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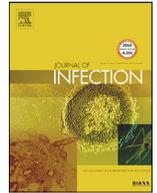
Four point-of-care lateral flow immunoassays for diagnosis of COVID-19 and for assessing dynamics of antibody responses to SARS-CoV-2

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SUMMARY

Objectives:: We aimed to evaluate the role of rapid serological tests in the management of coronavirus disease 2019 (COVID-19) patients.

Methods:: This retrospective study enrolled 16 real-time reverse transcription polymerase chain reaction-confirmed symptomatic patients with COVID-19 and 58 COVID-19 negative patients at a medical center in Taiwan over a 3-month period. Serial serum samples were collected and tested for antibody response using four point-of-care (POC) lateral flow immunoassays (LFIA) (ALLTEST 2019-nCoV IgG/IgM Rapid Test, Dynamiker 2019-nCoV IgG/IgM Rapid Test, ASK COVID-19 IgG/IgM Rapid Test, and Wondfo SARS-CoV-2 Antibody Test). Time-dependent detection sensitivity and timeliness of seroconversion were determined and compared between the four POC rapid tests.

Results:: The overall sensitivity and specificity of the four tests for detecting anti-SARS-CoV-2 antibodies after 3 weeks of symptom onset were 100% and 100%, respectively. There was no significant difference between the rapid tests used for detection of IgM and IgG separately and those used for detection of combined total antibody (mainly IgM/IgG). There was no significant difference between the four POC rapid tests in terms of time required for determining seroconversion of COVID-19. Patients with COVID-19 with pneumonia demonstrated shorter seroconversion time than those without pneumonia.

Conclusion:: Though the POC antibody rapid tests based on LFIA showed reliable performance in the detection of SARS-CoV-2-specific antibodies, the results of these tests should be interpreted and applied appropriately in the context of antibody dynamic of COVID-19 infection. COVID-19 patients complicated with pneumonia exhibited earlier anti-SARS-CoV-2 antibody response than COVID-19 patients without pneumonia.

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Introduction

Since the coronavirus disease 2019 (COVID-19) emerged at the end of 2019 in Wuhan, China, it has rapidly progressed to a pandemic status and has caused tremendous repercussions on human health and life.[1,2] As a newly emerged virus is normally assigned to a species based on its phylogeny and taxonomy,[3,4] the causative pathogen of COVID-19 was officially named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[2] SARS-CoV-2 has been found to be closely related to severe acute respiratory syndrome coronavirus (SARS-CoV), which was identified in 2003. Both SARS-CoV and SARS-CoV-2 may have originated from bats and have

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evolved to be capable of human-to-human transmission through accumulation of genetic mutations.[5] While facing an emerging infectious disease, rapid identification of the causative microorganism and analysis of its genome composition helps in the development of diagnostic tests, therapeutic drugs, and vaccines required to contain the spread of the disease and to mitigate the impact of the disease. It is especially true for a viral disease with high transmissibility, pathogenicity, and virulence, as observed in the current COVID-19 pandemic.

Viral culture, a gold standard for diagnosis of viral infections, is a time-consuming method and can only be performed in a high biosafety level laboratory specifically designed for handling biohazardous pathogens. Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assay, used for amplification and detection of specific viral nucleic acid sequences within few hours, has been an important and irreplaceable diagnostic tool in recent years. However, rRT-PCR assay remains labor-intensive and expertise-dependent and is only available in hospitals with a qualified microbiology laboratory.[6,7] The surge capacity of rRT-PCR assay has been limited by the mass influx of patients with COVID-19 due to the large-scale community outbreak of the disease. In addition, the site of specimen collection, technique, and timing of the disease course significantly affect the sensitivity and specificity of the rRT-PCR assay in the diagnosis of COVID-19. Thus, a simple, easy-to-use, and accurate diagnostic tool is needed to supplement the diagnosis of COVID-19 to improve patient outcome, resource allocation, and infection control interventions.

Serological tests based on point-of-care (POC) lateral flow immunoassays (LFIAs) have been developed to detect anti-SARS-CoV-2 antibodies simultaneously or separately for the diagnosis of COVID-19.[8-10] Compared to rRT-PCR, serological tests have the advantage of decreased technical requirement, short turnaround time, affordability, lower sampling and specimen preparation risk, and higher detection sensitivity and specificity. Serological testing is therefore an important diagnostic tool and can be used as an adjuvant to rRT-PCR for mass screening, clinical diagnosis, and epidemiological study of COVID-19.

Many rapid serological tests targeting anti-SARS-CoV-2 immunoglobulin A (IgA), immunoglobulin M (IgM), or immunoglobulin G (IgG) antibodies have been applied for clinical use in many countries.[8,9] However, the usefulness of these POC rapid serological tests based on lateral flow immunoassays (LFIAs) has not been elaborately studied. Therefore, the primary goal of this study was to evaluate and compare the usefulness of four POC antibody rapid tests in the diagnosis of COVID-19 infection based on different time courses of the disease. Further, we hypothesized that there might exist a difference in dynamic antibody response between patients with COVID-19 at different levels of severity. Therefore, the second goal of this study was to test our hypothesis by comparing the difference in time from symptom onset to seroconversion between patients with COVID-19 with and without pneumonia.

