# SARS-CoV-2 Serology Results in the First COVID-19 Case in California: A Case Report and Recommendations for Serology Testing and Interpretation

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**Case Report**

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# Abstract

**Background:** As countries in COVID-19 pandemic lockdown begin relaxation of shelter-in-place mitigation strategies, the role of serology testing escalates in importance. However, there are no clear guidelines as to when to use qualitative rapid diagnostic serology tests (RDTs) vs. SARS-CoV-2 viral RNA load (PCR) tests as an aid in acute diagnosis of patients presenting with flu-like symptoms, nor how to interpret serology test results in asymptomatic individuals or those with atypical COVID-19 symptomatology. Here we describe, in the context of the likely first case of COVID-19 in California, with an atypical presentation and not tested acutely, who nearly 3 months later was found to be IgM- and IgG+ positive for SARS-CoV-2 antibodies, highlighting the role of RDT-based serology testing and interpretation in retrospective diagnosis.

**Case Presentation:** A 62-year-old male practicing neurosurgeon had onset of flu-like symptoms on January 20 with fatigue, slight cough only on deep inspiration, intermittent pleuritic chest pain unrelated to exertion, dyspnea, and night sweats but without fever, sore throat or rhinorrhea. He had recently traveled abroad but not to China. CT scan revealed right lower lobe infiltrate and effusion. Because of atypical symptoms, and low prevalence of COVID-19 in January, community acquired pneumonia was diagnosed and one week of doxycycline was prescribed without relief, followed by a second week of azithromycin with symptom remission. Three months later the physician-patient (author THL), tested positive for SARS-CoV-2 antibodies by a serology point-of-care rapid diagnostic test (RDT).

**Conclusions:** Serology testing may be an aid in acute diagnosis of COVID-19, especially in patients with atypical presentations, as well as in assessment of asymptomatic higher-risk persons such as healthcare workers for prior infection. Recommendations for serology testing and interpretation are explicated.


# Background

As serology testing for COVID-19 immunoglobulins M and G (IgM/IgG) becomes more relevant further into the pandemic, many questions remain concerning test quality, criteria for test ordering, and interpretation of results. Positive qualitative rapid diagnostic serology tests to not assure lack of infectiousness, nor do they assure antibody effectiveness, i.e. immunity from SARS-CoV-2 infection [1]. As of April 17, the COVID-19 Tracking Project reported that 3.4 million RT-PCR viral RNA load (PCR) tests had been conducted in the U.S. [2], about 350,000 of which were conducted by the CDC and other U. S. public health laboratories [3], but information on the volume, results, and interpretation of serology testing results are lacking. Here we describe what appears to be the first case of COVID-19 in Los Angeles County, California, diagnosed based on retrospective clinical diagnosis and diagnostic testing for SARS-CoV-2 antibodies obtained almost three months post-infection. It suggests that COVID-19 was circulating earlier than widely thought. Serologic RDT testing may aid in both acute diagnosis and detection of prior infection, however test timing and symptom chronology, differences in RDT test performance, and pre-test prevalence of COVID-19 all affect serology test interpretation. Approaches and considerations regarding rapid, blood-based RDT testing for the presence of IgM and/or IgG antibodies, results

interpretation, and implications pertinent to both symptomatic and higher risk asymptomatic persons are discussed.

## Case Presentation

A 62-year-old neurosurgeon, otherwise healthy except for hypertension controlled with medication, presented on January 20 with onset of fatigue, slight cough only on deep inspiration, intermittent pleuritic chest pain unrelated to exertion, dyspnea, and night sweats but without fever, sore throat or rhinorrhea. Travel history was limited to a trip to Panama with return to Los Angeles airport on January 5th. A recent, unrelated CT coronary angiogram had revealed neither stenosis nor coronary artery calcifications. The previous fall the physician had annual compulsory influenza vaccination as well as pneumococcal conjugate vaccine (PCV13). He went hunting January 22-26, noticed worsening fatigue and dyspnea on exertion while walking the fields, and returned home January 26 with worsening intermittent chest pains characterized by deep aching and sometimes sharp stabbing pain related to deep breath. On January 26 he sought pulmonologist consultation and right lower lobe rales on auscultation and possible effusion on chest percussion were noted. A chest radiograph revealed right lower lobe (RLL) patchy infiltrate, while pulmonary function tests were unremarkable. Chest CT scan 29 January confirmed RLL pneumonia with pleural effusion (Fig. 1).

