



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com



Original Article

Multicenter evaluation of four immunoassays for the performance of early diagnosis of COVID-19 and assessment of antibody responses of patients with pneumonia in Taiwan



Yi-Chun Lin ^{a,1}, Yu-Lin Lee ^{b,1}, Chien-Yu Cheng ^{a,1},
Wen-Pin Tseng ^{c,1}, Jhong-Lin Wu ^c, Chien-Hao Lin ^c,
Ming-Yi Chung ^d, Chun-Min Kang ^d, Ya-Fan Lee ^d,
Cheng-Pin Chen ^a, Chien-Hua Huang ^c, Chun-Eng Liu ^b,
Shu-Hsing Cheng ^{a,**}, Shyr-Chyr Chen ^c, Shey-Ying Chen ^{c,e,***},
Po-Ren Hsueh ^{d,f,*}

^a Division of Infectious Diseases, Department of Internal Medicine, Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan, Taiwan

^b Department of Internal Medicine, Changhua Christian Hospital, Changhua, Taiwan

^c Department of Emergency Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan

^d Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan

^e Center for Quality Management, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan

^f Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan

Received 27 January 2021; received in revised form 5 February 2021; accepted 5 February 2021

Available online 23 February 2021

* Corresponding author. Departments of Laboratory Medicine and Internal Medicine, National Taiwan University College of Medicine, 7, Chung Shan South Road, Taipei 100, Taiwan.

** Corresponding author. Division of Infectious Diseases, Department of Internal Medicine, Taoyuan General Hospital, Ministry of Health and Welfare, 1492, Zhongshan Road, Taoyuan District, Taoyuan 330, Taiwan.

*** Corresponding author. Department of Emergency Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, 7, Chung Shan South Road, Taipei 100, Taiwan.

E-mail addresses: shuhsingcheng@gmail.com (S.-H. Cheng), erdrcsy@gmail.com (S.-Y. Chen), hsporen@ntu.edu.tw (P.-R. Hsueh).

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.jmii.2021.02.003>

1684-1182/Copyright © 2021, Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

KEYWORDS

COVID-19;
Immunoassays;
Antibody responses;
False positive;
Pneumonia

Abstract *Background/purpose:* Our study goals were to evaluate the diagnostic performance of four anti-SARS-CoV-2 antibodies tests and the differences in dynamic immune responses between COVID-19 patients with and without pneumonia.

Methods: We collected 184 serum samples from 70 consecutively qRT-PCR-confirmed COVID-19 patients at four participating hospitals from 23 January 2020 to 30 September 2020. COVID-19 pneumonia was defined as the presence of new pulmonary infiltration. Serum samples were grouped by the duration after symptom onset on a weekly basis for antibody testing and analysis. The four immunoassays: Beckman SARS-CoV-2 IgG/IgM (Beckman Test), Siemens (ADVIA Centaur®) SARS-CoV-2 Total (COV2T) (Siemens Test), SBC COVID-19 IgG ELISA (SBC Test) and EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research (EliA Test) were used for detecting the SARS-CoV-2 specific antibodies.

Results: The sensitivity of all tests reached 100% after 42 days of symptom onset. Siemens Test, the only test detecting total anti-SARS-CoV-2 antibodies, had the best performance in the early diagnosis of COVID-19 infection (day 0–7: 77%; day 8–14: 95%) compared to the other 3 serological tests. All tests showed 100% specificity except SBC Test (98%). COVID-19 patients with pneumonia had significantly higher testing signal values than patients without pneumonia (all *p* values < 0.05, except EliA IgM Test). However, Siemens Test and SBC Test had highest probability in early prediction of the presence of COVID-19 pneumonia.

Conclusion: Chronological analysis of immune response among COVID-19 patients with different serological tests provides important information in the early diagnosis of SARS-CoV-2 infection and prediction of the risk of pneumonia after infection.

Copyright © 2021, Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Novel coronavirus disease (COVID-19), caused by Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), has resulted in significant impacts on human health and life since 2019.^{1–4} The early identification and isolation of patients with COVID-19 are crucial to prevent the spread of the disease in the community due to the elusive clinical manifestations of COVID-19.^{5–8} In Taiwan, all real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) assay confirmed COVID-19 patients are mandatorily reported to the National Health Command Center and hospitalized in a negative pressure isolation room to prevent the transmission of SARS-CoV-2.^{3,9} However, the identification of patients with COVID-19 is challenging because of the broad spectrum of clinical manifestations, ranging from asymptomatic infection to acute respiratory distress syndrome.^{2,8} The qRT-PCR has been an important diagnostic tool for detecting SARS-CoV-2 infection. However, it is generally time-consuming and limited by the costs and the diagnostic sensitivity.^{10,11} Serological testing, an important diagnostic tool detecting responsive anti-SARS-CoV-2 antibodies, is more rapid and useful for studying the epidemiological seroprevalence of COVID-19 to obtain a more accurate estimate of the circulating dynamics and virulence of SARS-CoV-2.^{12,13}

To date, many point-of-care or fully automated immunoassays for COVID-19 diagnosis have been developed and launched.^{14–17} The performance of different serological tests should be comprehensively evaluated before its application into routine diagnostic protocols for patient management and pandemic control. Furthermore, previous studies showed a correlation between the high-level upsurge of the anti-SARSCoV-2 antibody response and tissue

injury among patients with COVID-19.^{18–20} Quantitative or semi-quantitative serological testing is therefore a potential diagnostic modality for early stratification of the risk of pulmonary involvement, in addition to retrospective diagnosis of SARS-CoV-2 infection.

The primary goals of this study were first to evaluate the performance of four serological immunoassays for the diagnosis of COVID-19. The second goal was to evaluate the dynamic immune responses among COVID-19 patients with and without pneumonia after SARS-CoV-2 infection by the four serological tests.

Materials and methods**Study design and patient enrollment**

In this retrospective, observational study, we collected 184 residual blood samples from 70 consecutively qRT-PCR-confirmed COVID-19 patients hospitalized at four participating hospitals from 23 January 2020 to 30 Sep 2020. The four participated hospitals were Tao Yuan General Hospital, Ministry of Health and Welfare, National Taiwan University Hospital, Changhua Christian Hospital and Nantou Hospital, Ministry of Health and Welfare. This study was approved by the institutional review board of all the participating hospitals, and the requirement for informed consent from each patient was waived (202003004RIND).

Clinical data collection and definitions

We retrieved the de-linked data of enrolled patients from electronic medical records of the participating hospitals. Patient data included age, sex, comorbid medical condition, date of symptom onset, initial presentation, date of

hospitalization, presence of pneumonia, intensive care unit (ICU) admission, length of hospital stay, and survival status on hospital discharge. Date of symptom onset was defined as the onset date of the first COVID-19-compatible symptom reported by patient. COVID-19 pneumonia was defined as the presence of new pulmonary infiltration on chest roentgenogram. COVID-19 patients were then classified into two groups according to the presence or absence of COVID-19 pneumonia.

Collection and grouping of serum samples

The residual blood samples of COVID-19 patients were collected from the regular medical care requirement. If a patient had multiple serum samples on the same date, only the first sample of that date was used for analysis. The serum of the collected blood samples was stored at -20°C before anti-SARS-CoV-2 antibody testing. Serum samples were further grouped on a weekly basis by the duration after symptoms onset: Day 0–7, Day 8–14, Day 15–21, Day 22–28, Day 29–35, etc. In addition, we included 200 control serum samples from 200 hospitalized patients who were tested negative ≥ 2 times using SARS-CoV-2 qRT-PCR to evaluate diagnostic specificity of the four serological tests in this study. None of these control group patients were subsequently confirmed to be COVID-19 infection after discharged from hospital.