Methods

National policy, study design, and patient enrollment

In Taiwan, samples from patients who meet the reporting criteria of COVID-19 have to be submitted to virology laboratories validated and contracted by the Centers for Diseases Control and Prevention of Taiwan (Taiwan CDC) for SARS-CoV-2 rRT-PCR testing.[11] The samples include oropharyngeal and nasopharyngeal swabs, oral gargling, and sputum. Three sets of primers and probes targeting SARS-CoV-2 envelope (E), nucleocapsid (N), and RNA-dependent RNA polymerase (RdRp) genes are used. In addition to SARS-CoV-2 RT-PCR testing, all respiratory samples are simultaneously evaluated for the presence of Influenza A/B viruses using

RT-PCR. If the results are negative for both SARS-CoV-2 and Influenza A/B viruses, an additional SARS-CoV-2 rRT-PCR test for another respiratory sample from the suspected COVID patient is performed.[12-14] All rRT-PCR confirmed patients with COVID-19 have to be reported to the National Health Command Center (NHCC) and are mandatorily hospitalized in a negative-pressure isolation room to prevent the spread of SARS-CoV-2 in the community. As of May 2, 2020, Taiwan has maintained a record of limited community transmission of COVID-19 and has 432 confirmed cases.[15]

Our hospital, National Taiwan University (NTUH), a 2500-bed teaching hospital in northern Taiwan, admitted a total of 16 rRT-PCR-positive and NHCC-confirmed patients with COVID-19 between January 23, 2020 and April 25, 2020. This retrospective and observational study was undertaken at NTUH where all the 16 rRT-PCR confirmed patients with COVID-19 were enrolled. This study was approved by the institutional review board of the hospital (NTUH202003004RIND) and the requirement for informed consent from each patient was waived.

Collection of serum samples and clinical data

We used the residual blood samples collected from attending physicians providing regular medical care. All blood samples were collected on the date of venipuncture. If multiple blood samples were collected from a patient with COVID-19 on the same day, only one sample was used for antibody rapid testing. The serum samples collected from the study patients were stored at −20 °C before use. Frozen samples were stored until further analysis with only a single freeze-thaw cycle.

In addition, 58 control serum samples collected from 58 hospitalized patients (median duration from symptom onset, 13 days; range, 1–59 days) with respiratory tract infection or fever but two negative results of SARS-CoV-2 rRT-PCR test were evaluated to validate the performance of the assay. None of these cases were confirmed to be positive for COVID-19 by rRT-PCR during or after hospitalization.

Patient data including sex, age, date of symptom onset, date of hospitalization, requirement of intensive care unit admission (ICU), and date of ICU admission were retrieved from electronic medical records. We also recorded the results of SARS-CoV-2 rRT-PCR test performed using respiratory tract specimens collected on the same day as blood samples. All the 16 confirmed COVID-19 patients had at least 2 chest roentgenographic survey during hospitalization. All of the chest roentgenograms were reviewed. Presence of pneumonia in chest roentgenogram of all confirmed patients with COVID-19 was judged by two investigators independently (Wu JL and Tseng WP). If there was discrepancy in the interpretation of initial chest roentgenogram, a third investigator's (Chen SY) opinion was sought to reach final consensus. For the 58 COVID-19-negative cases, OPD follow-up records and laboratory study results for infection etiology were further collected.

Point-of-care lateral flow immunoassays for detection of anti-SARS-CoV-2 antibodies

Four qualitative rapid tests for anti-SARS-CoV-2 antibody were evaluated in this study: (1) ALLTEST 2019-nCoV IgG/IgM Rapid Test (Hangzhou ALLTEST Biotech Co., Ltd., China), (2) Dynamiker 2019-nCoV IgG/IgM Rapid Test (Dynamiker Biotechnology (Tianjin) Co., Ltd., China), (3) ASK COVID-19 IgG/IgM Rapid Test (TONYAR Biotech Inc., Taiwan), and (4) Wondfo SARS-CoV-2 Antibody Test (Guangzhou Wondfo Biotech Co., Ltd., China). Product information of the four rapid tests used for detection of anti-SARS-CoV-2 antibodies is summarized in Table 1.[8,16-20] All the four rapid tests based on LFIA detect either anti-SARS-CoV-2 IgG and IgM antibodies separately (ALLTEST 2019-nCoV IgG/IgM Rapid Test, Dynamiker

Table 1
Product description of the four antibody rapid tests for diagnosis of coronavirus disease 2019 (COVID-19).

