

Performance of Severe Acute Respiratory Syndrome Coronavirus 2 Antibody Assays in Different Stages of Infection: Comparison of Commercial Enzyme-Linked Immunosorbent Assays and Rapid Tests

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We comparatively assessed sensitivities and specificities of 4 commercial enzyme-linked immunosorbent assays (ELISAs) and 2 rapid tests in 77 patients with polymerase chain reaction–confirmed severe acute respiratory syndrome coronavirus 2 infection, grouped by interval since symptom onset. Although test sensitivities were low (<40%) within the first 5 days after disease onset, immunoglobulin (Ig) M, IgA, and total antibody ELISAs increased in sensitivity to >80% between days 6 and 10 after symptom onset. The evaluated tests (including IgG and rapid tests) provided positive results in all patients at or after the 11th day after onset of disease. The specificities of the ELISAs were 83% (IgA), 98% (IgG), and 97% (IgM and total antibody).

Keywords. SARS; coronavirus; antibodies; immunoassay.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new betacoronavirus, is currently causing a massive pandemic with severe consequences for the health-care systems worldwide [1, 2]. Although polymerase chain reaction (PCR)–based tests quickly became the cornerstone of SARS-CoV-2 diagnosis, the potential of antibody tests has not been comprehensively evaluated. Depending on respective infection stages, antibody assays could nonetheless significantly complement PCR-based testing [3, 4].

Multiple commercial enzyme-linked immunoassays and rapid tests (lateral flow immunoassays) have recently become available, but their diagnostic ability has to be thoroughly evaluated and compared before they can be widely used in the clinical setting [5, 6]. In the current study, we compared the diagnostic

ability of 4 enzyme-linked immunosorbent assays (ELISAs), which assess SARS-CoV-2–specific antibodies of different immunoglobulin (Ig) classes (Euroimmun SARS-CoV-2 IgA and IgG and Wantai SARS-CoV-2 IgM and total antibody), and 2 rapid tests (Wantai SARS-CoV-2 Ab Rapid Test and Hangzhou AllTest Biotech 2019-nCoV IgG/IgM Rapid Test) in 77 patients with symptomatic SARS-CoV-2 infection.

METHODS

Patients and Samples

The study included serum/plasma samples from 77 symptomatic patients with acute SARS-CoV-2 infection (29 female, 48 male, median age, 63 years; age range, 15–92 years; 1 sample per patient) diagnosed by means of positive PCR from nasopharyngeal swab/respiratory secretion samples. In addition to swab/respiratory secretion samples, these serum/plasma samples were sent to the Center for Virology of the Medical University of Vienna between 27 February and 30 March 2020 for diagnostic testing and were subgrouped for this study by the interval between blood sample collection and initial onset of symptoms, as reported by the patients.

Occurrence of symptoms was evaluated using a study protocol based on the World Health Organization guidelines for diagnosing coronavirus disease 2019. The majority of patients reported fever, cough, headache, and general weakness. Notably, many patients did not have the subjective feeling of dyspnea, although hypoxemia was diagnosed. Written consent was obtained from the patients, and the study was approved by the ethics committee of the Medical University of Vienna (approval nos. EK 2156/2019 and EK 2283/2019).

Controls

Serum samples from 100 individuals without SARS-CoV-2 infection (60 female, 40 male; median age, 49 years; range, 2–93 years) served as controls. They comprised (1) symptomatic individuals whose samples were obtained during the same observational period as samples from infected patients, but in whom absence of SARS-CoV-2 was confirmed by PCR-negative swab samples ($n = 30$); (2) healthy volunteers with consecutive PCR-negative swab samples ($n = 30$); (3) stored serum samples from individuals with previous PCR-confirmed coronavirus OC43 infections ($n = 10$; median interval between infection and sampling, 306 days; range, 4–1452 days) and (4) serum samples from patients with pneumonia collected before December 2019 ($n = 30$).

PCR Testing

SARS-CoV-2 nucleic acid was extracted using the NucliSens EasyMag extractor, according to the manufacturer's instructions

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(Biomérieux). SARS-CoV-2 real time TaqMan PCR was performed with World Health Organization–recommended primers and probe located in the E-gene, as described elsewhere [1]. Sensitive detection was confirmed using a proficiency panel from Instand.

