CONFIRM BIOSCIENCES

A Case for Broadening both PCR (Molecular) and Antibody Testing of COVID-19

Cases of coronavirus disease 2019 (COVID-19) – caused by infection by the novel coronavirus SARS-CoV-2 – have been on the rise throughout 2020, and as of April 7, 2020, the disease has led to more than 78,000 deaths worldwide.1 Lack of means for timely diagnoses has limited leaders' abilities to slow the spread of COVID and prevent further carnage. There has been much media focus on a lack of COVID test availability, but a more complex issue related to test access is the quality and reliability of the extant tests. Tests for SARS-CoV-2 to date have used polymerase chain reaction (PCR) technologies to detect the relevant viral RNA, but these tests have produced false-negative results, which jeopardize efforts to contain COVID-19. With the rate of false-negatives reported to be as high as nearly 30%, the Centers for Disease Control and Prevention (CDC) has pointed to the potential for false-negatives, and case reports of false-negatives have been published. Researchers have therefore begun exploring other strategies to more reliably identify SARS-CoV-2.2–4

A new publication in Clinical Infectious Diseases describes how supplementing conventional RNA tests with antibody tests could enhance our ability to detect the virus.5 In their paper, Zhao et al. provide evidence that the seroconversion – or development of antibodies - that COVID-19 patients undergo provides an opportunity to improve testing sensitivity and avoid missing cases of COVID-19. The authors show that the nature of this seroconversion facilitates the identification of people currently suffering from viral infection as well as those who may have been infected weeks prior. This combination of capabilities could diminish the impact of the disease by improving patient management as well as endeavors to control the spread.

For their study, the researchers enrolled 173 of the 368 patients (Table 1) who had been admitted to the Shenzhen Third People's Hospital before February 9th of this year and who had tested positive for SARS-CoV-2 based on real-time reverse transcriptase PCR (rRT-PCR) performed on respiratory tract specimens.

TABLE 1 | Demographics and clinical characteristics of patients and sample cohort with COVID-19 in this study.

	Total	Non-critical	Critical
Number	173	141	32
GENDER, N(%)			
Male	84 (49)	63 (45)	21 (66)
Female	89 (51)	78 (55)	11 (34)
Age, median(IQR)	48 (35-61)	41 (33-56)	64 (58-66)
EPIDEMIOLOGICAL EXPOSURE (1 MONTH)			
Been to Wuhan	116 (67)	92 (65)	24 (75)
Been to other Cities of Hubei	10 (5.8)	8 (6.4)	1 (3.1)
Unclear or unknown	47 (27)	26 (28)	7 (22)
COMORBIDITIES, N(%)			
Hypertension	20 (12)	11 (7.8)	9 (28)
Diabetes	11 (6.4)	6 (4.3)	5 (16)
Coronary heart disease	3 (1.8)	0	3 (9.4)
Others*	13 (7.6)	10 (7.1)	3 (9.4)
Any	41 (24)	26 (18)	15 (47)
CLINICAL OUTCOME, N(%)			
Recovery	62 (36)	54 (38)	8 (25)
Still in hospital	109 (63)	89 (62)	22 (69)
Death	2 (1.2)	0	2 (6.3)
DAYS SINCE ONSET, MEDIAN(IQR)			
RNA confirmed †	4 (3-6)	4 (3-6)	6 (4-10)
1st sample for antibody test ‡	7 (5-10)	7 (5-9)	10 (6-16)
RNA (TS/NS) AT THE INVOLVED 1ST SAMPLE			
positive, n(%)	89 (51)	73 (52)	16 (50)
negative, n(%)	65 (38)	55 (39)	10 (31)
no data, n(%)	19 (11)	13 (9.2)	6 (19)
rRT-PCR CT, median(IQR)	29 (25-31)	29 (24-32)	29 (28-31)
No. of antibody tested samples			
Of each case, median(IQR)	3 (2-4)	3 (2-4)	4 (3-5)
Total	535	404	131