Highest temperature during the episode was 37.3°C. Laboratory tests excluded PCR testing for SARS-CoV-2 due to atypical presentation and low prevalence of COVID-19. Laboratory results ordered during the acute episode were remarkable only for modest elevations in high sensitivity C-reactive protein (hsCRP) 2.98 (normal range 0.20-1.00 mg/L) and D-dimer 2.22 (0.61-0.70 µg/mL), otherwise lab results were normal including WBC $5.1x10^3$ with normal differential, ESR, chemistry and liver functions tests, ß-natriuretic peptide, and troponin I. Repeat CT scan on February 13 showed mild residual patchy infiltrates RLL although symptoms had remitted. Community acquired pneumonia was diagnosed and treated empirically with doxycycline for one week without symptomatic relief, then azithromycin the following week accompanied by symptom remission. The physician-patient remained afebrile, continued to operate and work in clinic despite two weeks of fatigue and dyspnea.

Almost three months post-symptom onset a wealthy patient insisted that all her doctors undergo serological testing, and on April 10 the physician-patient's rapid diagnostic test (RDT) result was IgM-/IgG+ (Phamatech Lab COVID 19 RAPID Test IgM/IgG) (Fig. 2).

The same serology RDT was repeated April 13 and was negative, but the technician discarded the test strip in under two minutes despite kit instructions for use recommending waiting 8-10 minutes. A third serology test on April 17 was IgM-/IgG+ after 3 minutes 40 seconds, consistent with the initial RDT result.

## Discussion

This case may be the first patient to contract COVID-19 in California, and perhaps the third in the United States, given that the third and fourth cases in the country were diagnosed on January 26 in California [4]. Serology testing may have had utility in acute diagnosis, particularly in patients with atypical presentations such as this case. In addition, there is a rapidly growing need for serology testing as many patients appear to contract COVID-19 infection without becoming symptomatic. Guidelines remain unclear as to when to order a qualitative RDT serology test and how to interpret the results, therefore we propose the following recommended considerations and approach.

Pre-test probabilities should be factored into laboratory testing decisions and should appropriately influence physician interpretation of results [5]. The very low U.S. prevalence of COVID-19 in late January, combined with an atypical presentation (78% of Chinese patients describe fever as the primary symptom, followed by persistent cough) [6], and lack of risk factors such as travel to China, may have led to a result more likely to be a false positive than a true positive. The hsCRP and D-dimer level results could be considered non-specific given their modest specificities [7]. It should be noted, however, that D-dimer levels > 2.0 μg/mL have been reported as a strong predictor of COVID-19 in-hospital mortality, possibly reflecting activation of coagulation and fibrinolysis [8]. A positive finding of influenza hemagglutination inhibition (HAI) antibodies in a previously immunized person would not differentiate between acute influenza infection and HAI from the vaccination, and a negative result would not necessarily increase suspicion for COVID-19, given the limited 45% estimated efficacy rate of the influenza vaccine in 2019-2020 [9]. The CT scan finding of unilateral vs. bilateral infiltrate and effusion is another somewhat atypical finding, although approximately 20% of Chinese COVID-19 patients present with unilateral rather than bilateral radiological findings [10,11]. However, as the pre-test probability of COVID-19 infection has currently risen to levels many times higher than expected in the medical community, practitioners today should have a commensurately high index of suspicion for COVID-19 in the differential diagnosis. Two studies in April provide striking illustrations: first, studies a PCR-based screening of 3,300 patients in Santa Clara County, California found a demographic and test sensitivity adjusted prevalence of 4.2% [12], and second, a New York-based study reported 13.7% community prevalence based on universal screening of 215 pregnant women [13].