	ALLTEST 2019-nCoV IgG/IgM Rapid Test	Dynamiker 2019-nCoV IgG/IgM Rapid Test	ASK COVID-19 IgG/IgM Rapid Test	Wondfo SARS-CoV-2 Antibody Test
Targeting antibody	IgG and IgM	IgG and IgM	IgG and IgM	Total
Methodology	LFIA	LFIA	LFIA	LFIA
Qualitative analysis	Yes	Yes	Yes	Yes
Protein labeled	Nucleocapsid	Nucleocapsid	Spike	NA
Specimen type(s)	Whole blood (from venipuncture or fingerstick), serum or plasma	Whole blood (from venipuncture or fingerstick), serum or plasma	Whole blood (from venipuncture or fingerstick), serum or plasma	Whole blood, serum, or plasma
Specimen amount required	Whole blood: 20 µL Serum or plasma: 10 µL	10 µL	10 µL	10 µL
Turnaround time	10 min	5–10 min	10 min	15 min
Reported performance based on rRT-PCR results	Positive sera ($n=22$); Control sera ($n=100$)	Positive sera ($n=162$); Control sera ($n=300$)	NA	Positive sera ($n=361$); Control sera ($n=235$)
Sensitivity (95% CI)	IgG: >99.9% (82.5–100%)/IgM: 90.9% (71.0–98.7%)	IgG: 89.2% (80.1–94.4%)/IgM: 87.8% (78.5–93.5%)		86.4% (82.4–89.6%)
Specificity (95% CI)	IgG: 98.0% (92.6–99.9%)/IgM: 96.0% (91.8–99.4%)	IgG: 96.3% (90.8–98.5%)/IgM: 95.3% (89.5–98.0%)		99.6% (97.6–99.9%)
Accuracy (95% CI)	IgG: 98.4% (93.4–99.9%)/IgM: 95.9% (90.5–98.5%)	IgG: 93.4% (88.8–96.2%)/IgM: 92.3% (87.4–95.3%)		91.2% (89.1–93.6%)
Confirmed no cross reactivity with antibodies to non-coronaviruses	Influenza A and B viruses[a], adenovirus, RSV, HBV, HCV, HIV, *Treponema pallidum*, *Helicobacter pylori*	Influenza A viruses (new type A H1N1, seasonal H1N1, H3N2, H5N1, H7N9), influenza B virus (Yamagata, Victoria), rhinovirus (groups A, B, C), CMV, norovirus, mumps virus, VZV, measles virus, enterovirus (groups A, B, C, D), RSV, EBV, adenovirus (types 1, 2, 3, 4, 5, 7, 55), rotavirus, measles virus, *Mycoplasma pneumoniae*	NA	Influenza A and B viruses, adenovirus, RSV, EBV, CMV, VZV, measles virus, mumps virus, enterovirus type 71, HBV, HCV, HIV, *C. pneumoniae*, *M. pneumoniae*, *T. pallidum*
Cross reactivity with antibody to other coronaviruses	NA	NA	NA	NA
SARS and MERS Other seasonal coronaviruses	NA	No	NA	NA
Registration	CE-IVD	CE-IVD	No	CFDA, CE-IVD
Manufacturer	Hangzhou ALLTEST Biotech Co., Ltd. (China)	Dynamiker Biotechnology (Tianjin) Co., Ltd. (China)	TONYAR Biotech Inc. (Taiwan)	Guangzhou Wondfo Biotech Co., Ltd, (China)
Reference(s)	[16, 17]	[18]	[19]	[20]

[a] Cross reactivity was reported in sera samples with antibodies against influenza A, B viruses, adenovirus, and dengue virus [8].LFIA, lateral flow immunoassay; rRT-PCR, real-time reverse transcriptase polymerase chain reaction; RSV, respiratory syncytial virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; CMV, cytomegalovirus; VZV, varicella-zoster virus; EBV, Epstein-Barr virus; SARS. severe acute respiratory syndrome; MERS, Middle East respiratory syndrome; CE-IVD, Conformité Européenne in vitro diagnostic device; NA, not available; CFDA, China Food and Drug Administration of the United States.

2019-nCoV IgG/IgM Rapid Test, and ASK COVID-19 IgG/IgM Rapid Test) or total antibody (mainly IgM and IgG, Wondfo SARS-CoV-2 Antibody Test) within 5–15 min. Whole blood, serum, or plasma samples can be used as testing specimens. The tests require only 10–20 μL of sample volume. Weakly positive results (any shade of color in the test lines) of the POC antibody rapid tests were considered as positive according to the manufacturers' instructions.

Detection of anti-nucleocapsid antibody using western blot method

To validate the accuracy of the four POC rapid tests in detecting anti-SARS-CoV-2 antibodies, serial plasma samples from one COVID-19-positive patient with prolonged airway viral shedding were simultaneously tested for anti-nucleocapsid antibody using western blot (WB) method as previously described.[12,21]

Definitions

Seroconversion was defined as the earliest date on which a positive anti-SARS-CoV-2 antibody response was detected in a serum sample using a specific POC rapid test. Time to seroconversion detection was defined as the duration from the date of symptom onset to the date of seroconversion. Diagnostic sensitivity of a POC rapid test was defined as the percentage of serum samples from confirmed patients with COVID-19 found to be positive for antibodies against SARS-CoV-2. Diagnostic specificity of a POC rapid test was defined as the percentage of serum samples from control patients found to be negative for antibodies against SARS-CoV-2.

Statistical analysis

We calculated means and standard deviations (SDs) for age variables and percentages for categorical variables. The cumulative probabilities of detection of seroconversion for a specific rapid test were obtained using Kaplan–Meier method. The difference in the cumulative probability of detection of seroconversion between the four antibody rapid tests was evaluated using the log rank test. Further, the difference in cumulative probability of detection of seroconversion between patients with COVID-19 with and without pneumonia was investigated using the log rank test. Data were analyzed using SPSS for Windows (IBM SPSS Statistics v26). All p values are two-sided, and $p < 0.05$ was considered statistically significant.