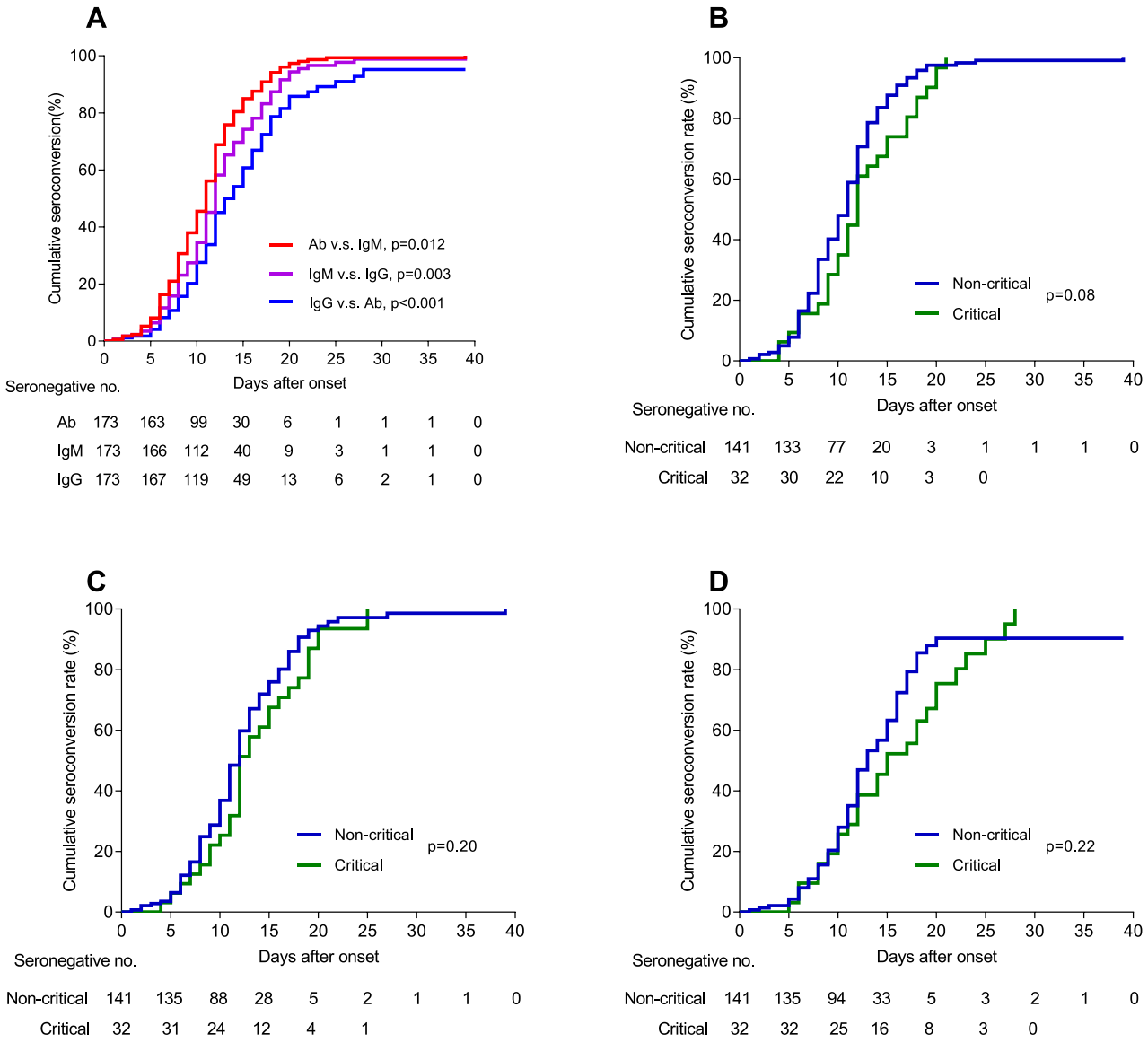
Notes: Data are presented as medians (interquartile ranges, IQR) and n(%). TS, throat swabs; NS, nasal swabs. * The other disease included chronic hepatitis B infection (n=5), tumors (n=2), obstructive sleep apnea syndrome (n=1), chronic bronchitis (n=1), hyperlipidemia (n=1), renal insufficiency (n=1), tuberculosis (cured, n=1) and fatty liver disease (n=1). † The data indicated the time of confirmation for positive for 2019-nCoV infection by using rRT-PCR on respiratory sample since illness onset. ‡ The data indicated the time since illness onset of the first plasma sample of patients involved for serological test in this study.

The scientists tested plasma from these patients for total antibody (Ab), Immunoglobulin M (IgM) antibody, and Immunoglobulin G (IgG) antibody against SARS-CoV-2 with enzyme linked immunosorbent assay (ELISA) kits. Some of their critical findings are as follows:

When tested approximately 1 month after disease onset, all COVID-19 patients had detectable antibodies

With respect to Ab and IgM, seroconversion eventually occurred in all patients whose samples were tested for at least one month following the onset of disease. In general, Ab became detectable first, followed by IgM and then IgG (Figure 1). The median time for seroconversion was 11, 12, and 14 days, respectively.