Serological Assays

Anti-SARs-CoV-2 antibodies were assessed using (1) Euroimmun SARS-CoV-2 IgA and IgG ELISAs (Euroimmun), (2) Wantai SARS-CoV-2 IgM and total antibody ELISAs (Beijing Wantai Biological Pharmacy), (3) the Wantai SARS-CoV-2 Ab Rapid Test (also from Beijing Wantai), and (4) the 2019-nCoV IgG/IgM Rapid Test (Hangzhou AllTest Biotech). All tests were performed as recommended by the manufacturers [3, 4].

The Euroimmun SARS-CoV-2 IgA and IgG ELISAs use the recombinant structural protein (S1 domain) of the spike protein as antigen. The Wantai SARS-CoV-2 total antibody ELISA is based on a double-antigen sandwich principle that detects total antibodies against the SARS-CoV-2 spike protein receptor binding domain. The recombinant protein is used as the immobilized and the horseradish peroxidase–conjugated antigen. The IgM ELISA uses the same horseradish peroxidase–conjugated receptor binding domain antigen as the total antibody ELISA.

Results by Euroimmun ELISA (IgA and IgG) were classified as negative when the antibody ratio was <0.8, as borderline with a ratio from 0.8 to 1.1, and as positive with a ratio >1.1. Wantai ELISA results (IgM, total antibody) were interpreted as negative with a ratio <0.9, borderline with a ratio from 0.9 to 1.1, and positive with a ratio >1.1.

Rapid tests were filled with 10 µL of serum/plasma using a pipette and interpreted by the same laboratory-experienced person after incubation for 10 minutes (2019-nCoV IgG/IgM Rapid Test) or 15 minutes (Wantai rapid test). All performed rapid tests provided positive control bands and were considered valid. In some patients, test bands were weak (with weaker band intensities than clearly positive tests with strong bands) or very weak (bands even weaker, but still recognizable with the naked eye). All tests with (still) visible bands were considered positive.

RESULTS

Patient Characteristics and Virus Concentration

Of the 77 patients with PCR-confirmed SARS-CoV-2 infection, 30 individuals (12 female, 18 male; median age, 58 years; age range, 15–83 years) provided serum/plasma samples that were obtained at symptom onset or 1–5 days after the onset of disease (group 1). Fifteen of these patients (50%) were hospitalized owing to moderate or severe illness severity, and 15 (50%) were dismissed to home care. From 25 patients (9 female, 16 male; median age, 68 years; range, 22–92 years), serum/plasma samples were obtained between 6 and 10 days after onset of disease (group 2). Twenty-three of these patients (92%) were

hospitalized, and 2 (8%) were dismissed to home care after blood sample collection. Finally, 22 patients (4 female, 18 male, median age, 64 years, range, 26–79 years) provided a serum/plasma sample at or after day 11 after the onset of symptoms (group 3). The median interval between onset of symptoms and sample acquisition in these patients was 15 days (range, 11–29 days). Except for 1 individual (a healthcare worker identified by a screening test), blood samples were obtained from all patients during hospitalization (95.4%).

Virus concentration in nasopharyngeal swab/respiratory secretion samples differed significantly among these groups ($P < .001$; Kruskal-Wallis test), with highest concentrations in group 1 (median cycle threshold [Ct], 26.0 [range, 16.8–36.1]; median viral load, 1.1×10^6 copies/mL [8.8×10^2 to 6.2×10^8 copies/mL]), followed by individuals from group 2 (median Ct, 32.2 [18.3–36.6]; median viral load, 1.3×10^4 copies/mL [5.4×10^2 to 2.1×10^8 copies/mL]) and with the lowest concentrations in group 3 (median Ct, 34.8 [28.6–36.8]; median viral load, 2.2×10^3 copies/mL [5.4×10^2 to 1.6×10^5 copies/mL]).

Sensitivity of ELISAs

As shown in Figure 1A, the Euroimmun IgA and IgG ELISAs tested positive in 9 (30%) and 1 (3.3%) of the 30 individuals from group 1, in 21 (84%) and 10 (40%) of the 25 from group 2, and in all 22 patients (100%) from group 3. The Wantai IgM and total antibody ELISAs tested positive in 8 (26.7%) and 11 (36.7%) of the 30 individuals from group 1. Both tests provided positive results in 23 of 25 patients (92%) from group 2 and in all 22 (100%) from group 3 (Figure 1A). Individual antibody concentrations among the different groups are also shown in Figure 1A.