Laboratory measurements

Four immunoassays were used for the detection of the SARS-CoV-2 specific antibodies in this study. This included Beckman SARS-CoV-2 IgG/IgM (Beckman Coulter Diagnostics, Inc., Brea, USA) (Beckman Test), Siemens (ADVIA Centaur®) SARS-CoV-2 Total (COV2T) (Siemens Healthcare Diagnostics Inc., NY, USA) (Siemens Test), SBC COVID-19 IgG ELISA (Schweitzer Biotech Company, Taipei, Taiwan) (SBC Test), and EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research (Thermo Fisher Scientific, Inc., MA, USA) (EliA Test). All the above four serological tests use the SARS-CoV-2 Spike protein (S protein) as labeling viral protein for the detection of anti-SARS-CoV-2 antibodies. Detail information of the four serological tests is summarized in Table 1.^{21–24} One experienced laboratory technician was responsible for all the serum samples preparation and testing. Test results were interpreted as positive if the signal value of the Beckman Test (S/CO) ≥ 1.0 for both IgG and IgM, the Siemens Test (index) ≥ 1.0 of total antibodies, the SBC Test (optical density [OD] ratio) ≥ 0.4 of IgG, and the EliA Test $> 20 \mu\text{g/L}$ for IgG or IgM and $> 5 \mu\text{g/L}$ for IgA, according to the manufacturers' instructions. All index values of Siemens Test > 10 were considered as 10 in the quantitative immune dynamic analysis.

Evaluation of false-positivity

Among the 200 control serum samples, any positive results by any of the four immunoassays were presumed to be falsely positive. For any sample with positive result by any of the four immunoassays, the sample was performed twice by the indicated assay. Serological tests for the presence of

cytomegalovirus (CMV) IgM/IgG antibodies and rheumatoid factor (RF) were performed to examine the possibility of cross reaction between RF or anti-CMV antibodies and anti-SARS-CoV-2 antibody as previously described.³ In addition, a Western blot analysis with *recomLine* SARS-CoV-2 IgG (MIKROGEN Diagnostik GmbH, Neuried, Germany), a line immunoassay specifically identifying IgG antibodies against the individual antigens of the nucleocapsid protein (NP) and spike protein: receptor binding domain (RBD) and S1 subunit, of SARS-CoV-2, and NPs of four seasonal human coronaviruses (HKU1, OC43, NL63, and 229E), was used as a confirmatory test to clarify the presence of anti-SARS-CoV-2 antibodies and antibodies against seasonal coronaviruses among control group patients with positive anti-SARS-CoV-2 antibody response of any of the serological tests in this study.^{25,26}

Statistical analysis

We calculated means and standard deviations (SDs) for age and serological test signal values, and percentages for the categorical variables. An independent Student's *t* test was used to compare continuous variables, whereas categorical variables were analyzed using Chi-squared or Fisher's exact tests. The diagnostic sensitivity was calculated as the percentage of serum samples from positive antibodies against SARS-CoV-2 in patients with COVID-19. The specificity was calculated as the percentage of serum samples from negative antibodies against SARS-CoV-2 in control patients. The dynamic immune responses between COVID-19 patients with and without pneumonia were further evaluated with the four serological tests. Serum samples in this section were also analyzed on a weekly basis after symptom onset. However, if a patient had multiple serum samples from different date of the same week, only the earliest serum sample of that week was used for immune dynamics analysis (week-representative sample). For example, if a patient had Day-8, Day-10, Day-13, Day-18, Day-23, Day-27 and Day-56 serum samples, only the Day-8 (representative of second week immune response), Day-18 (representative of third week immune response), Day-23 (representative of fourth week immune response), and Day-56 (representative of eighth week immune response) were used for analysis. It was to prevent the biased amplification of dynamic immune response from patients with multiple serum samples in the same week. Signal values between patients with and without pneumonia were then compared with the 4 serological tests. SPSS for Windows (IBM SPSS Statistics v26) was used for analysis. All *p*-values are two-sided, and a *p*-value < 0.05 was considered statistically significant.

Results

Clinical characteristics of patients with COVID-19

A total of 70 qRT-PCR-confirmed COVID-19 patients with 184 serum samples from different symptom onset date were finally enrolled in this study. The mean age was 42.6 years (standard deviation, 15 years). Thirty-four (48.6%) patients were men and 62 (88.57%) of them had no significant

Table 1 Information on the four immunoassays used for the diagnosis of coronavirus disease 2019 (COVID-19).

Parameter	Beckman SARS-CoV-2 IgG/IgM	Siemens (ADVIA Centaur®) SARS-CoV-2 Total (COV2T)	SBC COVID-19 IgG ELISA	EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research
Company (city, country)	Beckman Coulter Diagnostics, Inc. (Brea, USA)	Siemens Healthcare Diagnostics Inc. (Tarrytown, USA)	Schweitzer Biotech Company (Taipei, Taiwan)	Thermo Fisher Scientific, Inc. (MA, USA)
Targeting antibody	IgG and IgM (separated)	Total antibodies (including IgG and IgM)	IgG	IgG, IgM, and IgA (separated)
Immunoassay Analyzer	CLIA Beckman Coulter Access Immunoassay System (Access 2, UniCel DxI)	CLIA ADVIA Centaur® XP/XPT systems	ELISA Manual or automated ELISA workstation (Dy nex DS2, DYNEX Technologies, VA, USA)	FEIA Phadia 250
Qualitative analysis	Yes	Yes	Yes	Quantitative
Protein targeting	Receptor binding domain of the viral spike 1	Spike	Spike	Recombinant SARS-CoV-2 spike 1
Specimen type(s)	Serum, plasma	Serum, plasma	Serum	Serum, plasma
Specimen amount required	IgG: 20 µL IgM: 10 µL	50 µL	1.5 µL (manual) 5.0 µL (Dy nex DS2)	20 µL for each
Result interpretation	S/CO IgG: ≤0.8: non-reactive >0.8 - <1.0: equivocal ≥1.0: reactive IgM: <1.0: non-reactive ≥1.0: reactive	Index <1.0: nonreactive ≥1.0: reactive	OD ratio <0.3: negative 0.3≤<0.4: Inconclusive ≥0.4: positive	µg/L IgG • <14: negative • 14–20: equivocal • >20: positive IgM • >20: positive IgA • > 5: positive
Testing time	IgG: ~32 min IgM: ~37 min	18 min		114 min
Reported sensitivity or positive percent agreement (PPA) (95% CI) based on qRT-PCR results	IgG: Post qRT-PCR positive: • 0–6 days: 70.2% (56.0%–81.3%) • 7–14 days: 95.5% (88.9%–98.2%) • 14 days: 99.1% (95.0%–99.8%) • >18 days: 100% (93.8%–100%) IgM: Days post symptom onset • 0–7 days: 54.5% (34.7–73.1%) • 8–14 days, 91.7% (78.2%–97.1%) • 15–30 days: 98.3% (93.9%–99.5%)	Post qRT-PCR positive • 0–6 days: 61.05% (50.5%–70.89%) • 7–13 days: 97.50% (92.8%–99.48%) • ≥14 days: 100.00% (92.45%–100.00%)	Days post-symptom onset (PPA) • ≤ 7 days: NA • 8–14 days: NA • ≥ 15 days: 97.1% (85.5%–99.5%)	IgG Post qRT-PCR positive: • 0–8 days: 36.6% (22.1%–53.1%) • >8 days: 100.0% (85.8%–100.0%) IgM: NA IgA: NA
Reported specificity or NPA (95% CI)	IgG: • Specificity: 99.8% (99.4–99.9%) IgM: • NPA: 99.9% (99.5%–100%)	Specificity: 99.81% (99.45%–99.96%)	NPA: 100.0%	IgG: • Specificity: 100.0% (99.5%–100.0%) IgM: NA IgA: NA

(continued on next page)

Table 1 (continued)

