

A Persistent Positive Antibody Test in a Patient with No History of COVID-19 Infection

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Abbreviations: RT-PCR, reverse-transcription polymerase chain reaction; Ab, antibody; COI, cutoff index.

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ABSTRACT

Antibody testing for SARS-CoV-2 has been established as a tool with broad utility in the surveillance and control of the COVID-19 pandemic. However, because of limited knowledge about the duration of humoral immunity to COVID-19 and the existence of unique individual immune responses, the potential role of antibody testing in the diagnosis of current and past infections of COVID-19 remains ambiguous. Herein, we describe a unique case of an asymptomatic patient showing a persistent positive total antibody test for SARS-CoV-2 while testing negative for SARS-CoV-2 RNA and IgG-specific antibodies. This case study shows how a combination of tests can be employed to identify a false positive and draw conclusions about a patient's COVID-19 status. It also highlights the complexity of using antibody testing for the diagnosis of COVID-19.

The infectious disease COVID-19, caused by SARS-CoV-2, has spread rapidly around the world, causing a pandemic. Many recent studies have focused on the utility of SARS-CoV-2 antibody testing for identifying current and past infections.^{1,2} In addition, antibody testing has been explored as a tool for obtaining information on the stage of disease progression,²⁻⁴ for identifying undiagnosed infections past the point of viral shedding,^{5,6} and as a promising option for monitoring the portion of a population previously infected.^{7,8} However, despite the development of tests offering high levels of sensitivity and specificity for SARS-CoV-2 antibodies,⁹ many limitations exist for these potential uses. These include a lack of knowledge of the duration of SARS-CoV-2 antibodies after infection¹⁰ and the impact of low population seroprevalence on the ability to make accurate predictions using antibody testing.⁹ In addition to these limitations, multiple unique cases have been reported that illustrate the complexity and lack of clarity of the role that antibody tests should play in diagnosing COVID-19 infection.

^{6,11} Additional research is needed to explore the factors that influence antibody test accuracy and to clarify how to manage patients in whom the test results do not give a straightforward answer. Here, we report a unique case of a patient with no evidence of current or past infection with COVID-19 who persistently tested positive for SARS-CoV-2 antibodies on 1 test while testing negative on another.

Case Report

On June 10, 2020, a 45 year old woman and her husband and daughter presented to a testing clinic in Dallas, Texas to undergo testing for COVID-19. She reported a potential exposure to COVID-19 approximately 10 days prior through a close interaction with a family member who had direct exposure to someone diagnosed with COVID-19. The patient received a SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) test using a self-administered throat swab that returned negative. She was also evaluated using a plasma total antibody (Ab) Elecsys Anti-SARS-CoV-2 serology test supplied by Roche Diagnostics (Rotkreuz, Switzerland), completed according to the manufacturer's instructions. The total Ab test results came back positive with a cutoff index (COI) of 3.51. The patient's husband and daughter received negative results on both the SARS-CoV-2 RNA and total Ab tests. On June 12, 2020, the patient's 3 other children received the SARS-CoV-2 RNA and Roche total Ab tests, and all results came back negative.

Because the patient and her family showed no evidence of SARS-CoV-2 infection, the patient's immune response was re-evaluated on June 15, 2020 with a fresh serum specimen using the Abbott ARCHITECT i2000sr platform from Abbott Laboratories (Chicago, IL) to test for plasma IgG Abs. This test was performed according to the manufacturer's instructions and returned negative. The specificities of the total Ab assay and IgG assay to specimens with SARS-CoV-2 were determined as 99.7% (1151/1154) and 99.2% (1145/1154), respectively, by testing SARS-CoV-2-negative serum specimens collected before the outbreak.⁹

To minimize the probability of laboratory error for the Roche total Ab test, the serum from the June 10 blood draw was retested on June 17 and returned positive with a COI of 3.54, confirming the result of the previous test. Because a false positive was suspected for the Roche test, a third serum specimen was collected on June 19. Portions of this specimen were sent to 2 laboratories: One conducted a Roche total Ab test and the other conducted a Roche test and an Abbott IgG test. The Roche total Ab test returned positive for both laboratories, with COI values of 3.36 and 3.6. The repeated Roche tests with similar COI values confirmed that laboratory error was likely not the cause of the positive result. The Abbott IgG test returned negative with a COI of 0.1, confirming the original Abbott results. The median COI value for each type of antibody test was compared to characteristic COI values for different spec-

imen types, as shown in [TABLE 1](#). As of late August 2020, neither the patient nor her family had exhibited any symptoms of COVID-19.