FIGURE 1



There were 12 patients in the study for whom samples were only available from the early stage of the disease, up to 10 to 13 days following onset. Though these early samples were seronegative, these results are not inconsistent with the notion that antibodies develop in all patients within a month. Even in the case that antibodies to SARS-Co-V-2 could not have been detected in samples from these patients at one month after disease onset, the seroconversion rate for Ab would still prove to exceed 93%, and the seroconversion rate of IgM would exceed 82% (Table 1).

Antibody testing was superior to RNA testing for identifying SARS-Co-V-2 at certain time points

Unlike the RNA test, whose sensitivity maxed out at under 67%, the antibody test for Ab reached 100% sensitivity, and the sensitivities for IgM and IgG reached 94.3% and 79.8%, respectively (Table 2).

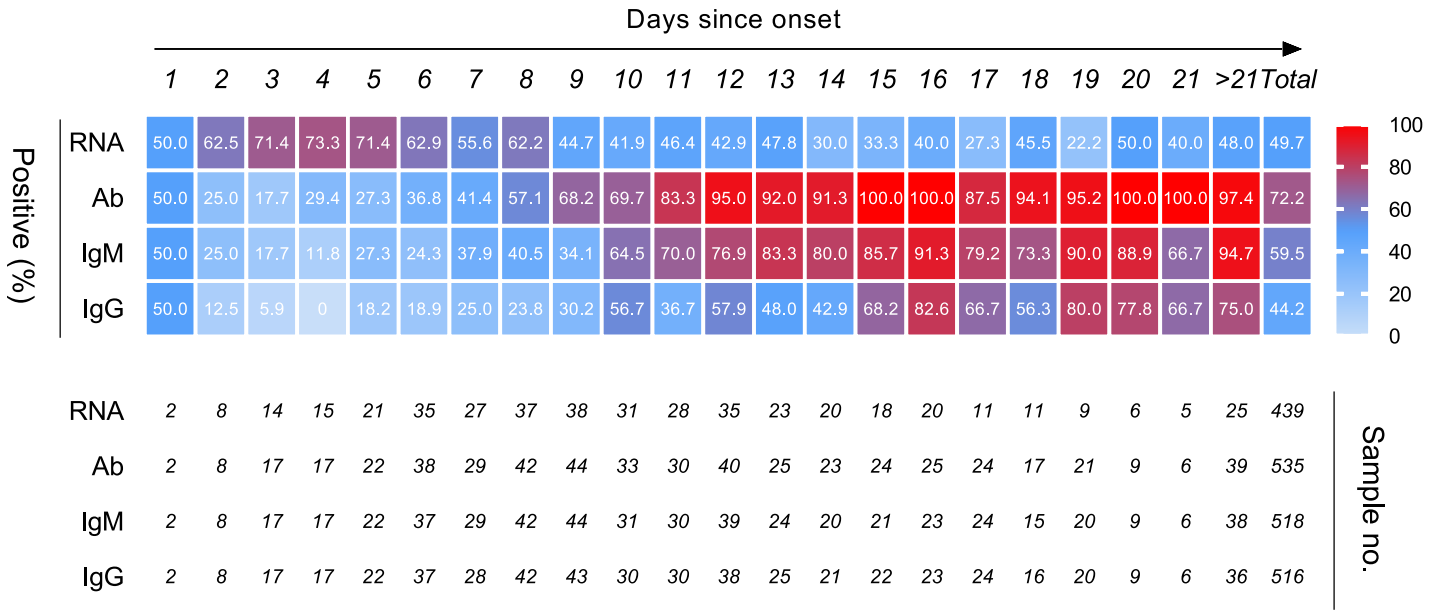
TABLE 2 | Performance of different detections in samples at different time since onset of patients.