Parameter	Beckman SARS-CoV-2 IgG/IgM	Siemens (ADVIA Centaur®) SARS-CoV-2 Total (COV2T)	SBC COVID-19 IgG ELISA	EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research
Confirmed cross-reactivity with antibodies against non-coronaviruses	IgG: NA IgM: anti-hepatitis C virus antibody	NA	NA	NA
Cross-reactivity with antibody against other coronaviruses	NA	NA	NA	No (no cross reactivity with sera from 20 patients with OC43, HKU1, 229E, or NL63 infection)
SARS and MERS				
Other seasonal coronaviruses				
Registration	CE-IVD, US FDA EUA	CE-IVD, USA FDA	No (Filed for application TFDA, CE-IVD, and US FDA)	IgG: CE-IVD IgM and IgA: no (RUO)
Reference	21	22	23	24

CE-IVD, Conformité Européenne *in vitro* diagnostic device; CLIA, chemiluminescent immunoassay; CMV, cytomegalovirus; ELISA, enzyme-linked immunosorbent assay; FEIA, fluorescence enzyme immunoassay; MERS, Middle East respiratory syndrome; NA, not available; NPA, negative percent agreement; OD, optical density; qRT-PCR, real-time reverse transcriptase-polymerase chain reaction; RUO, Research use only; SARS, severe acute respiratory syndrome; S/C, sample/control; TFDA, Taiwan Food and Drug Administration; US FDA, Food and Drug Administration of the United States.

comorbidities. Lower respiratory tract symptoms were the predominant symptom at the time of diagnosis (60.0%), followed by upper airway symptoms (52.9%), and fever (50%). Twenty-seven (38.6%) patients were diagnosed as having pneumonia during hospitalization, seven (25.9%) of them were admitted to intensive care unit and five (18.5%) required ventilator support (Table 2).

Clinical characteristics of COVID-19 patients with and without pneumonia were further compared (Table 2). COVID-19 patients with pneumonia were significantly older ($p < 0.001$) and had higher percentage of fever ($p < 0.027$) and requirement of ICU admission ($p = 0.001$) and ventilator support ($p = 0.007$). In contrast, COVID-19 patients without pneumonia were younger and had higher percentage with headache ($p = 0.022$) and upper airway symptoms ($p = 0.036$) as initial presentation.

Diagnostic performance of the four immunoassays

A total of 184 serum samples were collected from the 70 patients with SARS-CoV-2 infection at different time points after symptom onset. The number of samples collected from each individual patient ranged from one to 20 samples (median, two samples). Twenty-nine (15.8%) serum samples were collected after 42 days of symptom onset.

Of the 184 serum samples, testing for anti-SARS-CoV-2 antibodies was positive in 164 (89.1%) on using the Beckman Test, 174 (94.6%) on using the Siemens Test, 170 (92.4%) on using the SBC Test, and 164 (89.1%) on using the EliA Test. Fig. 1A shows the results of antibody responses at different time intervals after symptom onset on using the four serological tests. Siemens Test demonstrated the highest positive rate in early stage of disease course (day 0–7: 77%; day 8–14: 95%) than the other three serological tests.

For Beckman Test, which detects IgG and IgM antibody separately, IgG antibody was detected in 157 (85.3%)

samples and IgM antibody was detected in 144 (78.2%) samples, respectively. For EliA Test, which detects IgG, IgM, and IgA antibody separately, IgG antibody was detected in 154 (83.7%) samples, IgM antibody was detected in 151 (82.1%) samples and IgA antibody was detected in 133 (72.3%) samples, respectively. The class-specific antibody responses at different time intervals after symptom onset evaluated with Beckman Test and EliA Test are shown in Fig. 1B and C, respectively.

The dynamic signal values of the four immunoassays at different time intervals after symptom onset are shown in Fig. 2A–D. The IgM signal values were peaking at second week of symptom onset in Beckman Test (Fig. 2A) and at fourth week of symptom onset in EliA Test (Fig. 2D). The IgA signal values were peaking at third week of symptom onset in EliA Test (Fig. 2D).

Diagnostic sensitivity and specificity of the four serological tests were compared at different time intervals (Table 3). All tests demonstrated high diagnostic sensitivity of more than 93% after 21 days of symptom onset. All tests showed specificity of 100% except SBC Test (98%, 95% Confidence Interval 95.0–99.2%).

Samples of false-positivity

The signal values (OD ratio) by the SBC Test for the four samples (duplicate tests for each sample) were relatively low, ranged from 0.422 to 0.791 (Table 4). The four serum samples from control group patients with positive results for anti-SARS-CoV-2 IgG by SBC Test were negative for anti-SARS-CoV-2 IgG antibodies against SARS-CoV-2 NP, RBD, and S1 indicating the false-positivity of the SBC Test (Table 4). Three of the four serum sample were positive for antibodies against NPs of two or three seasonal coronaviruses and one was negative for all. All the four serum samples were positive for anti-CMV IgG and negative for anti-CMV IgM and RF.

Table 2 Clinical characteristics of the 70 patients with confirmed coronavirus disease 2019 (COVID-19), stratified based on the presence of pulmonary infiltration.

Characteristics ^a	All patients	With pulmonary infiltration	Without pulmonary infiltration	<i>p</i> value
No. of patients/no. of serum samples	70/184	27/91	43/93	
Age (years)	42.6 ± 15.0	51.3 ± 13.5	37.2 ± 13.4	< 0.001
Male sex	34 (48.6)	15 (55.6)	19 (44.2)	0.354
Comorbid medical condition				
Diabetes mellitus	4 (5.7)	3 (11.1)	1 (2.3)	0.291
Malignancy	2 (2.9)	2 (7.4)	0 (0)	0.145
Coronary artery disease	2 (2.9)	2 (7.4)	0 (0)	0.145
Initial presentation				
Fever	35 (50.0)	18 (66.7)	17 (39.5)	0.027
Headache	12 (17.1)	1 (3.7)	11 (25.6)	0.022
Myalgia	19 (27.1)	10 (37.0)	9 (20.9)	0.140
Malaise	21 (30.0)	10 (37.0)	11 (25.6)	0.309
Upper airway symptoms ^b	37 (52.9)	10 (37.0)	27 (62.8)	0.036
Low respiratory tract symptoms ^c	42 (60.0)	18 (66.7)	24 (55.8)	0.367
Diarrhea	16 (22.9)	6 (22.2)	10 (23.3)	0.920
Treatment outcome				
Length of hospital stay (days)	25.0 ± 14.6	28.4 ± 16.5	22.8 ± 13.0	0.123
Diagnosis of pneumonia	27 (38.6)	27 (100)	0 (0)	–
ICU admission	7 (10.0)	7 (25.9)	0 (0)	0.001
Ventilator required	5 (7.1)	5 (18.5)	0 (0)	0.007
ECMO support received	1 (1.4)	1 (3.7)	0 (0)	0.386
Hospital mortality	0 (0)	0 (0)	0 (0)	–
Sample collection				
Available sample number, mean ± SD	2.6 ± 2.8	3.4 ± 3.9	2.2 ± 1.8	0.081
No. of days between symptom onset and first sample collection, mean ± SD	20.1 ± 15.6	20.2 ± 14.9	20.1 ± 16.3	0.979

^a All values are expressed as mean ± SD or number (percentage).

^b Includes rhinorrhea, nasal stiffness, sore throat, and hoarseness.

^c Includes cough, productive sputum, dyspnea, and chest pain.

ICU, intensive care unit; ECMO, extracorporeal membrane oxygenation.

P values in boldface indicate those <0.05.

Association of anti-SARS-CoV-2 antibody responses with COVID-19 severity

Signal values of 78 week-representative serum samples from 27 COVID-19 patients with pneumonia were compared with 62 week-representative serum samples from 43 COVID-19 patients without pneumonia using different serological tests (Fig. 3A–G). COVID-19 patients with pneumonia showed significantly higher signal values than COVID-19 patients without pneumonia in different classes of antibodies, excepting IgM antibody of EliA Test (Fig. 3F). When signal values of COVID-19 patients with and without pneumonia were separately compared at different time intervals after symptom onset, Siemens Test (detecting total antibodies) (Fig. 3C) and SBC Test (detecting IgG antibody) (Fig. 3D) showed a better performance in early differentiation of the risk of pulmonary involvement than Beckman IgG Test (Fig. 3A), EliA IgG Test (Fig. 3E), and EliA IgA Test (Fig. 3G).