Sensitivity of Rapid Tests

The Wantai rapid test tested positive in 6 of 30 individuals (20%) from group 1, in 20 of 25 (80%) from group 2, and in all 22 (100%) from group 3 (Figure 1B). Of note, in group 1, 5 of 6 positive tests (83.3%) displayed only a weakly ($n = 3$) or very weakly ($n = 2$) positive band. In group 2, 12 of 20 positive tests (60%) originated from a weak ($n = 9$) or very weak ($n = 3$) band, whereas in group 3, 7 of 22 positive test results (31.8%) were weakly positive.

Using the 2019-nCoV IgG/IgM Rapid Test, 6 of the 30 (20%) patients from group 1 displayed a positive IgM and 4/30 (13.3%) a positive IgG band (all very weakly positive). Of the 25 individuals from group 2, 5 (20%) displayed weakly positive IgM and 12 (48%) clearly positive IgG bands. Of the 22 patients from group 3, 10 (45.5%) showed a weakly positive IgM band, and all 22 (100%) displayed a clearly positive IgG band.

Specificity

As shown in Figure 2, test specificities were determined in 100 non-SARS-CoV-2–infected controls. Specificities were

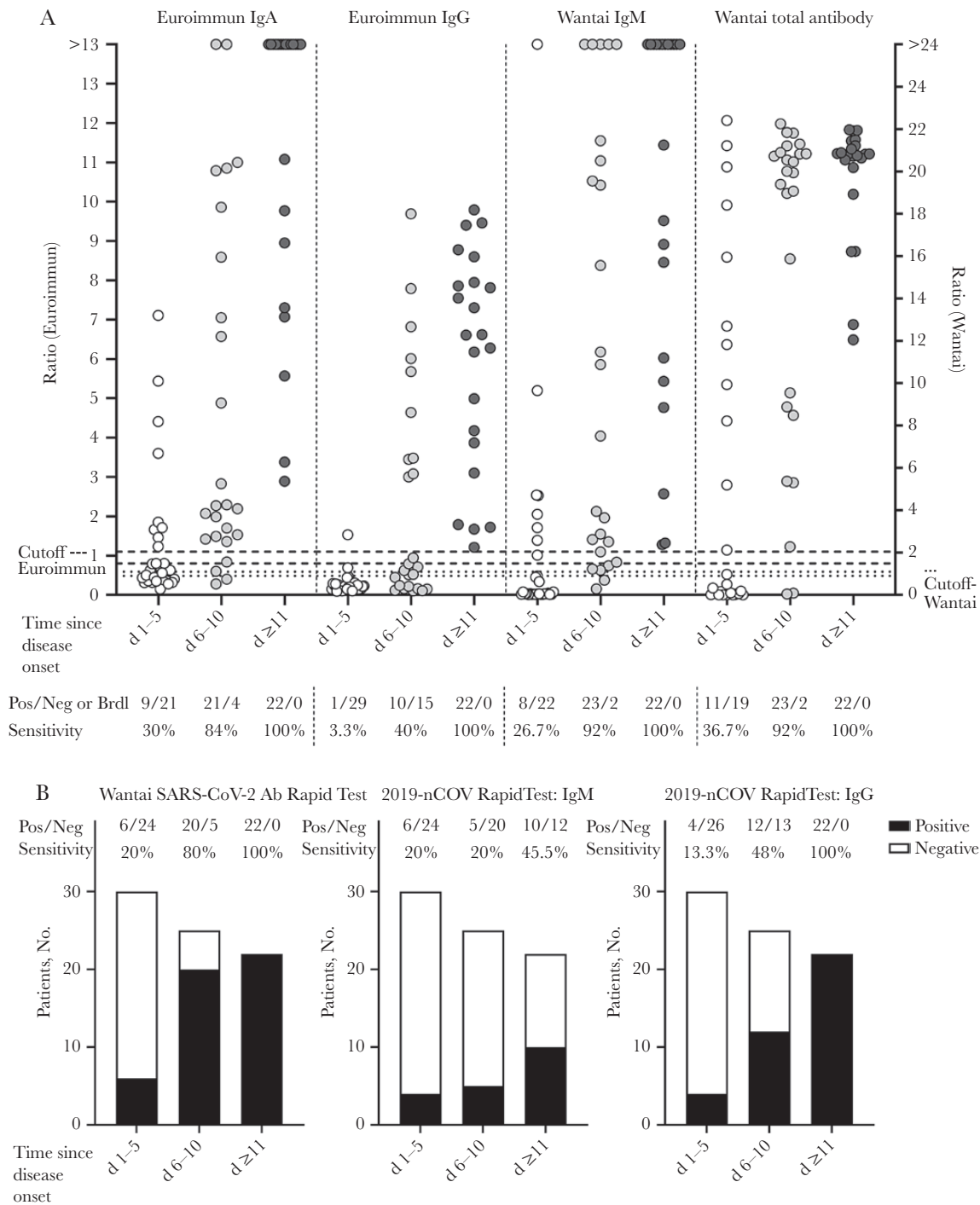


Figure 1. A, Comparison of individual antibody levels and sensitivities of enzyme-linked immunosorbent assays among 77 patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, subgrouped by the interval since onset of symptoms. B, Sensitivities of the Wantai SARS-CoV-2 Ab Rapid Test and the 2019-nCoV IgG/IgM Rapid Test (immunoglobulin [Ig] M and IgG bands; Hangzhou AllTest Biotech) in 77 patients with SARS-CoV-2 infection, grouped by interval since onset of symptoms. Abbreviations: Pos/Neg or Brdl, positive/negative or borderline results.

83% and 98% for the Euroimmun IgA and IgG and 97% for the Wantai IgM and the total antibody ELISAs, respectively (Figure 2A). The Wantai rapid test displayed a specificity of 98%, and the 2019-nCoV IgG/IgM Rapid Test a specificity of 99% for IgM and 100% for IgG, respectively (Figure 2B).

DISCUSSION

The current study provides the first comparative data on the sensitivity and specificity of 4 commercially available ELISAs and 2 rapid tests in 77 patients with SARS-CoV-2 infection. We demonstrate that the sensitivities of the evaluated