Retrospectively, the patient disclosed experiencing flu-like symptoms in early February 2020 that included 3 to 5 days of fatigue, weakness, runny nose, and chills. Two of the patient's children experienced similar symptoms approximately 1 week later and tested positive for the flu by point-of-care testing of viral RNA. The patient reported a quick recovery with no enduring symptoms. The patient reported no history of chronic medical conditions or serious illness, does not regularly take any medications other than melatonin, has no known allergies, and does not believe she was exposed to previous outbreaks of SARS-CoV-1 or MERS-CoV. The patient also reports no other potential exposures to COVID-19.

Discussion

We report a case of a patient who persistently tested positive for SARS-CoV-2 total Abs despite no evidence of previous COVID-19 infection, a negative SARS-CoV-2 RNA test, and a negative IgG Ab test. The combination of the repeated test results and the clinical presentation suggest that the patient did not have COVID-19 and that the Roche total Ab test returned a replicable false positive. Although it is currently unclear how many patients develop antibodies to SARS-CoV-2 after infection, some literature suggests it is possible for 100% of patients with COVID-19 to produce detectable antibodies.^{3,4} However, there is also evidence that some patients may not develop antibodies^{9,11} or may show a delayed antibody response.⁶ Although the immune response to SARS-CoV-2 infection is not uniform, it is unlikely that none of the patient's family would have tested positive for total Abs if some or all had contracted COVID-19 in February or June 2020. It is possible that the patient could have contracted COVID-19 independently of her family if she was able to successfully isolate herself from them during her symptomatic period in February 2020 or postexposure in June 2020. However, given that the patient had no symptoms of infection in June 2020 and was negative for viral RNA and IgG-specific Abs, the positive result on the total Ab test alone is not consistent with the unique patients in whom antibody response is delayed or undetectable.^{6,9,11} Furthermore, the lack of detectable IgG Abs for this patient indicates that infection in February 2020, when she experienced flu-like symptoms, was also reasonably unlikely. IgG Abs are indicative of the long-term immune response to an infection,² and recent data have shown that up to 90% of patients with a positive SARS-CoV-2 PCR test continue to exhibit detectable levels of anti-SARS-CoV-2 IgG Abs between 40 and 199 days after testing positive.¹²

In a typical patient with COVID-19, testing positive for total Abs—antibodies of any kind—but not IgG alone may indicate that the infec-

tion is active and recent.² However, median seroconversion times for total Abs and IgG Abs to SARS-CoV-2 have been reported at 11 and 14 days after symptom onset, respectively,³ making it unlikely that this patient would continue to test negative for IgG 9 days after the first positive total Ab test and 20 days after potential exposure. In addition, data reported by Roche Diagnostics shows that the COI (a ratio of a specimen readout to the cutoff value for a positive result) for the total antibody test, although not directly indicative of antibody concentration, continues to increase for up to at least 35 to 40 days after diagnosis.¹³ However, the patient's repeated Roche total Ab tests showed negligible change in COI value over time, and her result more closely matched the median COI for a false positive test than for a true positive, according to research conducted by Perkmann et al⁹ that compares the Roche and Abbott antibody tests ([TABLE 1](#)).

Notably, Perkmann et al⁹ also reported that of 12 specimens in their data set that returned a false positive on either the Abbott test or Roche test, none returned a false positive on both. Given that both antibody tests have comparable sensitivities and use the same viral protein,⁹ the discrepancy is not likely caused by differing abilities to detect low concentrations of antibodies. One possibility is that the replicable false positive is caused by some aspect of the composition of the Roche test, or the particular recombinant SARS-CoV-2 protein it employs, that is significant to the patient's serum and that the Abbott test does not share. For example, antibodies to a similar virus or nonspecific heterophile antibodies in the patient's serum could bind to the recombinant protein, leading to an apparent positive result despite SARS-CoV-2-specific antibodies being absent. Alternatively, anti-antibodies in the patient's serum could interfere by binding to any antibodies utilized in the immunoassay, although this mechanism of interference is only minimally likely for antibody tests; most use indirect detection methods, where serum is washed away before the addition of any secondary antibodies. The details of antibody test composition are not publicly available and thus cannot be analytically compared, precluding our ability to make a definitive conclusion on what could have caused the false positive result. However, this hypothesis would explain the discrepancy between manufacturers for the patient and the lack of other evidence of COVID-19 infection.