Discussion

In this study, we chronologically analyzed the antibody responses to SARS-CoV-2 infection with four viral spike

protein targeting anti-SARS-CoV-2 antibody tests to evaluate their performance in the early diagnosis and severity assessment of COVID-19 infection. There are four major findings of this study. First, diagnostic performances of serological tests for COVID-19 infection were highly disease time course dependent and consistently had high sensitivity after 21 days of symptom onset. However, Siemens Test showed the best performance in the early diagnosis of COVID-19 infection within 2 weeks of symptom onset. Second, IgG signal values reached plateau after 3 weeks of symptom onset, where the IgM and IgA score values peaked within 2–4 weeks of symptom onset. Knowing the dynamic differences between different classes of reactive immunoglobulin to SARS-CoV-2 infection helps the more accurate estimation of the time of infection for epidemiological study and infection control purposes. Third, COVID-19 patients with pneumonia had higher serum signal values than those values of patients without pneumonia with all the four serological tests in this study. Fourth, chronological analysis of signal values of week-representative serum samples revealed that Siemens Test (targeting total antibodies) and SBC Test (targeting IgG) effectively differentiated COVID-19 patients with pneumonia as early as in second week of symptom onset. Beckman Test and EliA Test

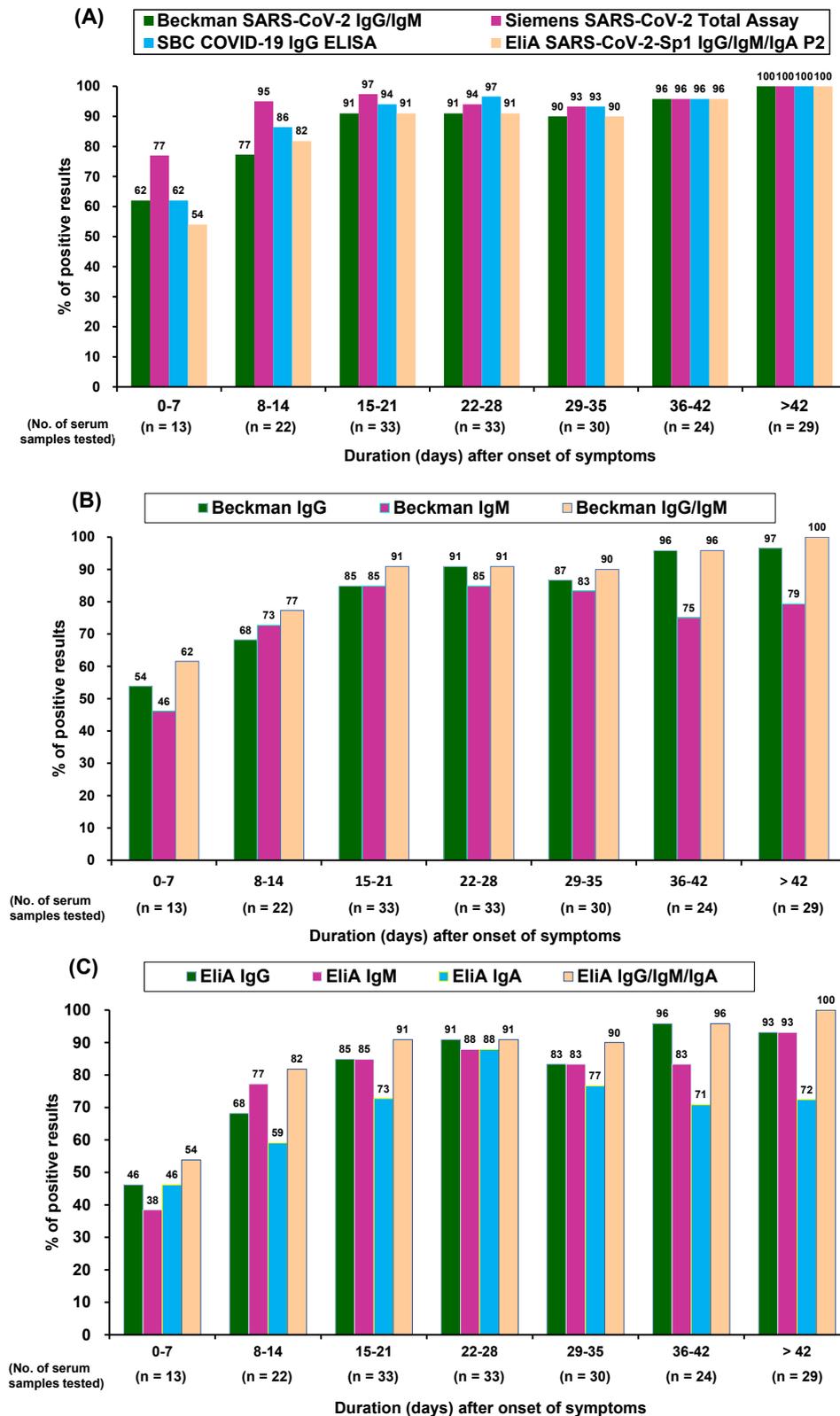
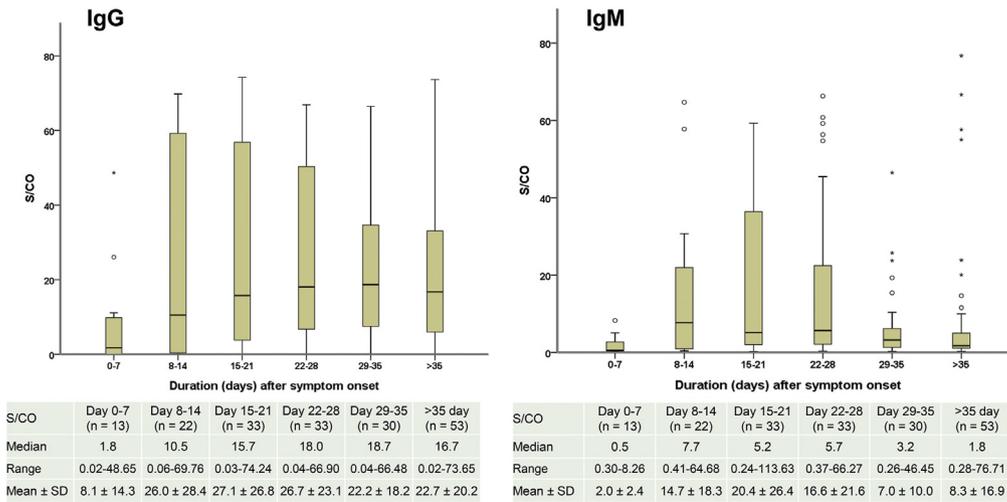
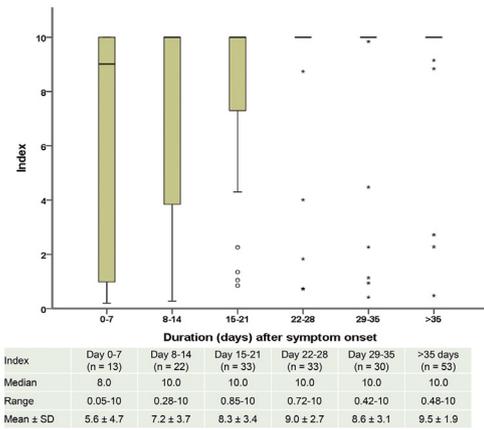


Fig. 1. Percentage of serum samples showing positive antibody findings when examined using the four studied serological assays after symptom onset. (A) Positive results of Beckman SARS-CoV-2 IgG/IgM (positive results of either IgG or IgM), Siemens SARS-CoV-2 Total (COV2T), SBC COVID-19 IgG ELISA Kit, and EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research (positive results of either IgG, IgM, or IgA), (B) Positive results of Beckman SARS-CoV-2 IgG, Beckman SARS-CoV-2 IgM, and Beckman SARS-CoV-2 IgG/IgM (positive results of either IgG or IgM), (C) positive results of EliA SARS-CoV-2-Sp1 IgG P2 Research, EliA SARS-CoV-2-Sp1 IgM P2 Research, EliA SARS-CoV-2-Sp1 IgA P2 Research, and EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research (positive results of either IgG, IgM, or IgA).