Results

Demographics and clinical characteristics of patients with COVID-19

All the 16 confirmed patients with COVID-19 had at least two (range 2–14, median 5) positive rRT-PCR results performed using either throat or lower respiratory specimens. The mean age was 45.6 years (standard deviation SD, 15.5 years) and was higher for male patients than for female patients (mean ± SD, 54.0 ± 11.4 vs. 34.9 ± 13.8, $p = 0.009$). Nine (56.3%) of the 16 patients with COVID-19 were men. One patient had human immunodeficiency virus (HIV) infection and one patient had colon cancer. All these patients developed clinical symptoms, including lower respiratory tract symptoms (12 patients, 75.0%), upper airway symptoms (10 patients, 62.5%) body temperature >38 °C (8 patients, 50.0%), headache or myalgia (5 patients, 31.3%), and gastrointestinal symptoms (3 patients, 18.8%). Ten (62.5%) patients had increased pulmonary infiltrate or hazy patch (pneumonia) on their chest radiograph. Three patients required intensive care and one of them received extracorporeal membrane oxygenation support.

Correlation of antibody response detected by western blotting and four rapid tests

Serial plasma samples from one patient with COVID-19 were simultaneously tested for anti-nucleocapsid antibody using WB method on days 1–5, 7, 9–11, 14–18, and 21 after symptom onset.[21] The anti-nucleocapsid antibody was persistently detected from day 10 onwards by WB method. The serum samples obtained after 8, 14, 30, 33, 35 days of symptom onset from the same patient were evaluated using the four POC rapid tests. The antibody response was found to be negative in sample obtained on day 8 but positive in samples obtained on day 14 and beyond using all the four rapid tests, which was consistent with the results obtained by WB method.[21]

Anti-SARS-CoV-2 antibody response among COVID-19 patients

A total of 99 serum samples were obtained from 16 patients with COVID-19 (number of samples obtained from individual patient ranged from 2 to 14 samples; median, 5 samples) at different points during the disease course (duration from symptom onset to sampling date ranged from 1 to 63 days; median, 16 days). The samples were used for the evaluation of four POC rapid tests for detection of anti- SARS-CoV-2 antibodies.

Of the 99 serum samples, IgM antibody was detected in 19 (19.2%), 65 (65.7%), and 48 (48.5%) samples using ALLTEST 2019-nCoV IgG/IgM Rapid Test, Dynamiker 2019-nCoV IgG/IgM Rapid Test, and ASK COVID-19 IgG/IgM Rapid Test, respectively. IgG antibody was detected in 74 (74.7%), 68 (68.7%), and 58 (58.6%) samples using ALLTEST 2019-nCoV IgG/IgM Rapid Test, Dynamiker 2019-nCoV IgG/IgM Rapid Test, and ASK COVID-19 IgG/IgM Rapid Test, respectively. Presence of either IgG or IgM was detected in 75 (75.8%), 69 (69.7%), 72 (72.7%), and 75 (75.8% for total antibodies) samples using ALLTEST 2019-nCoV IgG/IgM Rapid Test, Dynamiker 2019-nCoV IgG/IgM Rapid Test, ASK COVID-19 IgG/IgM Rapid Test, and Wondfo SARS-CoV-2 Antibody Test, respectively.

The antibody responses at different time points during the disease course after symptom onset were further evaluated using the found rapid tests (Fig. 1). Anti-SARS-CoV-2 antibody was detected in 100% serum samples collected after 3 weeks of symptom onset ($n = 30$) using all the four rapid tests (Fig. 1(A)–(D)). The Dynamiker 2019-nCoV IgG/IgM Rapid Test detected higher percentage and longer duration of IgM in serum samples than ALLTEST 2019-nCoV IgG/IgM Rapid Test and ASK COVID-19 IgG/IgM Rapid Test. All the three tests showed decreased percentage of IgM antibodies detection after 4th to 6th weeks of symptom onset (Fig. 1(A)–(C)). Overall, the diagnostic sensitivity of the four POC antibody rapid tests for early detection of COVID-19 infection, i.e. within 14 days of symptom onset, was 50.0% (95% Confidence Interval (CI), 34.9–65.1%), 41.3% (95% CI, 27.0–56.8%), 47.8% (95% CI, 32.9–63.1%), and 52.2% (95% CI, 37.0–67.1%) for ALLTEST 2019-nCoV IgG/IgM Rapid Test, Dynamiker 2019-nCoV IgG/IgM Rapid Test, ASK COVID-19 IgG/IgM Rapid Test, and Wondfo SARS-CoV-2 Antibody Test, respectively. The diagnostic sensitivity of the four POC antibody rapid tests for detection of COVID-19 infection increased to 95.7% (95% CI, 78.1–99.9%) (ALLTEST 2019-nCoV IgG/IgM Rapid Test), 87.0% (95% CI, 66.4–97.2%) (Dynamiker 2019-nCoV IgG/IgM Rapid Test & ASK COVID-19 IgG/IgM Rapid Test), and 91.3% (95% CI, 72.0–98.9%) (Wondfo SARS-CoV-2 Antibody Test) between 15 and 21 days after symptom onset, and reached to 100% (95% CI, 88.4–100%) after 3 weeks of symptom onset for all the four POC antibody rapid tests (Table 2).

