ALGORITHMS FOR TESTING COVID-19 FOCUSED ON USE OF RT-PCR AND HIGH-AFFINITY SEROLOGICAL TESTING: a consensus statement from a panel of Latin American experts

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ABSTRACT

The COVID-19 pandemic has caused an unprecedented public health, social, and economic crisis. Improving understanding on available tests for detecting COVID-19 is critical for effective management of the pandemic. We proposed that a multidisciplinary expert panel can establish recommendations on ideal use of diagnostic tools, with a focus on RT-PCR and serological high-affinity antibodies (both IgM and IgG) tests for the Latin America region. **STUDY DESIGN:** A collaborative multidisciplinary panel of 5 recognized experts in Latin America (an infectious disease specialist, three pathologists and an immunologist) was convened and supported by Roche Diagnostics to develop standard guidelines and an evidence-based document of best practices on the use of diagnostic tools for COVID-19. **RESULTS:** The authors reached consensus on the applicability of diagnostic tools to provide testing algorithms for the use of RT-PCR and serological high-affinity antibodies (both IgM and IgG) tests in three settings: 1) For asymptomatic subjects exposed to a SARS-CoV-2 infected person; 2) For epidemiological purposes and; 3) For symptomatic subjects.

CONCLUSION: The serological high-affinity SARS-Cov-2 antibodies (both IgM and IgG) tests play a key role in COVID-19 diagnosis. These tests can be applied for suspected false-negative RT-PCR results and for individual determination of response. The use of these tests can also contribute greatly to public health strategies, such as population screening and supporting vaccination planning. Serological status for high-affinity antibodies (both IgM and IgG) should be

performed ideally 21 days after potential infectious contact, given that the majority of exposed individuals will have seroconverted.

INTRODUCTION

In December 2019, atypical pneumonia cases caused by a new coronavirus were identified in Wuhan, a city of Hubei Province in China.[1] Within days, the virus had spread, resulting in an epidemic throughout China.[2] An increasing number of cases were reported in countries around the world in the ensuing weeks.[3] In February 2020, the World Health Organization (WHO) named the disease COVID-19, which stands for "coronavirus disease 2019".[4] The virus that causes COVID-19 was then named Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2).[5] COVID-19 has since been declared a global pandemic,[6] with 15,301,530 cases worldwide and 625,005 deaths globally. In the Americas, the numbers are also staggering: 11,667,196 confirmed cases with 419,995 deaths (as of August 17th, 2020).[7]

The initial stage involves an incubation period, when SARS-CoV-2 multiplies and establishes itself mainly in the respiratory system. During the second stage, localized inflammation can occur in the lungs. The third (and most severe) stage of the disease can cause the syndrome of extrapulmonary systemic hyperinflammation.[8]

RT-PCR is a test for diagnosing COVID-19, based on nasopharyngeal swab or other upper respiratory tract samples.[9] In symptomatic individuals, viral RNA can be detectable early on day one of symptoms and culminate within the first week of symptom onset. By week three, positivity of the test for detecting viral RNA starts to decline.[10] A downside of this sample collection approach involves false-negative results, largely due to inappropriate timing of sample collection relative to illness onset and poor sampling technique, especially for nasopharyngeal swabs. Given the design for the RT-PCR test is based on the genome sequence of SARS-CoV-2, its specificity is almost 100%, with few false-positive results.[10]

Another diagnostic tool for detecting SARS-CoV-2 infection is serological testing which evaluates the host immune response.[11] It is essential for patients with mild to moderate illness who may present two weeks after illness onset. Serological diagnosis is also becoming an important tool to help understand the extent of COVID-19 in the community.[10]

Antibodies start to increase from the second week of symptoms onset, constituting the earliest and most sensitive serological marker, with IgM and IgG levels peaking in the second and third weeks of illness. Subsequently, IgM decreases by week 5, while IgG remains high beyond 7 weeks.[10]

These findings together with the plethora of available testing methodologies,[11,12] evolving knowledge on the behavior of the virus, and the complexity of the human immune response, have led to the need for guidance on how to use and appropriately interpret results of the available tests.