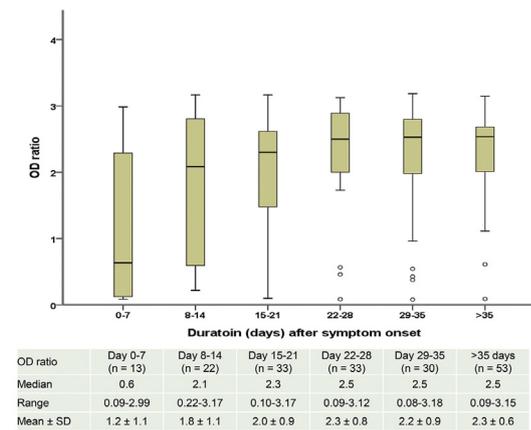
(A) Beckman SARS-CoV-2 IgG/IgM



(B) Siemens SARS-CoV-2 Total (COV2T)



(C) SBC COVID-19 IgG ELISA



(D) EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research

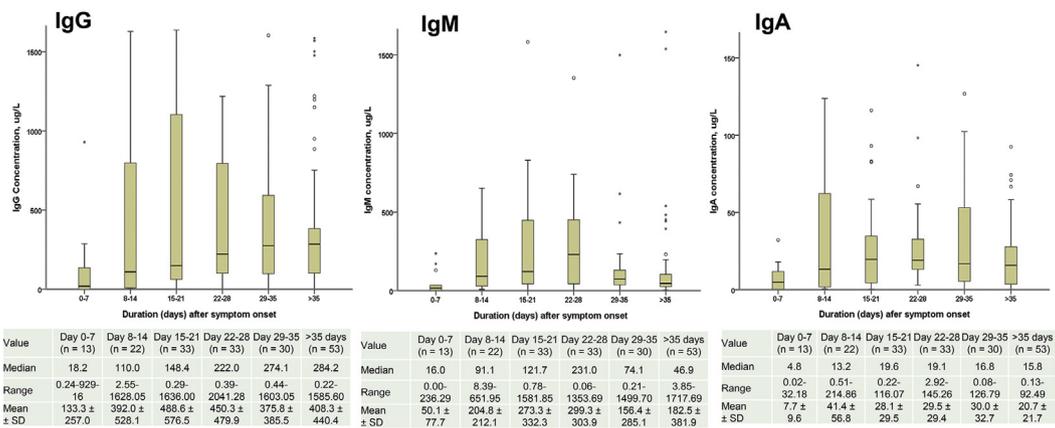


Fig. 2. Signal values of the four immunoassays for anti-SARS-CoV-2 antibodies detection after symptom onset (A) Beckman SARS-CoV-2 IgG/IgM, (B) Siemens SARS-CoV-2 Total (COV2T), (C) SBC COVID-19 IgG ELISA Kit, and (D) EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research.

Table 3 Diagnostic sensitivity and specificity of the four anti-SARS-CoV-2 antibody tests after symptom onset.

	Day 0–7		Day 8–21		After Day 21	
	COVID-19 specimens (n = 13)	Control specimens (n = 200)	COVID-19 specimens (n = 59)	Control specimens (n = 200)	COVID-19 specimens (n = 112)	Control specimens (n = 200)
BECKMAN SARS-CoV-2 IgG/IgM						
Positive ^a	8	0	51	0	105	0
Negative ^b	5	200	8	200	7	200
Sensitivity, % (95% CI)	61.5 (35.5–82.3)		86.4 (75.5–93.0)		93.8 (87.7–96.9)	
Specificity, % (95% CI)	100 (98.1–1.0)		100 (98.1–1.0)		100 (98.1–1.0)	
SIEMENS SARS-CoV-2 Total (COV2T)						
Positive	10	0	57	0	107	0
Negative	3	200	2	200	5	200
Sensitivity, % (95% CI)	76.9 (49.7–91.8)		96.6 (88.5–99.1)		95.5 (90.0–98.1)	
Specificity, % (95% CI)	100 (98.1–1.0)		100 (98.1–1.0)		100 (98.1–1.0)	
SBC COVID-19 IgG ELISA Kit						
Positive	8	4	54	4	108	4
Negative	5	196	5	196	4	196
Sensitivity, % (95% CI)	61.5 (35.5–82.3)		91.5 (81.7–96.3)		96.4 (91.2–98.6)	
Specificity, % (95% CI)	98.0 (95.0–99.2)		98.0 (95.0–99.2)		98.0 (95.0–99.2)	
EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research						
Positive ^c	7	0	52	0	105	0
Negative ^d	6	200	7	200	7	200
Sensitivity, % (95% CI)	53.9 (29.1–76.8)		88.1 (77.5–94.1)		93.8 (87.7–96.9)	
Specificity, % (95% CI)	100 (98.1–1.0)		100 (98.1–1.0)		100 (98.1–1.0)	

^a Presence of IgM and/or IgG antibodies.

^b Absence of IgM and IgG antibodies.

^c Presence of IgA and/or IgM and/or IgG antibodies.

^d Absence of IgA and IgM and IgG antibodies.

CI, confidence interval; COVID-19, coronavirus disease 2019.

targeting IgG and EliA Test targeting IgA differentiated COVID-19 patients with pneumonia in third week of symptom onset. Serological test targeting IgM, both in Beckman Test and EliA Test failed to show differences between COVID-19 patients with and without pneumonia. Collectively speaking, Siemens Test and SBC Test differentiated COVID-19 with and without pneumonia as early as the second week after symptom onset than the other two tests.

Many prior studies have demonstrated the detection sensitivities of anti-SARS-CoV-2 antibody tests are highly disease course timing dependent and increase as time elapsed from the date of symptom onset to the date of testing.^{3,18,27–29} Sensitivities in the early course of COVID-19 infection, especially within 2 weeks of symptom onset, were generally low in all kind of serological tests of either lateral flow or chemiluminescence immunoassays.^{3,18,27–31} Therefore, they are generally considered as laboratory tools useful for the diagnosis of acute or recent SARS-CoV-2 infection in adjuvant to qRT-PCR or for retrospective seroprevalence study of remote infection. However, though

prior studies did not show significant difference in the detection of time to seroconversion between various serological tests. Siemens Test in the current study did show a higher percentage of early detecting anti-SARS-CoV-2 antibodies within 2 weeks of symptom onset than other three serological tests. It is therefore that Siemens Test, a serological test targeting total antibodies against SARS-CoV-2 spike protein, might be a more useful laboratory tool in the early diagnosis of patient with COVID-19. A possible explanation for this result might be that Siemens Test detects total antibodies rather than single antibody class against SARS-CoV-2. Similar phenomenon has been observed in many prior COVID-19 serological studies.^{3,16,32} It is therefore a serological test detecting total antibodies might be more useful for the early diagnosis of COVID-19.

Presence of IgM in patient serum generally indicates acute or recent infection to a causative pathogen.³³ In contrast, IgG level increases later in the course of infection than the level of IgM. It is proposed a serological test detecting IgM in addition to IgG might increase the

Table 4 Summary of serological data for the four control samples with false-positive results by the SBC COVID-19 IgG ELISA test. Duplicate tests were performed for each sample by the SBC Test.