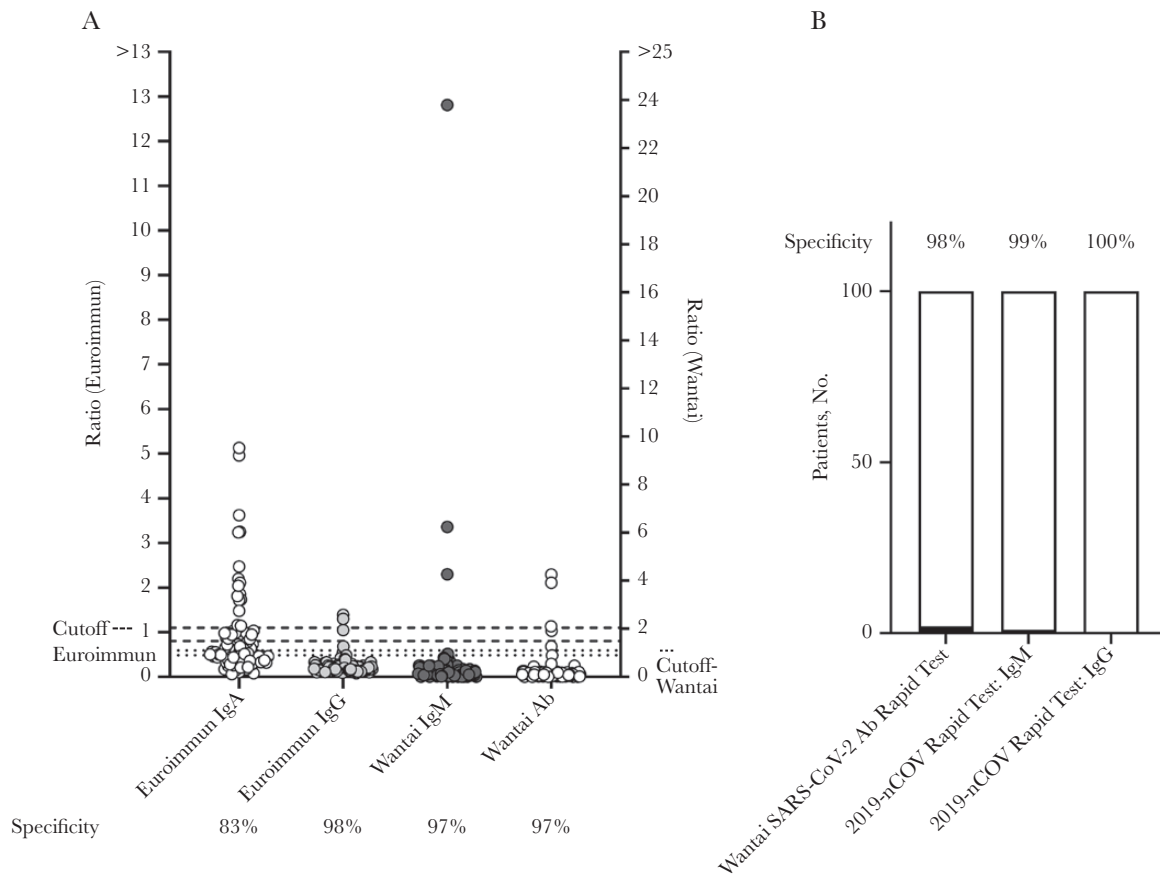


Figure 2. Specificities of the Euroimmun severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin (Ig) A and IgG and the Wantai IgM and total antibody enzyme-linked immunosorbent assays (A) as well as the Wantai SARS-CoV-2 Ab and the 2019-nCoV IgG/IgM Rapid Test (IgM and IgG bands; Hangzhou AllTest Biotech) (B), as assessed in 100 non-SARS-CoV-2-infected controls.

Anti-SARS-CoV-2 IgM and IgA ELISAs were low within 5 days after disease onset but subsequently increased to 84% for the Euroimmun IgA and 92% for the Wantai IgM ELISA between 6 and 10 days after onset of symptoms [3, 7, 8]. We furthermore observed very high sensitivities for all tests (including IgG and total antibody ELISAs) in the later phase of infection (beyond the 11th day after onset of symptoms, with a median interval of 15 days between onset of symptoms and sample acquisition). Of note, all samples from the later phase of the infection displayed significant IgM, IgA, and IgG titers (exceeding the respective cutoffs), and the majority (86%) of these samples were obtained within 21 days after the onset of disease (maximum, 29 days). However, although test specificities for IgM, IgG and total antibodies were $\geq 97\%$, specificity for the IgA assay was only 83%.

Although data provided by the study indicate that the evaluated IgM or IgA assays should not substitute for PCR-based diagnosis early after onset of symptoms, the high sensitivities we demonstrate for all evaluated tests beyond the 11th day after symptom onset highlights the possibility that these assays might significantly aid the diagnosis in later stages of infection—for example, in patients with pneumonia who have lower virus

concentrations in the upper respiratory tract (possibly causing false-negative PCR results from pharyngeal swab samples) [9].

Although we obtained comparable results for the rapid tests we evaluated, it should be considered that these tests were performed under optimal laboratory conditions (with pipetting of exact serum volumes and interpretation by an experienced laboratory technician under the same conditions), which might not necessarily reflect their ability in the point-of-care setting. Our observation, however, also indicates that rapid tests from different manufacturers may differ significantly in their diagnostic performance, especially in early stages of infection, and should therefore be particularly evaluated [1, 5].