In summary, we report a unique case in which a patient had a persistent test-specific false positive result for SARS-CoV-2 antibodies. This case study addresses the complexity of interpreting antibody test results and suggests that additional testing with another brand or manufacturer of antibody test would be a useful strategy for confirming a suspected false positive. It is important to consider several factors, including RT-PCR, clinical presentation, antibody test results, exposure timeline, population seroprevalence, and the progression of SARS-CoV-2 antibody se-

TABLE 1. SARS-CoV-2 Antibody Test COI Values for Positive and Negative Serum Specimens

Serology Specimen	Median COI Value	
	Roche T-Ab (cutoff, 1.0) ^a	Abbott IgG (cutoff, 1.4) ^a
Patient	3.54	0.1
Confirmed positive ^b	24.2 (7.22–52.90)	4.87 (2.77–6.78)
False positive ^b	1.65 (1.47–1.72)	2.21 (2.14–2.67)
Confirmed negative ^b	0.08	0.03

COI, cutoff index; T-Ab, total antibody.

^aCutoff represents the smallest COI value at which the specimen is considered positive for SARS-CoV-2 antibodies.

^bMedian COI data taken from Perkmann et al.⁹ Values in parentheses indicate the interquartile range for the COI values of the specimens in this category.

roconversion when attempting to make a diagnosis and issue guidance to a patient. False positive results on an antibody test can give patients a false sense of security that they are protected from future COVID-19 infection and thus can encourage behaviors that carry a higher chance of infection.⁸ Further research and surveillance are needed to address the causes of test-specific false positive results and to clarify the use of antibody tests for the diagnosis of COVID-19 in patients with ambiguous combinations of test results.

REFERENCES

1. Deeks JJ, Dinnes J, Takwoingi Y, et al.; Cochrane COVID-19 Diagnostic Test Accuracy Group. Antibody tests for identification of current and past infection with SARS-CoV-2. *Cochrane Database Syst Rev*. 2020;6:CD013652.
2. Jacofsky D, Jacofsky EM, Jacofsky M. Understanding antibody testing for COVID-19. *J Arthroplasty*. 2020;35(7S):S74–S81.
3. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. *Clin Infect Dis*. 2020;71(16):2027–2034.
4. Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med*. 2020;26(6):845–848.
5. Suhandynata RT, Hoffman MA, Kelner MJ, McLawhon RW, Reed SL, Fitzgerald RL. Longitudinal monitoring of SARS-CoV-2 IgM and IgG seropositivity to detect COVID-19. *J Appl Lab Med*. 2020;5(5):908–920.
6. Zhao J, Liao X, Wang H, et al. Early virus clearance and delayed antibody response in a case of coronavirus disease 2019 (COVID-19) with a history of coinfection with human immunodeficiency virus type 1 and hepatitis C virus. *Clin Infect Dis*. 2020;71(16):2233–2235.
7. Atchison C, Pristerà P, Cooper E, et al. Usability and acceptability of home-based self-testing for SARS-CoV-2 antibodies for population surveillance. *Clin Infect Dis*. 2021;72(9):e384–e393.
8. Peeling RW, Wedderburn CJ, Garcia PJ, et al. Serology testing in the COVID-19 pandemic response. *Lancet Infect Dis*. 2020;20(9):e245–e249.
9. Perkmann T, Perkmann-Nagele N, Breyer MK, 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):1405–1413.
10. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med*. 2020;26(8):1200–1204.
11. Zhang X, Li M, Chen T, Lv D, Xia P, Qian W. Persistent negative antibody test in COVID-19 patient: a case report. *Clin Infect Dis*. 2021;72(5):901–903.
12. Figueiredo-Campos P, Blankenhaus B, Mota C, et al. Seroprevalence of anti-SARS-CoV-2 antibodies in COVID-19 patients and healthy volunteers up to 6 months post disease onset. *Eur J Immunol*. 2020;50(12):2025–2040.
13. Elecsys Anti-SARS-CoV-2. Package insert. Roche Diagnostics. <https://www.fda.gov/media/137605/download>. Accessed May 5, 2021.