Test (values indicating positive results)	Specimen designation no. and test results			
	90,262	90,368	90,389	90,494
SBC COVID-19 IgG ELISA Kit (OD ratio, ≥ 0.4)	0.791/0.748	0.731/0.682	0.602/0.537	0.422/0.436
BECKMAN SARS-CoV-2 IgG/IgM				
IgG (S/CO, ≥ 1.0)	0.03	0.02	0.03	0.23
IgM (S/CO, ≥ 1.0)	0.23	0.49	0.24	0.27
SIEMENS SARS-CoV-2 Total (COV2T) (index, ≥ 1.0)	0.14	0.78	0.52	0.63
EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research				
IgG (>20 $\mu\text{g/L}$)	2.8	6.1	2.1	4.7
IgM (>20 $\mu\text{g/L}$)	4.1	3.6	11	0
IgA (>5 $\mu\text{g/L}$)	0.1	0.3	0	0.2
recomLine SARS-CoV-2 IgG				
S1 SARS-2	–	–	–	–
RBD SARS-2	–	–	–	–
NP SARS-2	–	–	–	–
Seasonal coronavirus				
NP HKU1	+	–	+	+
NP OC43	+	–	+	+
NP NL63	–	–	+	+
NP 229E	–	–	–	–
Anti-cytomegalovirus antibody				
IgG	+	+	+	+
IgM	–	–	–	–
Rheumatoid factor	–	–	–	–

usefulness of the test in early detection of seroconversion after SARS-CoV-2 infection. However, our study and prior studies all demonstrated that adding anti-SARS-CoV-2 IgM antibody detection did not improve the performance of a serological test in terms of early diagnosis of COVID-19 infection.^{3,18,20,27} In contrast, fewer studies provided information about the role of anti-SARS-CoV-2 IgA antibody in the diagnosis of COVID-19 infection. Prior studies showed a higher detection sensitivity of IgA than IgG antibody in early disease course of COVID-19 infection,^{20,34–36} our study failed to demonstrate the improvement of test performance in early COVID-19 diagnosis as the incorporation of IgA detection in EliA Test. Though adding IgM and/or IgA antibody detection did not improve diagnostic performance of serological tests in Beckman Test and EliA Test, information from the dynamic trend of IgM or IgA in relating to IgG helped a more accurate estimation of the time point (within or after 3 weeks of infection for example) during the disease course of COVID-19.

Previous studies found patients with COVID-19 complicated with pneumonia showed earlier seroconversion than those without pneumonia and thus early anti-SARS-CoV-2 antibody response might be a serological indicator of pulmonary injury.^{1,18} Subsequent studies demonstrated that a higher serum titer of antibody was associated with severity of COVID-19.^{19,37–39} Our previous study also found significant higher post-symptom second week chemiluminescent signal values in Roche Elecsys® Anti-SARS-CoV-2 Assay (Roche Test) and Abbott SARS-CoV-2 IgG Assay (Abbott Test) among COVID-19 patients with pneumonia than COVID-19 patients without pneumonia.³ Both Roche Test and Abbott Test detect antibodies targeting SARS-CoV-2 nucleocapsid

protein for the diagnosis of COVID-19. In the current study, we consolidated the phenomenon for serological tests detecting antibodies targeting SARS-CoV-2 spike protein. Furthermore, we chronologically elaborated the capabilities of different classes of anti-SARS-CoV-2 antibodies in the timing of differentiating COVID-19 patients with and without pneumonia in the current study. We found Siemens Test (targeting total antibodies) and SBC Test (targeting IgG antibody) predicted COVID-19 pneumonia earlier than Beckman Test and EliA Test (either targeting IgG or IgA antibody). Serological tests targeting IgM antibody were less effective and failed to differentiate COVID-19 pneumonia in this study. Our observation therefore provides important information for first-line physicians in their risk assessment decision for COVID-19 patient management when using different serological tests.

This study has several limitations. First, this multicenter study might have information bias due to different investigator in four hospitals even we used a standardized patient reporting form. Second, this was a retrospective study and serum samples tested at different duration were not at the same day; therefore, information bias due to laboratory data might exist. Third, the significant differences between study groups may not be observed due to small populations. Fourth, we only use plain chest roentgenogram in the diagnosis of pneumonia. It is less sensitive than computed tomography and some patients might be misclassified as non-pulmonary infection. Finally, the plausible reasons for the false-positive anti-SARS-CoV-2 IgG (2%) by the SBC Test but not by the other three immunoassays were not further investigated and therefore remain unclear.

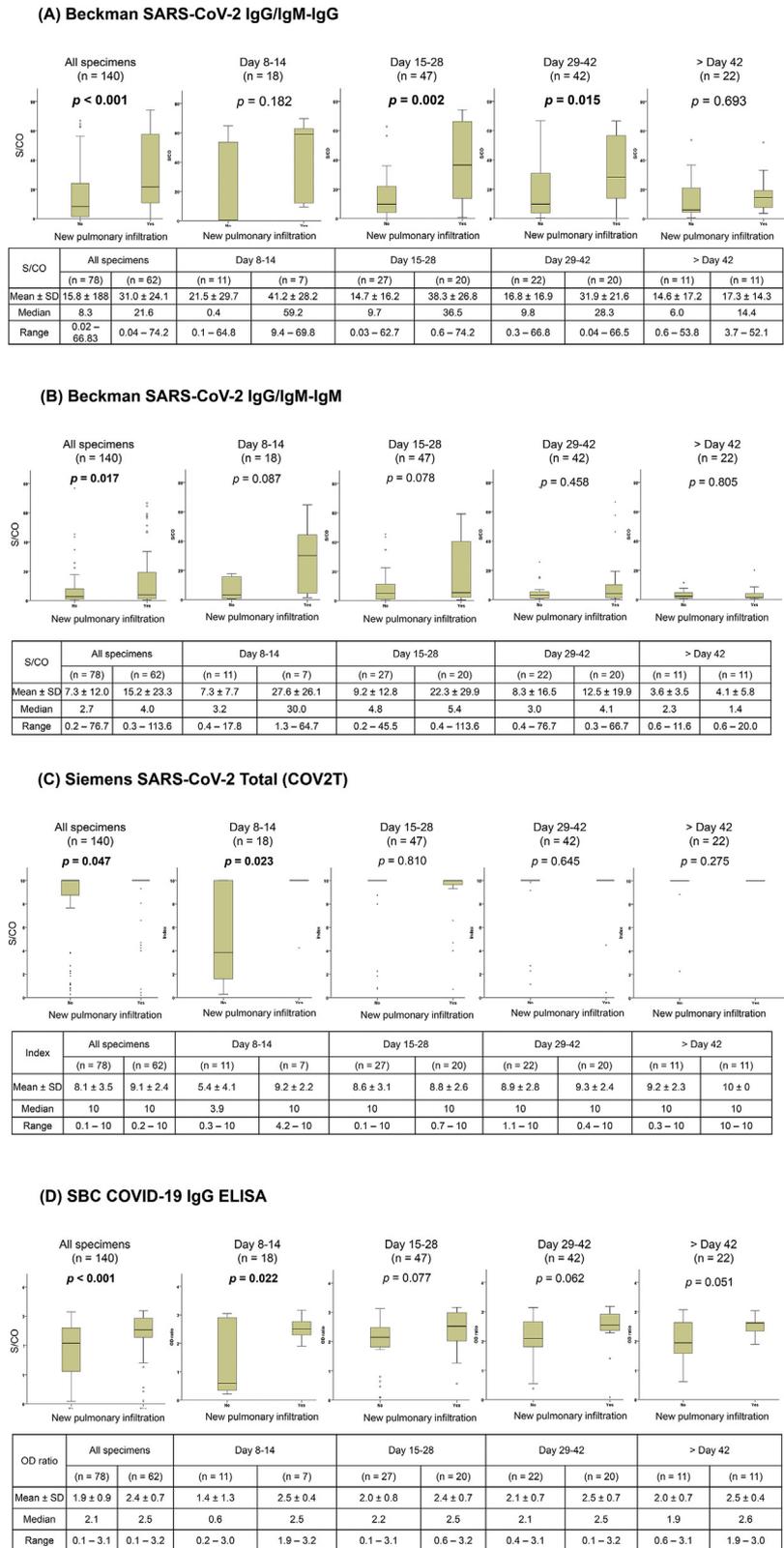
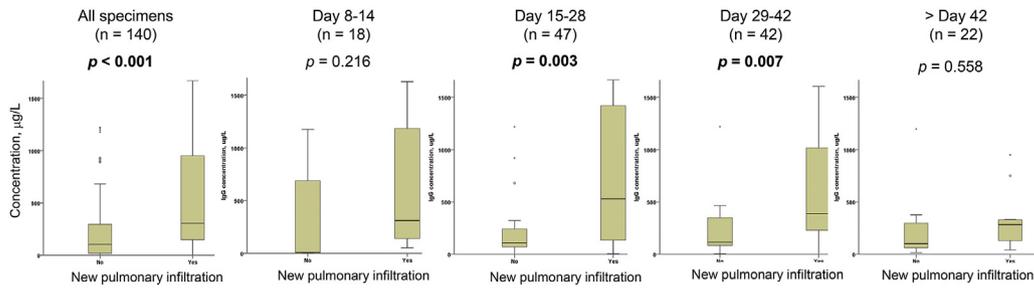