As the community prevalence of COVID-19 explodes, the question as to whether asymptomatic or mildly symptomatic healthcare professionals were previously infected has become more acute. Practitioners live with high states of anxiety related to surviving in a work environment with high levels of exposure to infected patients. Knowledge of prior infection might ease these concerns, with the important caveat that qualitative RDT serology tests do not provide quantitative IgG titers as enzyme linked immunosorbent assays (ELISA) do, and neither test guarantees immunity. To establish immunity, a 3-5 day neutralization assay would have to show that the patient's antibodies can inhibit viral growth in a cell culture system. However, there are no FDA-approved/authorized neutralization assays for SARS-CoV-2 in the U.S, these assays may return false negative results if they miss antibodies to viral proteins not involved in replication, and a recent Chinese study found poor correlations between high titers of neutralizing antibodies and efficacy retarding virus growth in cell culture [14]. Although IgM-/IgG+ results indicate past infection, it is not established that such patients cannot continue to transmit the virus. Current CDC

guidance for healthcare workers after positive COVID-19 diagnosis (which may be based on clinical impression or test result), include a test-based strategy of obtaining two negative PCR tests 24 hours apart to rule out SARS-CoV-2 viral loads in the nasopharynx, or a non-test based strategy in lab-test confirmed COVID-19 positives who are asymptomatic to wait ten days after their test result so long as they do not develop symptoms during that interval [15].

Positive serology results may indicate either current and recent infection (IgM titer elevated) or prior infection (IgM-/IgG+). Because SARS-CoV-2 viral RNA loads rise before IgM levels, PCR tests are preferred in early acute infection, and serology tests may have a significant false negative rate as illustrated in Fig. 3.

After an incubation period averaging 5 days (range 1-2 days to 14 days), COVID-19 symptoms develop [16]. The exact time course for the rise in viral RNA vs. IgM/IgG titers is not yet broadly studied and may vary if different serology assay have different limits of detection. Guo et al. found that 22% of serology tests were negative in symptomatic COVID-19 patients positive by PCR, and that IgM peaked 5 days after symptoms onset vs. IgG at 14 days [17]. The false negative rate for serology testing may also be related to symptom severity, with Wu et al. reporting that 30% of COVID-19 recovered patients never developed high levels of neutralizing IgG antibodies [14]. In the Guo et al. study there was no cross-reactivity against the “benign” coronavirus strains NL63, 229E, OC43 and HKU1 which are responsible for one-third of common colds [18]. Although false positives could be caused by cross-reactivity of SARS-CoV-2 test antibodies to SARS-CoV-1, this is unlikely to cause a false positive in the U.S., given its very low incidence of SARS cases in 2003-2004 [19]. Other serology tests may not achieve the same specificity as the assay cited above, thus clinicians should be alert to potential assay differences in cross-reactivity to common cold coronavirus strains [20].

In order to expedite test availability, The FDA has not insisted on reviewing and authorizing all serology tests until May 4 [21]. Of the many dozens of different tests currently promoted commercially, only three serology tests achieved FDA Emergency Use Authorization (EUA) as of April 15. Importantly, FDA EUAs for both PCR and serology tests do not require reporting of clinical sensitivity and specificity based on COVID-19 samples split and compared to a reference standard. Instead EUAs are issued based on analytic performance using contrived samples in test tubes. This approach has important limitations, for example analytic performance review only for a PCR test may not reveal limitations in clinical sensitivity caused by inadequate sampling of the nasopharynx, and contrived samples used to evaluate a serology test that lack common cold coronavirus antigens may not reveal problems with clinical specificity. Published test performance would not have predicted the likely false negative 2$^{nd}$ serology test in this physician-patient where the lab technician did not wait the recommended 8-10 minutes for the test to turn positive. In general, we expect clinical test performance to be lower than the analytical sensitivity and specificity reported in FDA Instructions for Use for COVID-19 tests.

Clinical index of suspicion for COVID-19 should now be high as prevalence has risen quickly, and as it has been systematically underestimated due to narrow criteria limiting testing to the sickest patients. As

many as 44%-79% of cases may be transmitted by asymptomatic patients who would never be selected for PCR testing because of the lack of symptoms of a viral syndrome [22,23]. Many more, like the case here, may present with atypical or mild symptoms and serologic testing could facilitate retrospective diagnosis of COVID-19. PCR vs. serology test selection should be related to duration of symptoms, with the PCR test reserved for acute cases ill for ≤ 7 days, especially in a country where access to PCR testing is limited because of test kits or reagents, inadequate staffing, or supplies of nasal swabs. Antibody testing has been shown to be more sensitive than viral nucleic acid detection after approximately eight days of COVID-19 illness duration in two separate studies [17,24]. Patients with symptoms for more than 7 days might be confirmed with positive IgM titers on a serology test. However, symptomatic IgM-/IgG- patients may be falsely negative, and we would recommend PCR testing in this scenario. Persistence of IgM elevation in either IgM+/IgG- patients or IgM+/IgG+ serology indicates active infection and these patients should be isolated and their contacts traced. IgM-/IgG+ results indicate past infection and isolation is unnecessary. However, IgM-/IgG+ results on a qualitative RDT serology test should not be considered a guarantee of SARS-CoV-2 immunity, and contagion safety precautions should be maintained. In IgM-/IgG+ cases by qualitative RDT testing, a follow-up quantitative IgG ELISA with a high antibody titer might support the argument for immunity, similarly to the way higher rubella antibody titers in pregnant women indicate immunity [25], however this recommendation requires further study. However, this recommendation, like others above, are limited by the general lack of available information on COVID-19, as well as inadequate information on test performance, even for FDA-authorized tests.