Analysis of the ability of the four POC antibody rapid tests in the early diagnosis of COVID-19 infection was evaluated with Kaplan–Meier cumulative probability of seroconversion detection (Fig. 2). There was no statistical difference in the results after 7
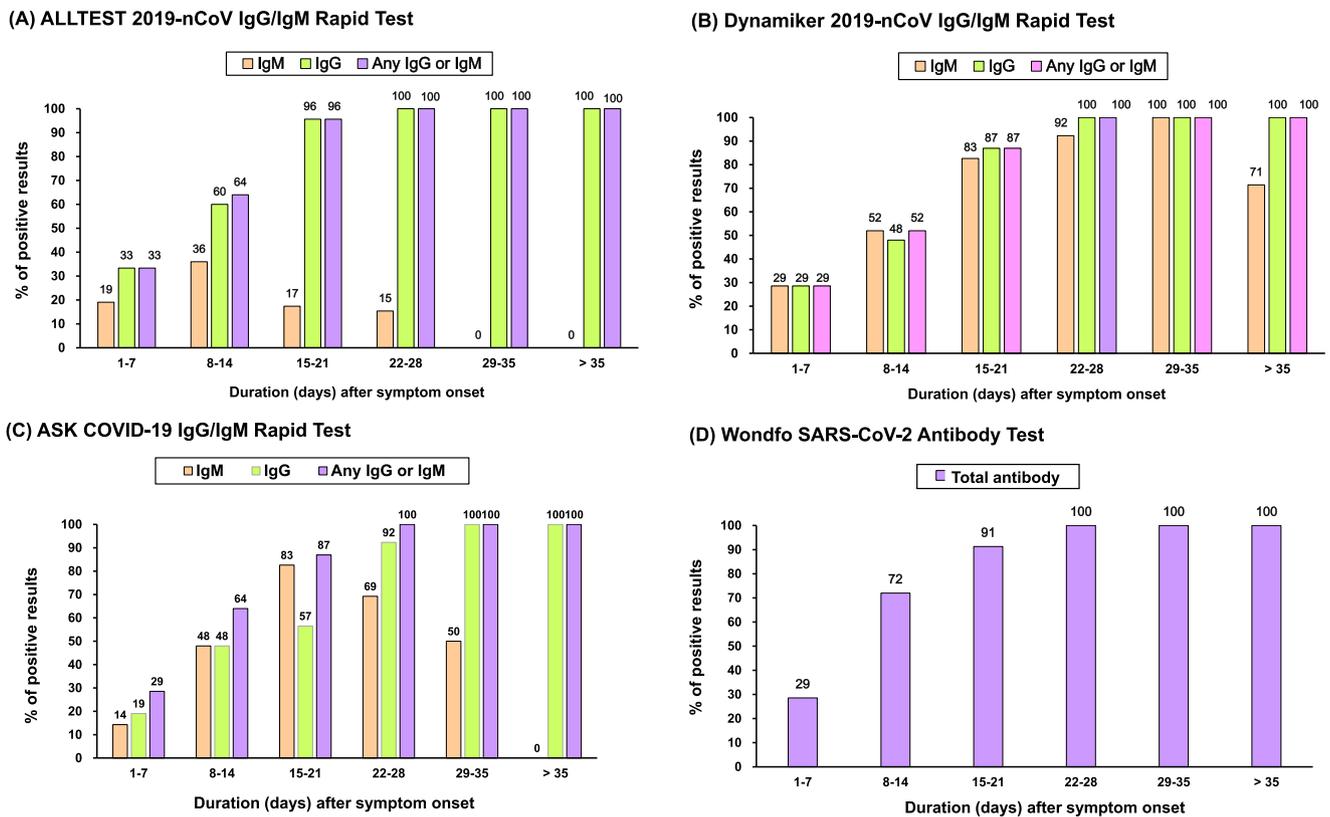
Fig. 1. Percentage of samples with positive antibody response determined using the four studied point-of-care antibody rapid tests after symptom onset. A. ALLTEST ASK COVID-19 IgG/IgM Rapid Test. D. Wondfo SARS-CoV-2 Antibody Test.
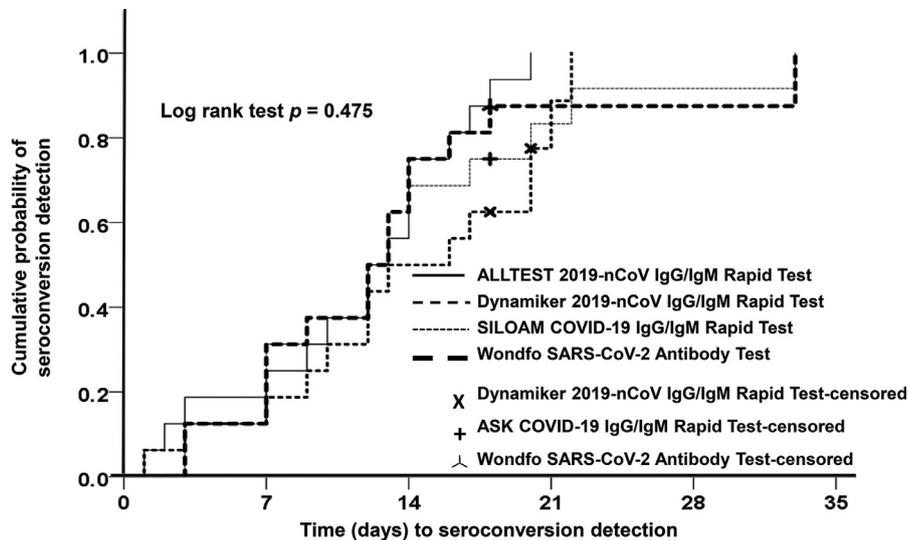
Fig. 2. Parallel comparisons of cumulative probability of detection of seroconversion detection between the four studied point-of-care antibody rapid tests.

days ($p = 0.894$), 10 days ($p = 0.976$), 14 days ($p = 0.632$), and 35 days ($p = 0.475$) of symptom onset based on log rank test.