As the pandemic progresses, it has become clear that the primary transmission pathway is via respiratory aerosols[13], as well as through direct contact of eyes, nose, or mouth with contaminated surfaces.[14] The virus has also been detected in non-respiratory samples, such as stools, urine, blood, ocular secretions, and semen.[9] The risk of transmission of SARS-CoV-2 from an infected person to another appears to vary and depends on the type and duration of exposure, use of preventive measures and other individual factors.[15]

Latin America is a large and heterogeneous territory, including well-developed and poor areas with limited resources, in which the pandemic rapidly spreads. The aim of this paper is to provide Latin American clinicians with guidance on the use of RT-PCR and serological high-affinity antibodies (both IgM and IgG) tests.

METHODS

Five recognized experts in Latin America joined an online expert panel and worked collaboratively on an online application (Within3®) from June 12th to 24th, 2020, supported by Roche Diagnostics. Panel members had either clinical or scientific experience in infectious disease or immunology and serological tests. Adopting standard guideline development processes,[16] a literature review was performed on serological diagnosis and panelists shared these articles on COVID-19.[1-39]

Based on the papers retrieved, an infectious disease specialist prepared nine questions (Chart 1) and drafts of algorithms testing for SARS-CoV-2, focusing on the use of RT-PCR and serology testing in different settings. Panelists had the opportunity to suggest modifications to these algorithms and were required to propose evidence-based best practices for the RT-PCR and serological diagnosis of COVID-19.

CHART 1 – QUESTIONS TO BE DISCUSSED BY THE PANELISTS WITH RATIONALE.

1. If an asymptomatic person has had continuous contact (i.e. living in the same house) with someone diagnosed with COVID-19 (by the PCR test) for the preceding 14 days or longer, which test would you recommend to the asymptomatic person?

2. Considering the previous scenario, if the contact person has done the PCR test within the last14 days, which test would you consider for the asymptomatic person?

3. In the event a person has only had a clinical diagnosis of COVID-19 (not confirmed by PCR test), for less than 2 weeks, which test would you recommend for follow-up, if any?

4. Considering the previous scenario, if an asymptomatic person has had close contact with the clinically diagnosed patient, which test would you recommend for the person, if any?

5. In the case of front—line healthcare professionals, which test would you recommend for screening? And which actions would you recommend in response to the results of this screening test?

6. Is there any other situation where you would consider the use of a mature antibodies serology test for screening an asymptomatic population?

7. What is the role of the mature antibodies serology test in COVID-19 diagnosis for PCR-negative symptomatic patients?

8. What is the role of the mature antibodies serology test for COVID-19 diagnosis where there is no access to the PCR test?

9. How frequently would you suggest repeating the serological test (mature antibodies), if testing negative, for an asymptomatic person with a history of contact with an infected patient? And for an individual without a history of contact with an infected person (for the epidemiological purposes of a company for example)?

These preliminary efforts served as the basis for discussion and to establish the guidelines. The participants explained the rationale for their recommendations until a final consensus was reached. Lastly, panelists had the opportunity to make further reviews and remarks using the online platform, in reaching the consensus for the guidelines presented.