# Conclusions

This case of an atypical COVID-19 presentation, highlights the importance of maintaining a high clinical index of suspicion and ordering SARS-CoV-2 viral load or antibody testing as an adjunct to diagnosis. Serologic RDTs can serve as a rapid adjunct for acute diagnosis, with point-of-care results available within ten minutes. Novel immunoassays may have higher sensitivity than PCR-based testing which may miss 1/4 of cases [26,27], although RDT test performance is assay-specific and variable today. For now, PCR-based nasal swab or saliva testing should be ordered instead in the first week of symptoms, with RDT used after 7 days. RDT-based serology testing also serves as an indicator of past-COVID-19 infection, identifying donor candidates for antibody harvesting and potentially relieving demands for personal protective equipment, as well as improving our understanding of COVID-19 pandemic epidemiology. However, whether IgM-/IgG+ patients are immune, will likely require further evaluation with high quantitative IgG ELISA-based antibody titers or neutralization assays that are well studied and characterized.

# Declarations


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- Ethics approval and consent to participate not applicable for this standard of care case report.
- Consent for publication: Author THL consents for use of his personal health information, and will provide consent form upon request.
- All data generated or analyzed during this study are included in this published article.
- The authors declare that they have no competing interests.
- RBL drafted the initial manuscript. Both RBL and THL analyzed and interpreted the patient data regarding the case. All authors read and approved the final manuscript.
- Acknowledgements are found below the main article.

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# Figures

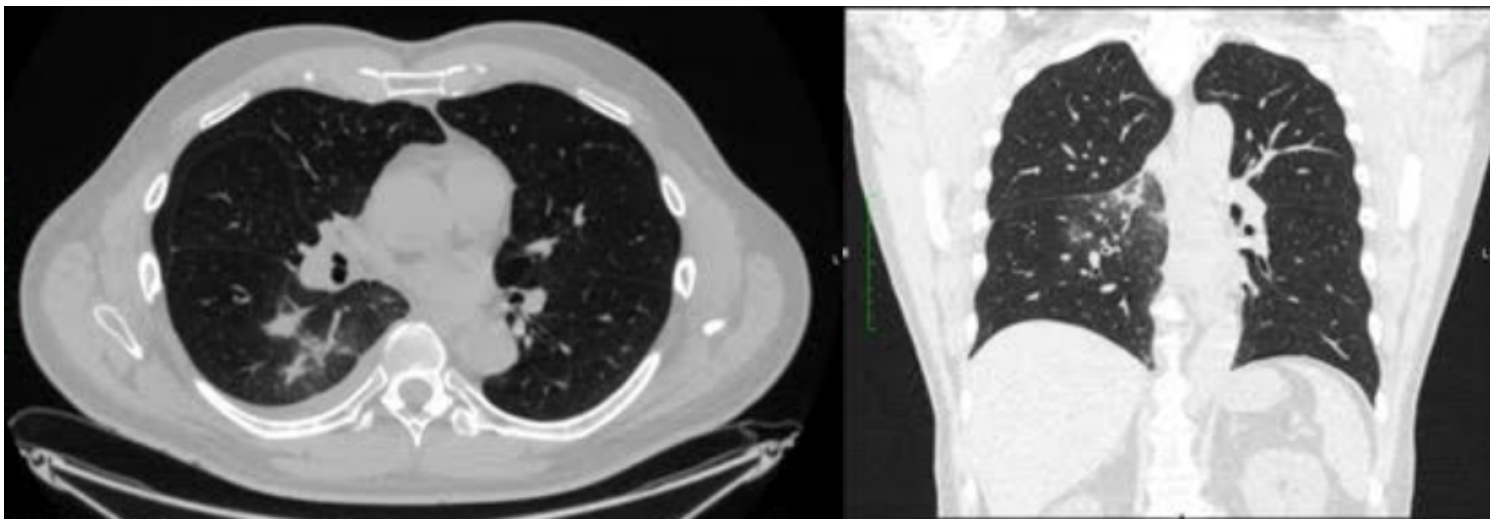

**Figure 1**

Chest computerized tomography (CT). CT (while supine) showing patchy infiltrates and pleural effusion in right lower lobe (RLL).