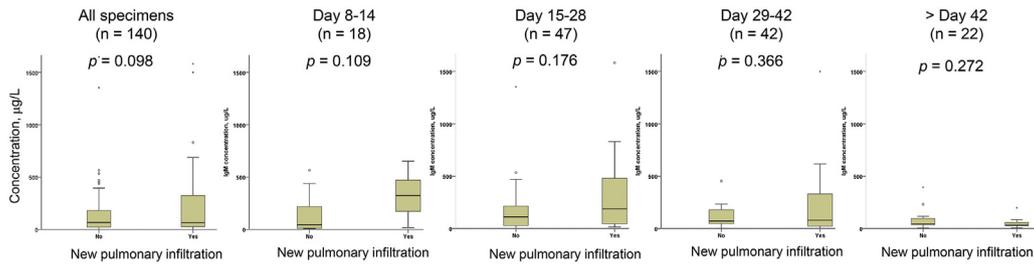
Fig. 3. Comparison of signal values of the four immunoassays in patients with COVID-19 with or without pneumonia (A) Beckman SARS-CoV-2 IgG/IgM-IgG, (B) Beckman SARS-CoV-2 IgG/IgM-IgM, (C) Siemens SARS-CoV-2 Total (COV2T), (D) SBC COVID-19 IgG ELISA Kit, (E) EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research-IgG, (F) EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research-IgM, (G) EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research-IgA.

(E) EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research - IgG



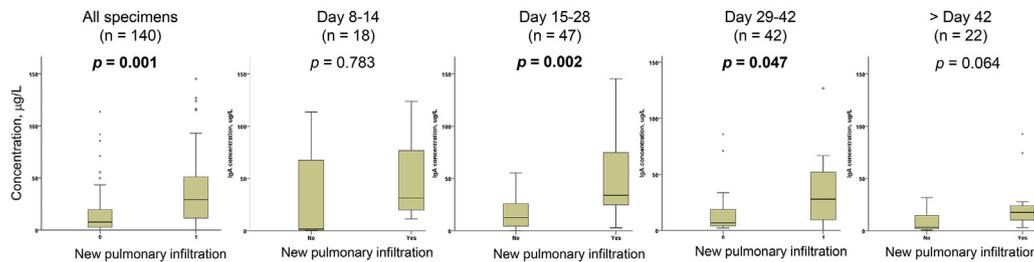
Conc., µg/L	All specimens		Day 8-14		Day 15-28		Day 29-42		> Day 42	
	(n = 78)	(n = 62)	(n = 11)	(n = 7)	(n = 27)	(n = 20)	(n = 22)	(n = 20)	(n = 11)	(n = 11)
Mean ± SD	232.5 ± 316.7	579.3 ± 568.4	317.6 ± 459.3	662.8 ± 685.2	212.5 ± 287.2	747.0 ± 666.1	229.3 ± 267.0	614.3 ± 534.1	240.8 ± 341.7	320.5 ± 283.6
Median	105.0	306.4	8.4	310.5	109.6	530.2	116.1	389.4	101.7	283.5
Range	0.2 – 1219.2	0.4 – 2041.3	2.6 – 1175.7	52.1 – 1628.1	0.3 – 1218.0	6.2 – 2041.3	5.2 – 1219.2	0.4 – 1603.1	15.4 – 1197.5	42.5 – 949.5

(F) EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research - IgM



Conc., µg/L	All specimens		Day 8-14		Day 15-28		Day 29-42		> Day 42	
	(n = 78)	(n = 62)	(n = 11)	(n = 7)	(n = 27)	(n = 20)	(n = 22)	(n = 20)	(n = 11)	(n = 11)
Mean ± SD	152.7 ± 259.5	246.2 ± 374.5	148.4 ± 198.6	325.9 ± 242.8	187.0 ± 275.2	320.2 ± 389.2	170.1 ± 345.3	288.6 ± 488.3	97.1 ± 116.4	53.7 ± 52.3
Median	67.1	66.3	43.8	325.1	110.6	187.7	70.6	79.4	46.9	31.9
Range	0 – 1646.8	0 – 1717.7	8.4 – 565.5	16.5 – 652.0	0.1 – 1353.7	14.4 – 1581.9	1.6 – 1646.8	0.2 – 1717.7	4.5 – 394.6	5.8 – 197.6

(G) EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research - IgA



Conc., µg/L	All specimens		Day 8-14		Day 15-28		Day 29-42		> Day 42	
	(n = 78)	(n = 62)	(n = 11)	(n = 7)	(n = 27)	(n = 20)	(n = 22)	(n = 20)	(n = 11)	(n = 11)
Mean ± SD	18.6 ± 31.1	38.0 ± 35.5	43.0 ± 70.1	51.4 ± 47.5	17.1 ± 14.8	48.6 ± 38.8	16.9 ± 21.8	33.7 ± 30.9	9.0 ± 10.1	27.1 ± 28.9
Median	7.7	29.2	1.8	31.5	12.7	34.0	6.8	27.9	3.4	17.8
Range	0.1 – 214.9	0.1 – 145.3	0.5 – 214.9	11.5 – 123.8	0.2 – 55.5	3.0 – 145.3	2.2 – 85.7	0.1 – 126.8	0.9 – 31.9	3.1 – 92.5

Fig. 3. Continued

In conclusion, performances of different serological tests in detecting anti-SARS-CoV-2 antibodies vary significantly. Targeting antibody selected, viral protein labelled, immunoassay method adopted, and analyzer system used might all influence the performance of a serological test for COVID-19 diagnosis. A serological test detecting total anti-SARS-CoV-2 antibodies is more sensitive in early diagnosis of COVID-19 infection. Quantitative or semiquantitative analysis of IgG and IgA signal values of serological tests better differentiates COVID-19 patient with pneumonia than IgM signal values. Chronological analysis of immune response among COVID-19 patients with different serological testing provides important information for first-line clinicians in the early diagnosis of SARS-CoV-2 infection and the assessment of the risk of pulmonary involvement after infection.

Declaration of competing interest

All authors declare no conflict of interest.