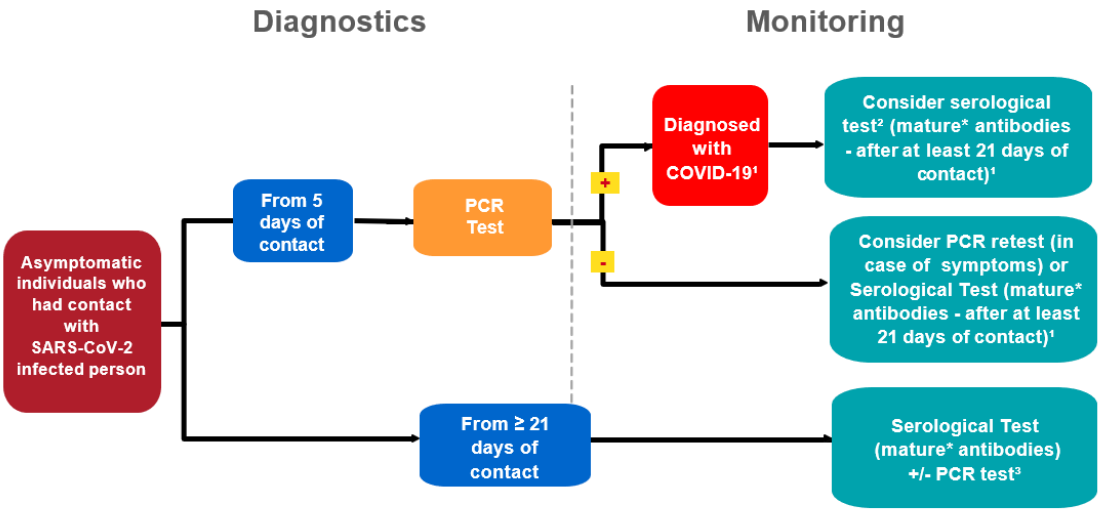
RESULTS

Algorithms emerged in a consensus of the panelists on serologic testing for COVID-19 in Latin America in three different settings:

1. **Asymptomatic individual exposed to Sars-Cov-2 infected patients – Algorithm 1.**

For asymptomatic individuals who had contact with a confirmed case of COVID-19 (Algorithm 1), RT-PCR should be performed preferably after 5 days of contact, while a serology test can be performed to detect mature antibodies against SARS-CoV-2, high-affinity antibodies (both IgM and IgG), ideally 21 days after contact.
Accordingly, due to the lower sensitivity of antibody tests in detecting infection during earlier phases,[17] the panelists proposed adoption of a minimum cut-off period after potential infectious contact, for performing serological evaluation of asymptomatic individuals (Figure 1).[18,19]

Figure 1. Asymptomatic individual exposed to Sars-Cov-2 infected patients – Algorithm 1.

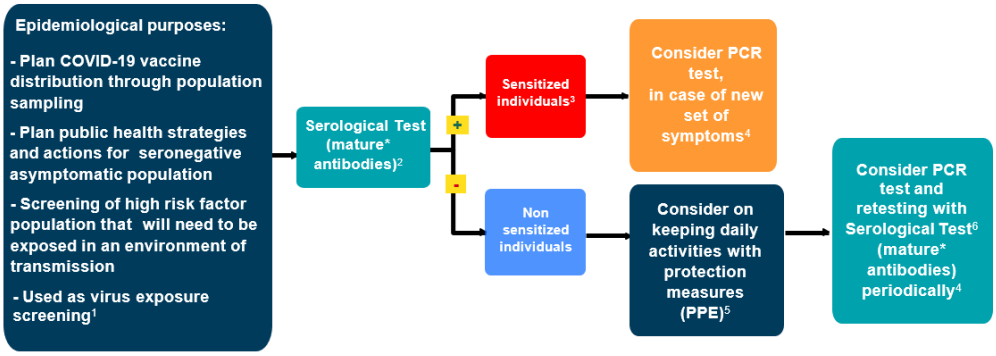


* Late-onset/high-affinity antibodies
1-Social distancing and other precautionary measures recommended, according to local health authority decisions.
2-According to medical decision (case by case), to better understand the immune response.
3-According to PCR test availability and costs.
Note: "This represents a consensus among experts and does not necessarily reflect individuals' opinions. Findings are based on scientific and personal information available up to June 24th, 2020 and are not yet endorsed by any guidelines or medical society."

2. Epidemiological purposes – Algorithm 2.

Serological testing also has utility for epidemiological purposes, such as: virus exposure screening, especially of high-risk populations (police and military personnel, food market suppliers, traffic agents, medical personnel), planning public health strategies and actions for seronegative asymptomatic populations, and planning vaccine distribution through population sampling. In these situations, the use of mature antibodies serological tests can help understand those that are SARS-CoV-2 sensitized. For those, PCR testing should be considered in case of a new set of symptoms. In such cases, social distancing, and other precautionary measures according to local health authority decisions, should be taken. For non-sensitized individuals, performing daily activities using personal protection equipment (PPE) should be considered, as well as regular retesting with PCR and serological tests. (Figure 2).

Figure 2. Epidemiological purposes – Algorithm 2.