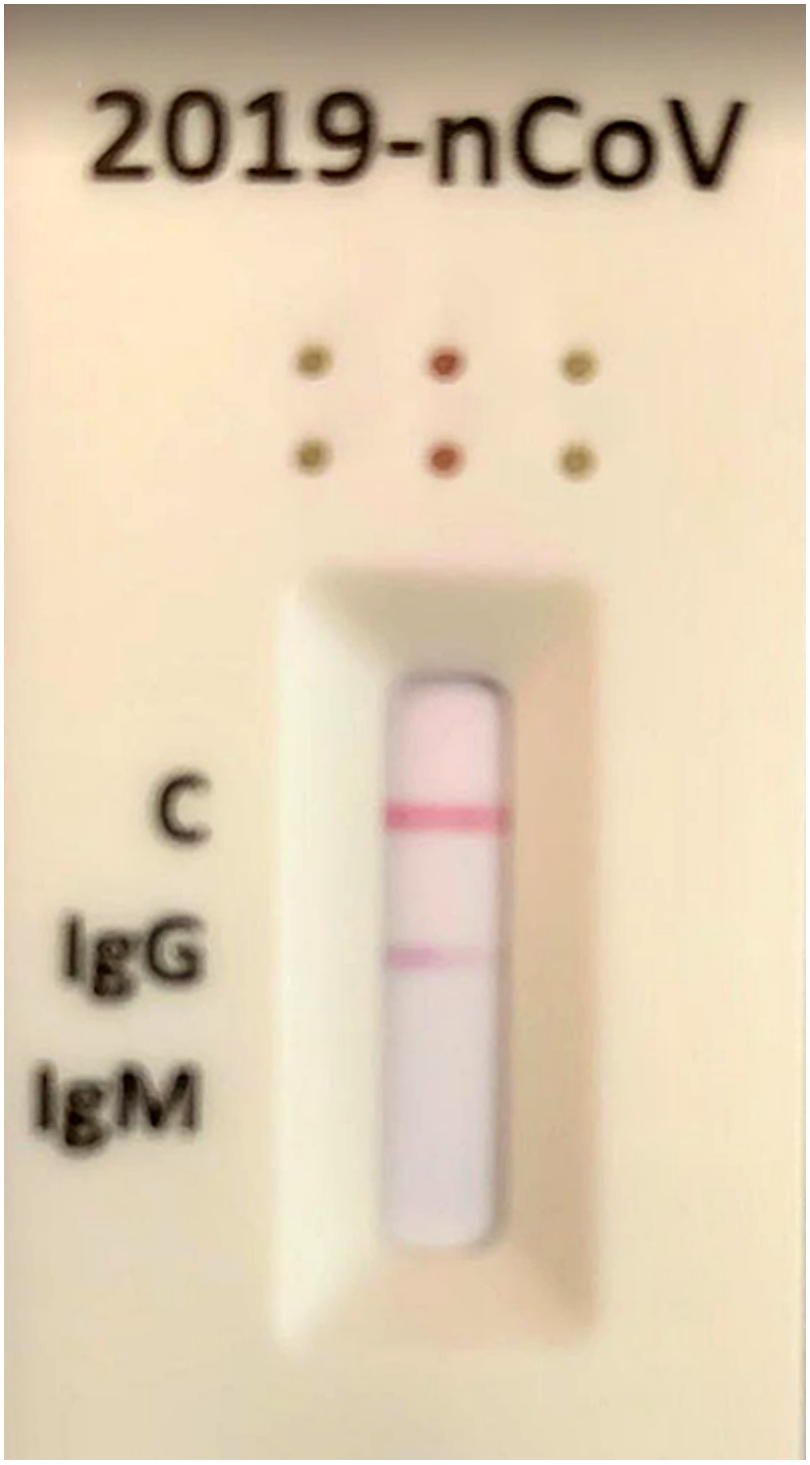


**Figure 2**

Serology rapid diagnostic test (RDT) for COVID-19 IgM and IgG antibodies. Shows IgM- and IgG+. C = Control.