Days after onset		RNA			AB			IGM			IGG			RNA+AB		
	n	n(+)	Sensitivity	+%, CI	n(+)	Sensitivity	+%, CI	n(+)	Sensitivity	+%, CI	n(+)	Sensitivity	+%, CI	n(+)	Sensitivity	+%, CI
Total	173	112$	67.1	(59.4, 74.1)	161	93.1	(88.2, 96.4)	143	82.7	(76.2, 88)	112	64.7	(57.1, 71.8)	172	99.4	(96.8, 100.0)
1-7	94	58$	66.7	(55.7, 76.4)	36	38.3	(28.5, 48.9)	27	28.7	(19.9, 39.0)	18	19.1	(11.8, 28.6)	74	78.7	(69.1, 86.5)
8-14	135	67$	54	(44.8, 63.0)	121	89.6	(83.2, 94.2)	99	73.3	(65.0, 80.6)	73	54.1	(45.3, 62.7)	131	97	(92.6, 99.2)
15-39	90	25$	45.5	(32.0, 59.5)	90	100	(96.0, 100.0)	83*	94.3	(87.2, 98.1)	71#	79.8	(69.9, 87.6)	90	100	(96.0, 100.0)

NOTES: * Two patients missed IgM tests due to inadequate plasma samples. # One patient missed IgG tests due to inadequate plasma samples. $ There were 7, 11 and 35 patients had not been performed RNA testing during the 1-7 onset day, 8-14 onset day and 15-39 onset day, respectively.

While the antibody test was superior to the RNA test in identifying SARS-CoV-2 from 8 days after disease onset and thereafter, the RNA test was more sensitive than the antibody test during the week following disease onset (Figure 2).

FIGURE 2



Nonetheless, by identifying seroconversion, the antibody test was able to pick up false negative results produced by the RNA test, even in samples collected as early as day 1 post-onset. In samples from days 1 through 3 the antibody test produced positive results for 28.6% of cases that had been deemed negative through the RNA test (Table 3).

TABLE 3 | Serological presence of antibodies against SARS-CoV-2 in patients with undetectable viral RNA at different time since onset of disease.

DAYS AFTER ONSET	NO. OF PATIENTS WITH UNDETECTABLE RNA*	DETECTABLE ANTIBODY IN PLASMA, N (%)		
		AB	IGM	IGG
1-3	7	2 (28.6)	2 (28.6)	2 (28.6)
4-7	28	15 (53.6)	12 (42.9)	8 (28.6)
8-14	57	56 (98.2)	45 (78.9)	40 (70.2)
15-39	30	30 (100)	28 (93.3)	22 (73.3)

* RNA was tested using throat/nasal swab sample.

The ability of the antibody test to point to a COVID-19 diagnosis in false negative results from the RNA jumped to 53.6% when the samples were collected between days 4 and 7 (Table 3). These results suggest that even in the first week of disease onset, when RNA testing is superior – identifying 66.7% of COVID-19 cases, compared to only 38.3% with the antibody test (Table 2) - a significant number of false negatives could be avoided by incorporating antibody testing into diagnostic procedures.

Though the antibody test can identify COVID-19 patients who tested negative with the RNA test during the early stage of infection, the results it produces during later time points may provide an even more compelling case for the use of the antibody test in the COVID-19 crisis. At 15 to 39 days after disease onset, when antibody sensitivities peaked, reaching 100% for Ab and IgM, RNA could be detected in only 45.5% of samples, and of those who tested negative for COVID-19 using the RNA test during this interval, 100% had detectable Ab (Table 3). Even those who tested negative using the RNA test between days 8 and 14 after disease onset tested positive using the antibody test in 98.2% of cases. Given that RNA testing and antibody testing each showed superior sensitivity at distinct time points, the authors argue that combining these techniques will improve our ability to diagnose COVID-19.

Total antibody levels were associated with COVID-19 case severity

Data from this study showed that rising antibody levels were not always associated with RNA clearance (Figure 3), suggesting that seroconversion did not effectively rid COVID-19 patients of the virus in all cases. It may instead be the case with COVID-19 that antibodies continue to rise in those for whom lower levels of antibodies have not enabled recovery. While no significant difference in Ab levels in critical versus non-critical patients was observed in the first 12 days following disease onset, the data showed that critical patients had higher levels of Ab after day 12 than did non-critical patients (Figure 4). These findings suggest that in addition to older age and male sex, higher Ab levels may also provide predictive value for classifying the clinical severity of COVID-19 cases (Table 4).