Ten of the 16 patients with COVID-19 developed clinical and radiographic evidence of pneumonia. Comparison of time required for detection of seroconversion between patients with COVID-19 with and without pneumonia is shown in Fig. 3. Patients with COVID-19 with pneumonia demonstrated shorter seroconversion time than those without pneumonia when dynamic antibody response was evaluated with ALLTEST 2019-nCoV IgG/IgM Rapid Test (log rank test $p = 0.005$) and Dynamiker 2019-nCoV IgG/IgM Rapid Test (log rank test $p = 0.016$). Of the 10 patients with COVID-19 and

pneumonia, three patients had increased oxygen demand after seroconversion and two of them progressed to respiratory failure requiring intensive care.

Correlation between rRT-PCR RNA results for SARS-CoV-2 and anti-SARS-CoV-2 antibody response among patients with COVID-19

The results of rRT-PCR performed for detection of SARS-CoV-2 in respiratory tract specimens were positive in 93 (93.9%) of the 99 blood samples collecting date. The respiratory tract specimens obtained from 16 patients with COVID-19 remained SARS-CoV-2 rRT-

Table 2
Diagnostic sensitivity and specificity of the four point-of-care antibody rapid tests after symptom onset.

	Day 1–14 COVID-19 specimens (n=46)	Control specimens (n=37)	Day 15–21 COVID-19 specimens (n=23)	Control specimens (n=11)	> Day 21 COVID-19 specimens (n=30)	Control specimens (n=10)
ALLTEST 2019-nCoV Rapid Test						
Positive[a]	23	0	22	0	30	0
Negative[b]	23	37	1	11	0	10
Sensitivity,% (95% CI)	50.0 (34.9–65.1)		95.7 (78.1–99.9)		100.0 (88.4–100)	
Specificity,% (95% CI)	100.0 (90.5–100)		100.0 (71.5–100)		100.0 (69.2–100)	
Dynamiker 2019-nCoV Rapid Test						
Positive[a]	19	0	20	0	30	0
Negative[b]	27	37	3	11	0	10
Sensitivity,% (95% CI)	41.3 (27.0–56.8)		87.0 (66.4–97.2)		100.0 (88.4–100)	
Specificity,% (95% CI)	100.0 (90.5–100)		100.0 (71.5–100)		100.0 (69.2–100)	
ASK COVID-19 IgG/IgM Rapid Test						
Positive[a]	22	0	20	0	30	0
Negative[b]	24	37	3	11	0	10
Sensitivity,% (95% CI)	47.8 (32.9–63.1)		87.0 (66.4–97.2)		100.0 (88.4–100)	
Specificity,% (95% CI)	100.0 (90.5–100)		100.0 (71.5–100)		100.0 (69.2–100)	
Wondfo SARS-CoV-2 Antibody Test						
Positive[a]	24	0	21	0	30	0
Negative[b]	22	37	2	11	0	10
Sensitivity,% (95% CI)	52.2 (37.0–67.1)		91.3 (72.0–98.9)		100.0 (88.4–100)	
Specificity,% (95% CI)	100.0 (90.5–100)		100.0 (71.5–100)		100.0 (69.2–100)	

CI, confidence interval; COVID-19, coronavirus disease 2019.
[a] Indicates the presence of either IgM or IgG antibody or both.
[b] Indicates the absence of IgM and IgG antibodies.

PCR positive irrespective of the positive anti-SARS-CoV-2 antibody response detected in blood samples. For the 11 patients who had 3 sequential negative rRT-PCR results and were discharged from hospital isolation, the average duration of persistent positive rRT-PCR results after seroconversion was 16.8 ± 12.9 days (range, 7–53 days; median, 14 days).

Anti-SARS-CoV-2 antibody response among non-COVID-19 patients

Serum samples from 58 hospitalized patients with two negative results of SARS-CoV-2 rRT-PCR tests were used as control group. Among the 58 control group patients, 10 (17.2%) had comorbid condition or received therapy that potentially predisposed them to an impaired immunity. This included malignancy ($n=7$), HIV infection ($n=2$), and post renal transplantation receiving immunosuppressant agents ($n=1$). All these patients had epidemiological risk for contracting SARS-CoV-2 infection, including recent travel history to country with community outbreak of COVID-19 or contact with symptomatic people who had traveled back from a country with community outbreak of COVID-19. The 58 serum samples from the control group patients were tested for SARS-CoV-2 antibody response using the four antibody rapid tests in this study. All yielded negative results. Among the 58 control group patients, causative pathogens were documented in only 18 patients (31%). The pathogens include *Mycoplasma pneumoniae* ($n=6$, 33.3%), *Pneumocystis jirovecii* ($n=3$, 16.6%), *Klebsiella pneumoniae* ($n=2$, 11.1%), *Streptococcus pneumoniae* ($n=1$, 5.5%), *Chlamydophila pneumoniae* (IgG positive) ($n=1$, 5.5%), *Legionella pneumophila* ($n=1$, 5.5%), cytomegalovirus (IgM and IgG positive) ($n=1$, 5.5%), and Epstein-Barr virus (VCA-IgM positive) ($n=1$, 5.5%). The sputum sample of one patient with pneumonia was found to be positive for both methicillin-susceptible *Staphylococcus aureus* and *Pseudomonas aeruginosa*. One febrile patient was diagnosed with urinary tract infection caused by *Enterococcus* species.