Importantly, the majority of patients in our cohort who provided serum/plasma samples during the later phase of infection (at or beyond the 11th day after onset of disease) were hospitalized at this time point. Because antibody titers have been shown to be correlated with disease severity, the sensitivity of the evaluated tests could thus differ significantly in individuals with mild or asymptomatic courses of infection, calling for further studies with asymptomatic individuals and patients with mild disease [3, 7]. High antibody levels, which we observed in

this cohort of mainly hospitalized patients during the late phase of the infection, might also have affected the good test performance of the rapid tests we evaluated.

In summary, although our study has the limitation of a relatively small sample size, it nonetheless provides comparative data on the early available commercial ELISAs, indicating a high potential of the evaluated tests for SARS-CoV-2 diagnosis, especially in symptomatic patients and progressed stages of infection.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR [published correction appears in Euro Surveill 2020;25:20200409c]. *Euro Surveill* **2020**; 25:2000045.
2. Eurosurveillance Editorial Team. Note from the editors: World Health Organization declares novel coronavirus (2019-nCoV) sixth public health emergency of international concern. *Euro Surveill* **2020**; 25:200131e.
3. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clin Infect Dis* doi:10.1093/cid/ciaa344.
4. Okba NMA, Muller MA, Li W, et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients. *Emerg Infect Dis* **2020**; 26. doi:10.3201/eid2607.200841.
5. Cassaniti I, Novazzi F, Giardina F, et al. Performance of VivaDiag COVID-19 IgM/IgG Rapid Test is inadequate for diagnosis of COVID-19 in acute patients referring to emergency room department. *J Med Virol* doi:10.1002/jmv.25800.
6. Li Z, Yi Y, Luo X, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *J Med Virol* doi:10.1002/jmv.25727.
7. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* **2020**; 581:465–9.
8. Guo L, Ren L, Yang S, et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). *Clin Infect Dis* doi:10.1093/cid/ciaa310.
9. To KK, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* **2020**; 20:565–74.