FIGURE 3

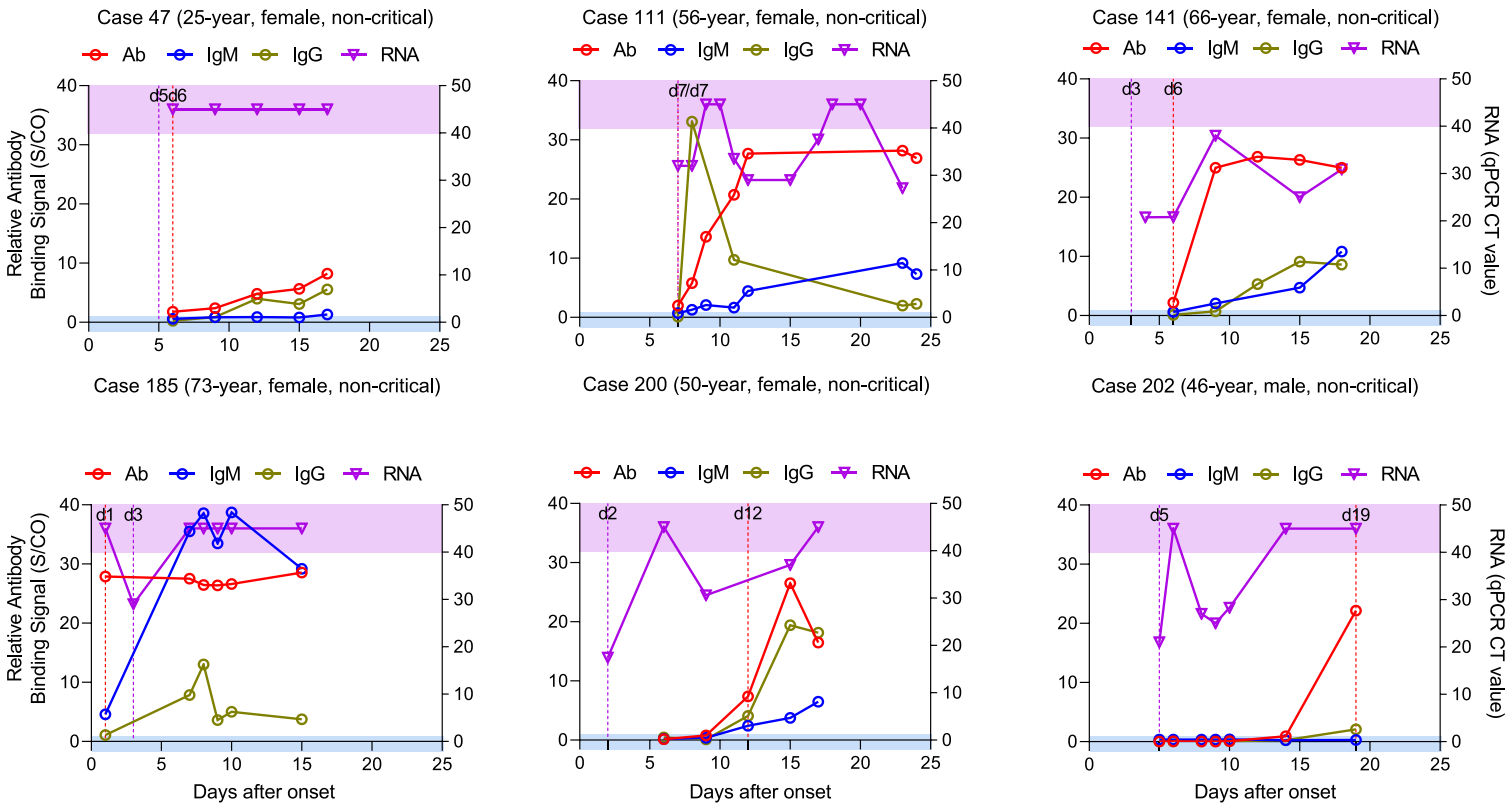


FIGURE 4

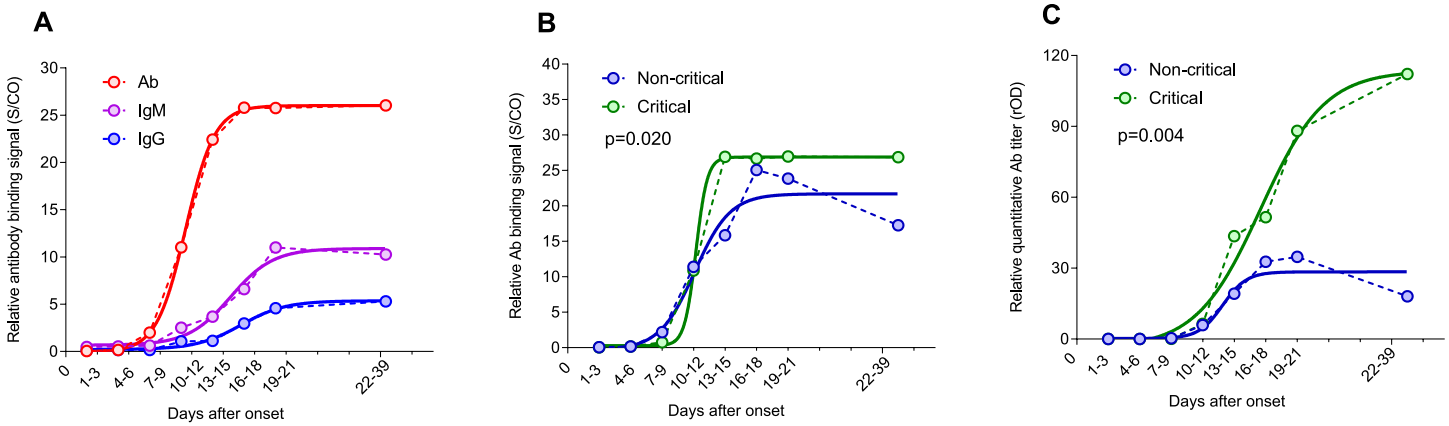


TABLE 4 | Univariate and multivariate analysis of factors associated with patients in critical

CHARACTERISTICS	UNIVARIATE	MULTIVARIATE			
	P VALUE	COEFFICIENT	STANDARD ERROR	95% CI	P VALUE
Age	<0.001	0.138	0.026	0.087-0.190	<0.001
Gender	0.056	1.415	0.512	0.412-2.418	0.006
Comorbidities	0.001	0.06	0.536	-0.991-1.112	0.91
Relative Ab titer	0.004	0.336	0.123	0.095-0.576	0.006

NOTES: p value <0.05 indicates significant differences between critical patients and non-critical patients as determined by GEE model analysis.

Takeaways

The results of a new study on how to identify the virus causing COVID-19 suggest that combining viral nucleic acid testing with serological testing may improve our ability to diagnose the disease and prevent the spread of the virus, especially when that spread is attributable to false negative test results. The data provide evidence that compared to the RNA tests, antibody tests are superior in identifying SARS-Co-V-2, particularly at certain time points. As noted by the authors, antibody tests also generally have some advantages over RNA tests that require PCR, such as a faster turn-around time, higher-throughout, and less laborious workload.

This study investigated samples from 173 COVID-19 patients, 32 of whom were critically ill and required mechanical ventilation. The median age of study participants was 48 years, and just over half of the participants were females (Table 1).