Discussion

This study assessed the diagnostic performance of different POC anti-SARS-CoV-2 antibody rapid tests in different chronological stages and severity of COVID-19 infection. There are three major findings of this study. First, usefulness of POC antibody rapid tests in the diagnosis of COVID-19 infection highly depends on the timing of the disease course; in this study, the tests reached 100% sensitivity after 3 weeks of symptom onset. Second, no difference in the diagnosis of COVID-19 could be observed among POC antibody rapid tests obtained different manufacturers. Compared to detection of all antibodies, detection of IgM and IgG separately using rapid tests did not improve the performance of the tests in terms of early diagnosis of COVID-19 infection. Third, patients with pneumonia showed an earlier immune response to SARS-CoV-2 than patients without pneumonia and did not implicate the eradication of virus from the respiratory tract of a patient with COVID-19 based on the presence of RNA detected by rRT-PCR. These findings are important for appropriate application and interpretation of results of POC SARS-CoV-2 antibody rapid tests by first-line physicians for screening, diagnosis, and treatment of patients during the COVID-19 pandemic.

To date, only two studies have investigated the usefulness of POC antibody rapid tests in the diagnosis of COVID-19. The studies reported variable results for diagnostic sensitivity (83–97.5%) and specificity (87–100%).[8,22] In this study, the overall diagnostic sensitivity ranged from 69.7% to 75.8% and specificity was consistently 100% for the four POC antibody rapid tests. The performance was lower than observed in previous two studies. However, the
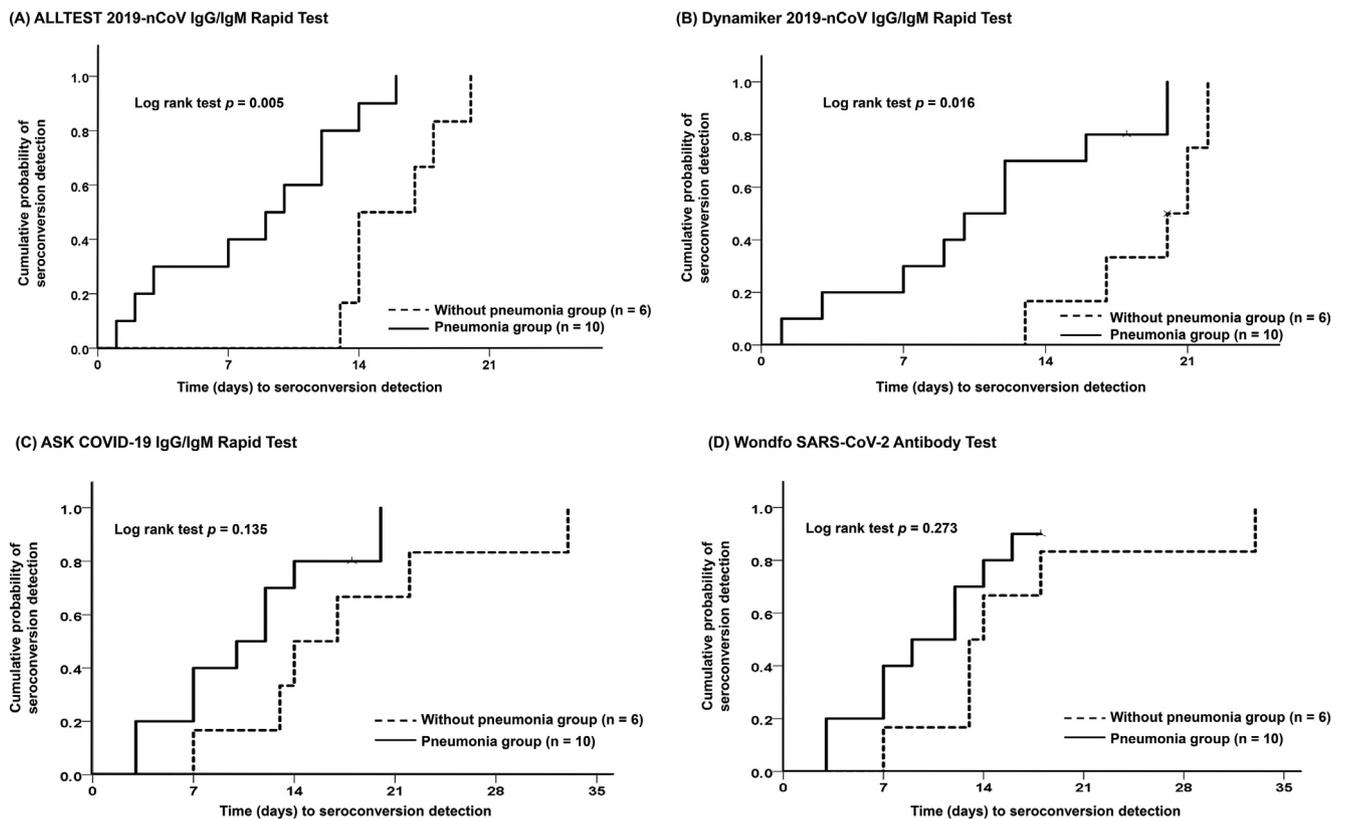
Fig. 3. Detection of seroconversion in patients with COVID-19 with or without pneumonia using the four point-of-care antibody rapid tests. A. ALLTEST 2019-nCoV IgG/IgM Rapid Test. B. Dynamiker 2019-nCoV IgG/IgM Rapid Test. C. ASK COVID-19 IgG/IgM Rapid Test. D. Wondfo SARS-CoV-2 Antibody Test.