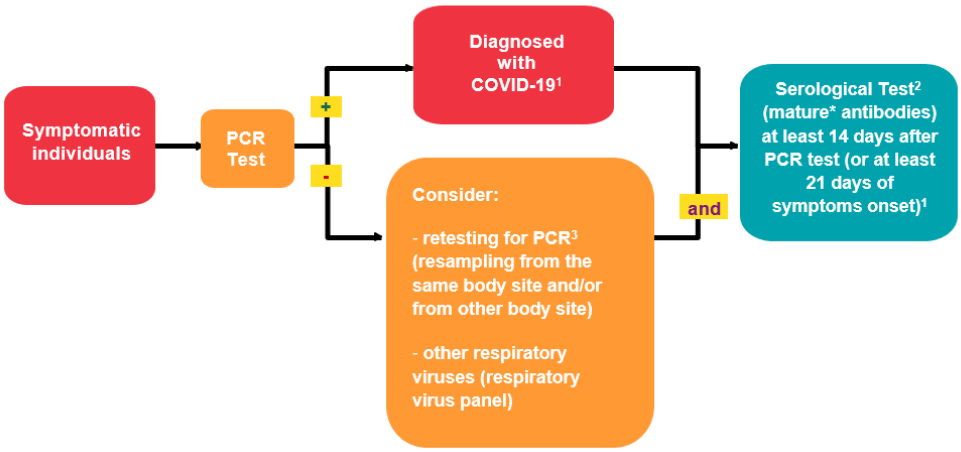


* Late-onset/high-affinity antibodies
1-Asymptomatic individuals with an epidemiological history of increased risk of exposure to people with COVID-19 (such as first responders, police and military personnel, food market suppliers, traffic agents, medical personnel).
2-Consider testing strategy by sampling the group (sample size defined by statistical methodology).
3-Sensitized denotes persons who have demonstrated the development of antibodies against SARS-CoV-2 on the assay, but there is currently no scientific evidence for immunity.
4-Social distancing and other precautionary measures recommended, according to local health authority decisions.
5-PPE=Personal Protective Equipment.
6-Consider for populations with a prevalence of less than 3%, the serological algorithm using the orthogonal method (CDC interim guideline).
Note: "This represents a consensus among experts and does not necessarily reflect individuals' opinions. Findings are based on scientific and personal information available up to June 24th, 2020 and are not yet endorsed by any guidelines or medical society."