Though the results provide a compelling case for adding antibody testing to the diagnostic toolbox for combating COVID-19 and its spread, the authors also pointed to some limitations. For instance, given that the RNA tests were performed exclusively on specimens produced from patients' upper respiratory tracts, it is possible that these tests' sensitivity may prove higher if specimens are retrieved from the lower respiratory tract. Additionally, because specimen analysis was restricted to only about one month following disease onset, the study does not provide information on the persistence of antibodies in those who have been infected by SARS-Co-V-2.

More research into the seroconversion that occurs in those with COVID-19 and additional comparative data on the sensitivity of RNA tests and antibody tests in identifying the virus will help to elucidate the best ways that these tests can be used alone or in combination to diagnose COVID-19 and mitigate its impact on mankind.

References

1. Calfas, J, Ping, CH, & Hinshaw D. Global coronavirus death toll passes 78,000 as some lockdowns tighten. Wall Street Journal. https://www.wsj.com/articles/global-coronavirus-death-toll-nears-75-000-as-lockdowns-tighten-11586254730. Published 2020. Accessed April 7, 2020.

2. Krumholz H. If you have coronavirus symptoms, assume you have the illness, even if you test negative. New York Times. https://www.nytimes.com/2020/04/01/well/live/coronavirus-symptoms-tests-false-negative.html. Published 2020. Accessed April 9, 2020.

3. Fact sheet for patients: CDC-2019-nCOV real time RT-PCT diagnostic panel. CDC. https://www.cdc.gov/coronavirus/2019-ncov/downloads/Factsheet-for-Patients-2019-nCoV.pdf. Published 2020. Accessed April 9, 2020.

4. Feng, H, Liu, Y, Lv, M, & Zhong J. A case report of COVID-19 with false negative RT-PCR test: necessity of chest CT. Jpn J Radiol. 2020.

5. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis. March 2020. doi:10.1093/cid/ciaa344