time point of serum sample collection is crucial for the evaluation of the diagnostic performance of these POC rapid tests targeting host immune responsive antibodies. Studies have shown an increase in serum antibody levels against nucleocapsid protein (NP) or surface spike protein receptor binding domain (RBD) in samples from patients with COVID-19 obtained after 10–17 days of symptom onset and analyzed using enzyme linked immunosorbent assay (ELISA) or magnetic chemiluminescence enzyme immunoassay (MCLIA).[22-26] In the current study, the diagnostic sensitivity was high and increased to more than 87% between 15 and 21 days after symptom onset and reached to 100% after 3 weeks of symptom onset for all four POC antibody rapid tests. Our study has provided further supportive evidence with a detail chronological analysis and has validated this observation to POC antibody rapid tests based on LFIA. Combining the results of our and previous studies, diagnosis of COVID-19 infection with serological responsive antibodies is most valuable after 2 weeks of symptom onset. Furthermore, a POC rapid test detecting IgM separately from IgG antibodies against SARS-CoV-2 did not add an early and overall diagnostic value compared a POC rapid test detecting total or mixed IgG and IgM antibodies. A similar result was also observed in studies by To et al. and Long et al., which showed an earlier onset and higher overall seroconversion rate of anti-NP IgG than anti-NP IgM antibody among patients with COVID-19.[23,26]

It was observed that anti-SARS-CoV-2-NP or anti-SARS-CoV-2-RBD IgG levels correlated with virus neutralization titer. The viral load also seemed to be related inversely to serum antibody response.[23,24] Therefore, presence of virus-specific antibodies is expected to be associated with rapid virus eradication and clinical improvement. However, prolonged viral shedding with a median duration of up to 14 days after seroconversion was observed in the current study. We also found that the 10 patients with COVID-19 and pneumonia exhibit earlier seroconversion than the six pa-

tients with COVID-19 who did not develop pneumonia. Among the 10 patients with COVID-19 and pneumonia, three patients had evidence of clinical deterioration despite of the presence of anti-SARS-CoV-2 antibodies. In earlier studies by Zhao et al. and Long et al., it was found that a higher titer of Ab was independently associated with clinical severity of COVID-19 infection.[25,26] Previous studies on SARS, which emerged in 2003, have also showed anti-spike IgG antibody stimulated pulmonary pro-inflammatory response and injury in one animal model,[27] and a correlation between a high level upsurge of IgG antibodies in response to SARS-CoV with progression to acute respiratory distress syndrome in another clinical study.[28] As both SARS and COVID-19 are caused by novel coronaviruses, which have similar genetic components and clinical manifestations, it is plausible that both diseases possess the same pathogenic effect. Based on these observations, we propose that the presence of anti-SARS-CoV-2 antibodies does not necessarily indicate the rapid eradication of SARS-CoV-2. Furthermore, early anti-SARS-CoV-2 antibody response might be a surrogate serological indicator for patients with COVID-19 with risk of subsequent pulmonary injury. Therefore, the implications of a positive test for a patient in terms of being a correlate of protective immunity remain not clearly established at this time.

This study has several limitations. First, this was a single-center study. Thus, generalization of our findings requires further confirmation. Second, inadequate case number might have failed to demonstrate the statistical difference in the diagnostic performance between different POC antibody rapid tests. Third, nine serum samples collected before March 1 (seven from the first and two from the second enrolled patient with COVID-19) were stored at −20 °C for more than 9 days before testing them for antibody response.[20] Two of the nine samples were negative for antibody response in all four POC rapid tests. The two samples had been obtained after days 2 and 7 of symptom onset. We could

not exclude the possibility of false negative results for these two serum samples due to prolonged storage. Fourth, laboratory tests for other concomitant respiratory tract viral pathogens, especially other coronaviruses, were inadequate in this retrospective study. Therefore, we could not exclude the possibility of co-infection in our patients with COVID-19. Cross reactivity with non-SARS-CoV-2 coronaviruses by the four POC rapid antibody tests was not well investigated (Table 1). Fifth, pneumonia diagnosis of the COVID-19 patients was primarily based on chest roentgenogram in this study. Because plain chest roentgenogram is less sensitive than computed tomography scan in detecting parenchymal change of viral pneumonia, we could not exclude the possibility of misclassification of a COVID-19 pneumonia patient with subclinical pulmonary infiltration to non-pneumonia patient. Finally, as a non-protocolized retrospective study, serum samples tested in different post-symptom stages were not consistent; therefore, this study is subject to information bias in both clinical and laboratory data. Because of above limitations, findings in the current study need to be further confirmed in a prospectively recruited, adequately powered diagnostic accuracy study.

In conclusion, serological testing may be a useful diagnostic tool in addition to rRT-PCR for the diagnosis of patients with COVID-19. In this study, the detection sensitivity of POC rapid tests corresponded with the antibody dynamics and reached 100% after 3 weeks of symptom onset. COVID-19 patients complicated with pneumonia exhibited earlier seroconversion than those without pneumonia. However, the presence of anti-SARS-CoV-2 antibodies neither indicated the rapid eradication of the virus nor provided immune protection from disease deterioration. Our study findings provide supportive evidence for the appropriate application and interpretation of POC antibody tests in the diagnosis and management of patients with COVID-19.

Declaration of Competing Interest

The authors declare no conflict of interest.

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