3. Symptomatic individuals – Algorithm 3.

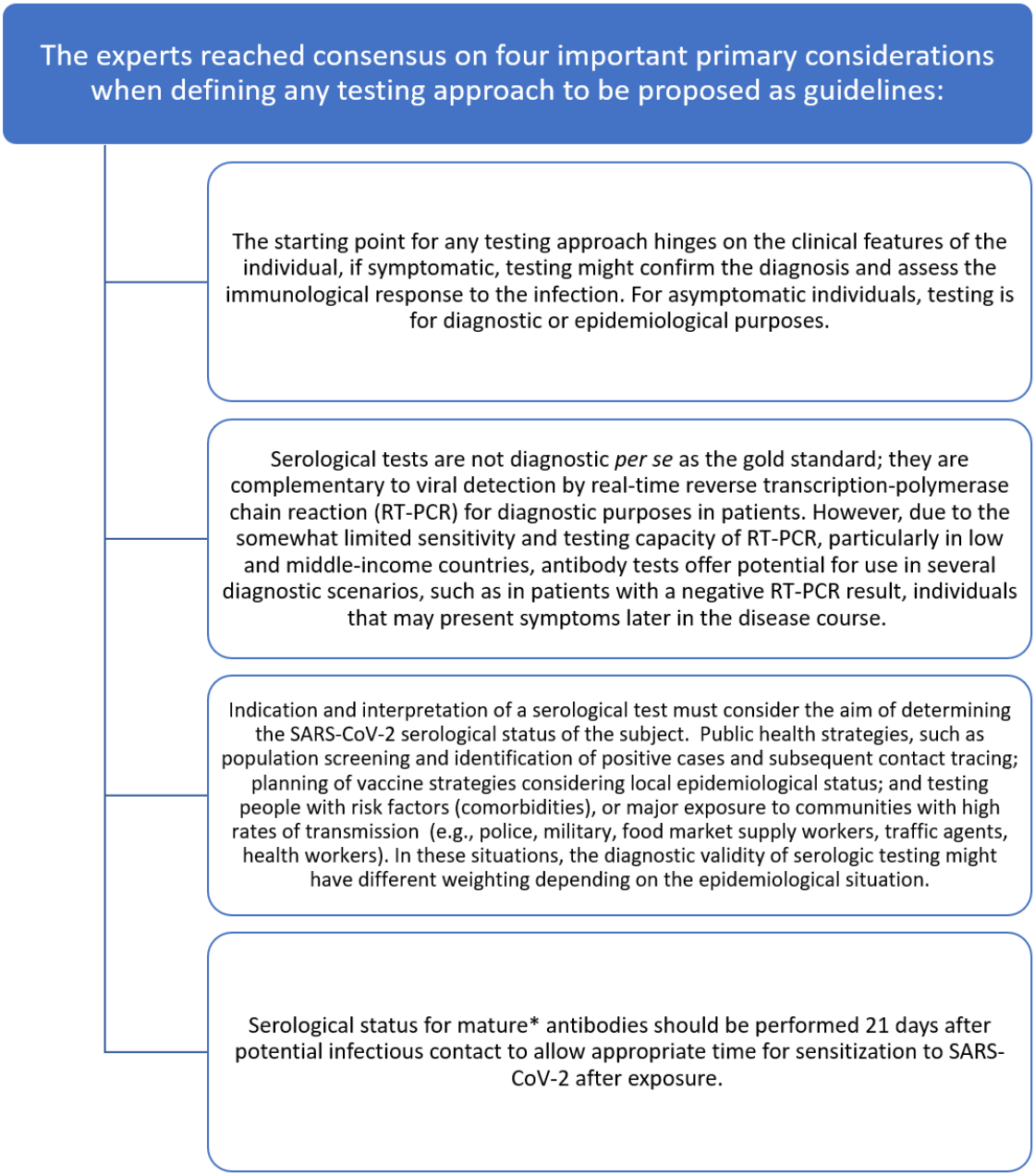
For symptomatic individuals, the gold standard is the RT-PCR test performed predominantly on nasopharynx and/or oropharyngeal swab samples. If patients present with a negative PCR test, retesting for PCR (same and/or other body site) should be considered, as well as performing a respiratory virus panel. In all symptomatic people, a serology test to measure high-affinity antibodies (both IgM and IgG), could be performed at least 14 days after PCR test or 21 days after symptoms onset. (Figure 3)
The rationale for this timeframe relies on a study that reported the proportion of patients with positive virus-specific IgG reached 100% approximately 17 to 19 days after the onset of symptoms.[20]

Figure 3. Symptomatic individuals – Algorithm 3.



* Late-onset/high-affinity antibodies
1- Social distancing and other precautionary measures recommended, according to local health authority decision.
2- According to medical decision, and to better understand the immune response.
4- Consider retest, particularly for high clinical suspicion of COVID-19 cases.
Note: "This represents a consensus among experts and does not necessarily reflect individuals' opinions. Findings are based on scientific and personal information available up to June 24th, 2020 and are not yet endorsed by any guidelines or medical society."

Figure 4 - The experts reached consensus on four important primary considerations when defining any testing approach to be proposed as guidelines



*late-onset/high-affinity

DISCUSSION

RT-PCR for SARS-CoV-2, based on samples obtained preferably from the upper nasopharynx[11], remains the gold standard for diagnosis of the acute phase of COVID-19.[9,11] However, there are some drawbacks of this technique, namely its variability in accuracy depending on the specimen,[21] hazards in collecting samples,[22] and sensitivity concerns.[22,24] For instance, negative tests in patients with SARS-CoV-2 lower respiratory tract infection and minimum upper respiratory symptoms are not uncommon.[23,24] Given the limitations of the RT-PCR, in the

context of urgent need for accurate detection of infected subjects and their subsequent isolation as a pivotal step for effective prevention of the spread of the SARS-CoV-2 virus,[25] serological testing plays an essential role in different diagnostic and epidemiological settings.[12,26,27]

Nevertheless, relying solely on IgM serological detection for diagnosis of acute disease is not a suitable strategy, particularly for the early acute phase. In one study,[11] all 39 patients had both IgM and IgG after 5-7 days of symptoms onset. In another Chinese study of 285 patients, three seroconversion patterns were observed: synchronous seroconversion of both IgM and IgG, IgM seroconversion earlier than IgG (expected pattern), and IgM seroconversion after IgG. The proportion of patients with virus-specific IgM peaked at 94.1% in approximately 20-22 days, whereas for IgG, 100% reached a peak 17-19 days after symptoms onset.[20] However, antibody responses against SARS-CoV-2 are not fully understood,[28] and the neutralizing activities of detected IgG antibodies have yet to be determined.[26]

