XL SARS-CoV-2 S1/S2 IgG CMIA**
The Diasorin Liaison® XL SARS-CoV-2 S1/S2 IgG CMIA was conducted in accordance to the recommendations of the manufacturer (Diasorin, Saluggia, Italy). RLU were converted to arbitrary units (AU/ml) based on a standardized master curve. If the value was >15 AU/ml, the test was considered to be clearly positive. Values below 12 AU/ml indicated the absence of recognizable SARS-CoV-2-specific IgG antibodies binding to S1/S2.

**RealStar® SARS-CoV-2 RT-PCR**
For direct SARS-CoV-2 detection, the RealStar® SARS-CoV-2 RT-PCR kit (Altona, Hamburg, Germany), which targets the SARS-CoV-2 genes S and E, was used.

**SARS-CoV-2 in-cell-ELISA-based neutralization test (icNT)**
The SARS-CoV-2 icNT was recently described in an open access journal including a detailed laboratory protocol (Scholer et al., 2020). For the analysis of neutralizing antibodies, serum samples were inactivated at 56°C for 30 min. Briefly, defined doses of SARS-CoV-2 were incubated with different serum dilutions for 1 h at 37°C prior to Vero E6 infection in a 96-well plate. At 16 to 24 h p. i., cells were fixed with 4% (w/v) paraformaldehyde/PBS. Cells were permeabilized with 1% (v/v) Triton-X-100/PBS and blocked with 3% (v/v) FCS/PBS. The primary antibody was added and incubated for 2 h at room temperature or overnight at 4°C. Peroxidase-labelled secondary antibody was incubated for 1 to 2 h. Washing steps were performed with 0.05% (v/v) Tween-20/PBS. Tetramethylbenzidin (TMB) substrate was added to visualize the enzyme reaction. The reaction was stopped with 0.5 M HCl. Subsequently, the absorbance was measured using a microplate multireader (Mithras2 LB 943; Berthold Technologies, Bad Wildbad, Germany). An anti-N mAb (ABIN6952435) and POD-coupled secondary antibodies (Dianova) were used.

**Senova rapid SARS-CoV-2 IgG antibody test**
To perform the rapid test, 10 µl of the virus transport medium (VTM) derived from the clinical nasopharyngeal swab specimens was pipetted onto the IgG antibody test strip. Subsequently, 2 drops of the buffer from the kit were added to the strip. The test was evaluated after 15 minutes. Positive, negative and intermediate results could be distinguished, whereby the test line was visible with a weak appearance in the case of intermediate.

**QUANTIFICATION AND STATISTICAL ANALYSIS**
Quantification of optical density was performed by use of the microplate multireader Mithras2 LB 943 and MicroWin software (Berthold Technologies). The resulting data were analyzed using GraphPad Prism software. Statistical significance was determined using Student’s t-test. A p-value of <0.05 was considered statistically significant.