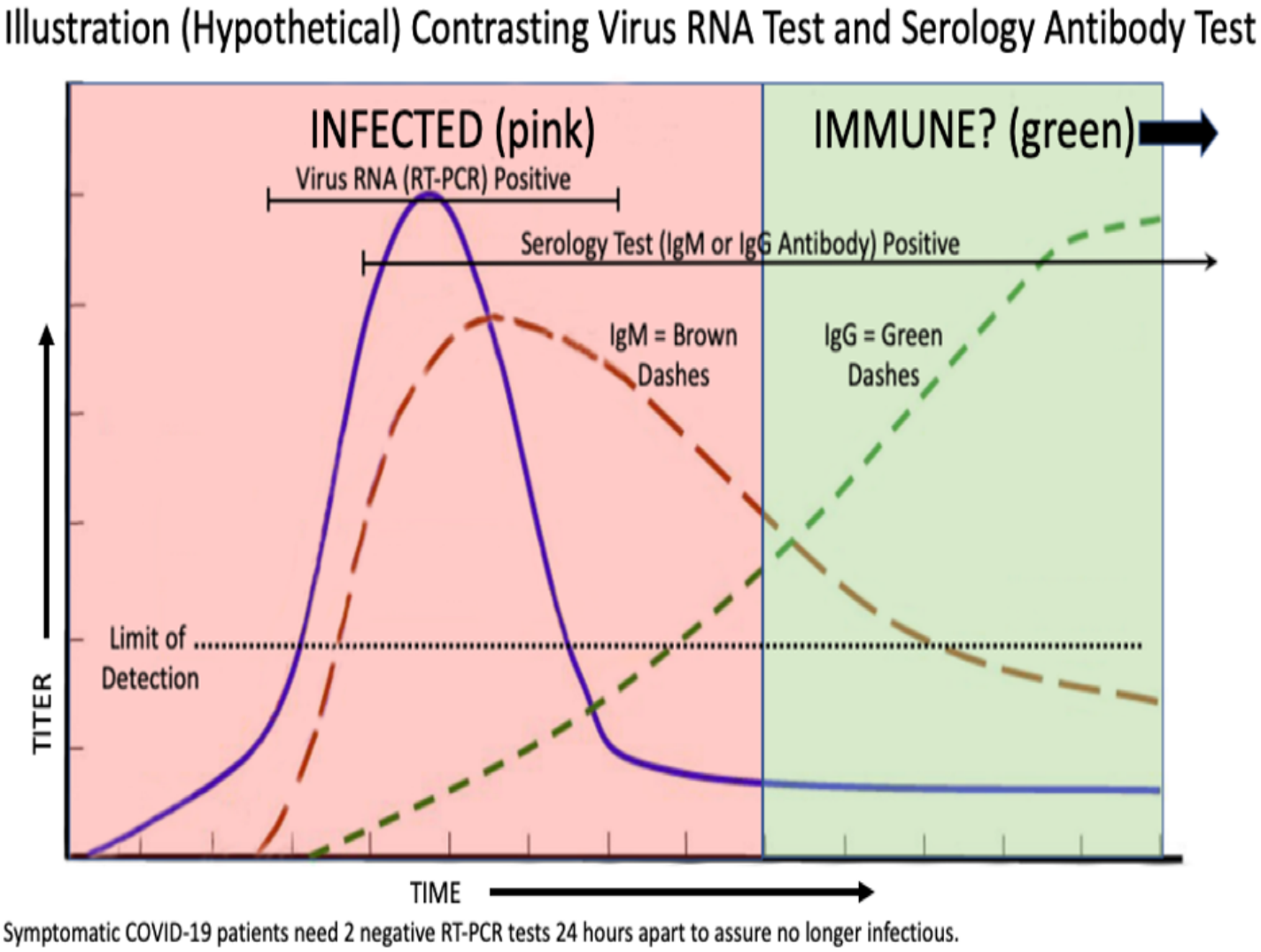


**Figure 3**

Hypothetical illustration of SARS-CoV-2 virus RNA load versus antibody levels. After an incubation period, PCR-detected viral load rises before IgM antibody, with IgG rising later. IgM-/IgG+ indicates post-infection.