According to the North American Centers for Disease Control (CDC-USA), results of serological tests should not be used as a single diagnostic test for an acute infection, excluding, or diagnosing SARS-CoV-2 infections.[12] Moreover, the US Food and Drug Administration (FDA) recommends use of serological tests to detect SARS-CoV-2 antibodies by health professionals, as this may help identify individuals exposed to or who have recovered from COVID-19 infection.[29] In Latin America, the Brazilian Ministry of Health recommends the use of laboratory tests, RT-PCR until the eighth day of symptom onset and immunological, which detects, or not, the presence of antibodies in samples collected from the eighth day of symptom onset, in patients presenting with a flu-like syndrome or Severe Acute Respiratory Syndrome (SARS).[30] Despite the increased knowledge on the utility of serological tests, a recent survey by the Royal College of Physicians highlighted misinterpretation issues, where 40% of respondents considered patients to have “cleared COVID-19” in cases with active symptoms and IgM–IgG+ serologies.[31]

Serological testing for SARS-CoV-2 high-specificity antibodies can also be used as an additional diagnostic tool for suspected false-negative RT-PCR results,[32] or for individual determination of antibody levels to trace who has been infected in the past.[27,32] In some situations, the use of serological testing may also be applied to determine the immunity status of asymptomatic subjects with an epidemiological history of a high risk of exposure to people with COVID-19.[33] In such settings, serologic testing at appropriate intervals following contact with infected subjects might result in relatively fewer false-positive results.[34]

Serological testing also plays a pivotal role in population-based seroepidemiological studies. It provides essential data about SARS-CoV-2 transmission dynamics and allows interventions to reduce transmission of the disease. Moreover, this testing can be used to assess seroprevalence overall or in specific groups, thereby helping to estimate core characteristics of the pandemic and to plan intervention measures such as vaccination of populations.[27,35,36]

The World Health Organization (WHO) states that seroepidemiological investigation can help understand and provide robust estimates of clinical, epidemiological, and virological characteristics of COVID- 19.[37]

In Latin America, given the high prevalence of SARS-CoV-2 infection, a serological test can be used to determine the level of exposure and identify people who may be sensitized. The latter tests should ideally provide high specificity, with a small confidence interval, detection of high-affinity antibodies and no cross-reactivity with other coronaviruses.[38,39]

Finally, the CDC Interim Guidelines for COVID-19 Antibody Testing,[12] that recommend the use of serological assays in some other scenario: (a) as a method to support the diagnosis of acute COVID-19 illness for persons who present late onset, for whom serologic testing is offered in addition to RT-PCR; (b) as a method to support establishing a diagnosis when patients present with late complications of COVID-19 illness; and (c) as a method to reduce false-positive results in high prevalence settings.

LIMITATIONS

Although based on well-established consensus formation techniques and drawing on panelists´ expertise, these recommendations do not constitute a statement from the institutions or associations to which these professionals are affiliated. The main limitations of this expert panel consensus are selection bias, observer bias, confirmation bias, publication bias and cohort effects (different features and pace of the COVID-19 pandemics in each country of Latin America).

IMPLICATIONS

This expert panel consensus can help clinicians to apply testing for SARS-CoV-2 on an individual level. Moreover, the guidance can also support decision-making stakeholders when acting on public health measures, such as seroprevalence studies and business reopening. Lastly, the consensus can support payers from both private and public settings with a more straightforward tool for evaluating the use of a specific test (or sequence of tests).

CONCLUSION

In conclusion, serological testing and studies are of great importance for public health strategies, such as population screening, and will prove pivotal to support planning of vaccination strategies. Serological status for high-affinity antibodies (both IgM and IgG) should be determined 21 days after potential infectious contact to allow appropriate time for sensitization to SARS-CoV-2 following exposure.

ACKNOWLEDGMENT

This paper was supported by Roche Diagnostics. The data were collected by the sponsor and analyzed in conjunction with the authors. All of them contributed to writing the article and approved to submit for publication. Medical writing assistance was provided by CoreBox Medical Communications.

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