References

- Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;**382**(8):727–33.
- Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 2020;**8**(5):475–81.
- Chen SY, Lee YL, Lin YC, Lee NY, Liao CH, Hung YP, et al. Multicenter evaluation of two chemiluminescence and three lateral flow immunoassays for the diagnosis of COVID-19 and assessment of antibody dynamic responses to SARS-CoV-2 in Taiwan. *Emerg Microb Infect* 2020;**9**(1):2157–68.
- Lee PI, Hsueh PR. Emerging threats from zoonotic coronaviruses—from SARS and MERS to 2019-nCoV. *J Microbiol Immunol Infect* 2020;**53**(3):365–7.
- Lai CC, Liu YH, Wang CY, Wang YH, Hsueh SC, Yen MY, et al. Asymptomatic carrier state, acute respiratory disease, and pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): facts and myths. *J Microbiol Immunol Infect* 2020;**53**(3):404–12.
- Yen MY, Schwartz J, Chen SY, King CC, Yang GY, Hsueh PR. Interrupting COVID-19 transmission by implementing enhanced traffic control bundling: implications for global prevention and control efforts. *J Microbiol Immunol Infect* 2020;**53**(3):377–80.
- Chintalapudi N, Battineni G, Amenta F. COVID-19 virus outbreak forecasting of registered and recovered cases after sixty day lockdown in Italy: a data driven model approach. *J Microbiol Immunol Infect* 2020;**53**(3):396–403.
- 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;**48**(4):543–51.
- Yang CJ, Chen TC, Chen YH. The preventive strategies of community hospital in the battle of fighting pandemic COVID-19 in Taiwan. *J Microbiol Immunol Infect* 2020;**53**(3):381–3.
- Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* 2020;**25**(3):2000045.
- Klein D. Quantification using real-time PCR technology: applications and limitations. *Trends Mol Med* 2020;**8**(6):257–60.
- Lin Q, Zhu L, Ni Z, Meng H, You L. Duration of serum neutralizing antibodies for SARS-CoV-2: lessons from SARS-CoV infection. *J Microbiol Immunol Infect* 2020;**53**(5):821–2.
- 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**(8). e00797-20.
- 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**(8). e00941-20.
- Muench P, Jochum S, Wenderoth V, Ofenloch-Haehnle B, Hombach M, Strobl M, et al. Development and validation of the Elecsys Anti-SARS-CoV-2 immunoassay as a highly specific tool for determining past exposure to SARS-CoV-2. *J Clin Microbiol* 2020;**58**(10). e01694-20.
- Perkmann T, Perkmann-Nagele N, Breyer MK, Breyer-Kohansal R, Burghuber OC, Hartl S, et al. Side-by-side comparison of three fully automated sars-cov-2 antibody assays with a focus on specificity. *Clin Chem* 2020;**66**(11):1405–13.
- Lippi G, Salvagno GL, Pegoraro M, Militello V, Caloi C, Peretti A, et al. Assessment of immune response to SARS-CoV-2 with fully automated MAGLUMI 2019-nCoV IgG and IgM chemiluminescence immunoassays. *Clin Chem Lab Med* 2020;**58**(7):1156–9.
- Wu JL, Tseng WP, Lin CH, Lee TF, Chung MY, Huang CH, et al. Four point-of-care lateral flow immunoassays for diagnosis of COVID-19 and for assessing dynamics of antibody responses to SARS-CoV-2. *J Infect* 2020;**81**(3):435–42.
- Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. *Clin Infect Dis* 2020;**71**(16):2027–34.
- Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* 2020;**26**(6):845–8. <https://doi.org/10.1038/s41591-020-0897-1>.
- Beckman SARS-CoV-2 IgG/IgM. <https://www.beckmancoulter.com/search?query=covid>.
- Siemens (ADVIA Centaur®) SARS-CoV-2 total (COV2T). <https://doclib.siemens-healthineers.com/document/710513?forceLogin=true>.
- SBC COVID-19 IgG ELISA. <http://covid19.sbc-biotech.com/>.
- EliA SARS-CoV-2-Sp1 IgG/IgM/IgA P2 Research. <https://dfu.phadia.com/Data/Pdf/5eeb0e5b89c2320a102997be.pdf>.
- recomLine SARS-CoV-2 IgG. <https://www.mikrogen.de/produkte/produktuebersicht/testsystem/sars-cov-2-igg-aviditaet-ruo.html>.
- Tseng WP, Wu JL, Wu CC, Kuo KT, Lin CH, Chung MY, et al. Seroprevalence surveys for anti-SARS-CoV-2 antibody in different populations in Taiwan with low incidence of COVID-19 in 2020 and severe outbreaks of SARS in 2003. Data submitted.
- To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020;**20**(5):565–74. [https://doi.org/10.1016/S1473-3099\(20\)30196-1](https://doi.org/10.1016/S1473-3099(20)30196-1).
- Kweon OJ, Lim YK, Kim HR, Kim MC, Choi SH, Chung JW, et al. Antibody kinetics and serologic profiles of SARS-CoV-2 infection using two serologic assays. *PLoS One* 2020;**15**(10):e0240395. <https://doi.org/10.1371/journal.pone.0240395>.
- Marlet J, Petillon C, Ragot E, Abou El Fattah Y, Guillon A, Marchand Adam S, et al. Clinical performance of four immunoassays for antibodies to SARS-CoV-2, including a prospective analysis for the diagnosis of COVID-19 in a real-life routine care setting. *J Clin Virol* 2020;**132**:104633. <https://doi.org/10.1016/j.jcv.2020.104633>.

30. Harley K, Gunsolus IL. Comparison of the clinical performances of the Abbott Alinity IgG, Abbott Architect IgM, and Roche Elecsys total SARS-CoV-2 antibody assays. *J Clin Microbiol* 2020; **59**(1):e02104–20. <https://doi.org/10.1128/JCM.02104-20>.
31. Van Elslande J, Decru B, Jonckheere S, Van Wijngaerden E, Houben E, Vandecandelaere P, et al. Antibody response against SARS-CoV-2 spike protein and nucleoprotein evaluated by four automated immunoassays and three ELISAs. *Clin Microbiol Infect* 2020; **26**(11). <https://doi.org/10.1016/j.cmi.2020.07.038>. 1557.e1-1557.e7.
32. Manthei DM, Whalen JF, Schroeder LF, Sinay AM, Li SH, Valdez R, et al. Differences in performance characteristics among four high-throughput assays for the detection of antibodies against SARS-CoV-2 using a common set of patient samples. *Am J Clin Pathol* 2021; **155**(2):267–79. <https://doi.org/10.1093/ajcp/aqaa200>.
33. Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. *J Am Med Assoc* 2020; **323**(22):2249–51.
34. Beavis KG, Matushek SM, Abeleda APF, Bethel C, Hunt C, Gillen S, et al. Evaluation of the EUROIMMUN anti-SARS-CoV-2 ELISA assay for detection of IgA and IgG antibodies. *J Clin Virol* 2020; **129**: 104468. <https://doi.org/10.1016/j.jcv.2020.104468>.
35. Pape C, Remme R, Wolny A, Olberg S, Wolf S, Cerrone L, et al. Microscopy-based assay for semi-quantitative detection of SARS-CoV-2 specific antibodies in human sera: a semi-quantitative, high throughput, microscopy-based assay expands existing approaches to measure SARS-CoV-2 specific antibody levels in human sera. *Bioessays* 2020:e2000257. <https://doi.org/10.1002/bies.202000257>.
36. Iyer AS, Jones FK, Nodoushani A, Kelly M, Becker M, Slater D, et al. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. *Sci Immunol* 2020; **5**(52):eabe0367. <https://doi.org/10.1126/sciimmunol.abe0367>.
37. Schlesinger T, Weißbrich B, Wedekink F, Notz Q, Herrmann J, Krone M, et al. Biodistribution and serologic response in SARS-CoV-2 induced ARDS: a cohort study. *PLoS One* 2020; **15**(11):e0242917. <https://doi.org/10.1371/journal.pone.0242917>.
38. Yongchen Z, Shen H, Wang X, Shi X, Li Y, Yan J, et al. Different longitudinal patterns of nucleic acid and serology testing results based on disease severity of COVID-19 patients. *Emerg Microb Infect* 2020; **9**(1):833–6.
39. Wolf J, Kaiser T, Pehnke S, Nickel O, Lübbert C, Kalbitz S, et al. Differences of SARS-CoV-2 serological test performance between hospitalized and outpatient COVID-19 cases. *Clin Chim Acta* 2020; **511**:352–9. <https://doi.org/10.1016/j.cca.2020.10.035>.