Serologic Test for SARS-CoV-2: Mechanism, Assays, Concerns, Suggestions

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Abstract: The COVID-19 pandemic and the severe acute respiratory syndrome coronavirus 2 virus present important diagnostic challenges. Serology tests detect SARS-CoV-2 infection and use to confirm the presence of current infection. Serological tests can be categorized into lab-based and cassette-based assays. Generally, while lab-based assays are more sensitive and specific, cassette-based assays can be more easily handled and produced at much lower costs, rendering the two types of serological tests with distinct applications during the pandemic. Serology of the novel COVID-19 virus is still an under-researched area. The development of serologic tests requires comprehension of several aspects, including the structural basis of the virus and the mechanism of antibody tests. These tests results will provide scientific instruction to design; evaluate vaccines and therapeutic antibodies in the future. CCS CONCEPTS• Applied computing • Life and medical sciences • Health care information systems.

1. Introduction

1.1 COVID19, the need for quick and accurate antibody tests

SARS-CoV-2 can be transmitted from human to human as well [1]. To detect the symptoms, WHO provided some methods like antibody testing that includes 8 for neutralizing antibodies, and 1 immunoassay for simultaneous antigen at the beginning of January 2021 [1].

Antibody testing, or also known as serology testing, is a blood test that can detect whether someone is or has been infected with COVID-19 or not. The existence of this test indicates if the body has been exposed to the infection. After exposure, it normally takes 1 to 3 weeks for antibodies to form in the blood. Antibodies can work by blocking the entrance of the virus or by interfering with viral transcription [2]. By using antibody tests, researchers can use the results to understand the immunopathology of the disease to provide instructions to design and evaluate future vaccines and therapeutic antibodies. In addition, his testing is less cumbersome and subjective than immunofluorescence assay and can be used for mass screening in times of epidemics [2]. However, scientific tests cannot replace clinical observation and hands-on experience. A negative test result cannot determine the presence of the disease under clinical suspicion.

1.2 Structure basis of SARS-CoV2

Understanding the structural basis of the coronavirus is essential for the development of serologic tests. To begin with, the coronavirus is characterized by its capability to expand its host ranges and high infection speed. The mechanisms that make these traits possible are recombination, mutator alleles, and mutational robustness [3].

Structure wise, the coronavirus contains both structural and non-structural proteins. Structural proteins are responsible for host infection, membrane infusion, as well as viral envelope assembly. Whereas non-structural proteins are responsible for viral replication and transcription [4]. Structural

proteins of the coronavirus include the membrane (M), the envelope (E), and the spike protein (S). In particular, the spike protein is essential in the development of antibodies, serology, and vaccination. Its role is to mediate the virus' attachment, fusion, and entry into the host cell. Nucleocapsid proteins (N) is both a structural and nonstructural protein. It is essential in antigen recognition in serologic testings. These spike proteins contain a receptor-binding domain (RBD), which binds to receptor angiotensin-converting enzyme 2 (ACE2). The RBD is composed of a core and a receptor-binding motif (RBM), and the RBM is responsible for mediating contact with ACE2 [5, 6]. The spike protein's appearance is "corona-like" due to its trimers on the virion surface. These spike proteins are composed of two regions: S1 and S2 (See Figure 1.). S1 contains two domains - the N-terminal domain (NTD) and C-terminal domain (CTD) - and binds to receptors. Whereas S2 is responsible for fusion.

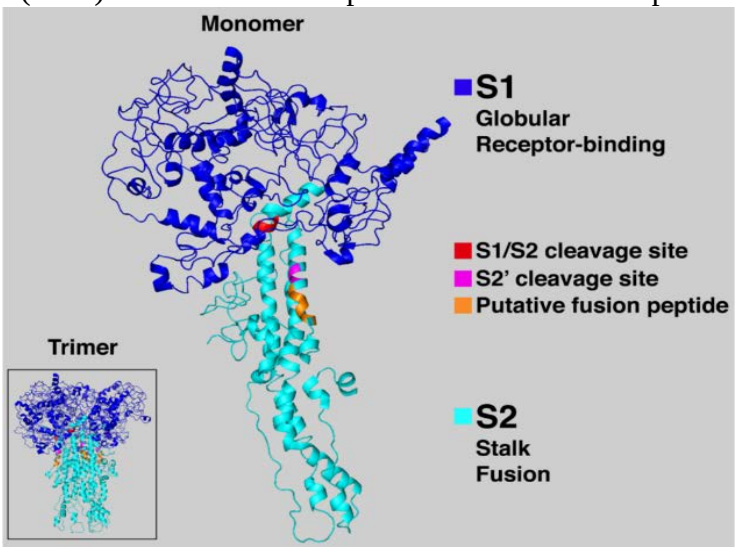

Figure 1. Three-dimensional SARS-CoV spike protein structure. S1, S2, cleavage site, fusion peptide are highlighted [7].

1.3 Mechanism basis of serologic test

The objective of the serologic test is to identify the presence of antibodies in the blood. Antibodies are Y-shaped proteins that are developed when spike proteins, in the case of COVID-19, comes in contact with the immune system. The spike protein will attach to B cells, which will lead to the production of corresponding antibodies. The shape of the antibodies makes them capable of attaching to the spike protein to prevent SARS-CoV antigens from binding to other cells [8].

Serologic tests detect the presence or absence of either active or inactive antibodies in blood serum, depending on the type of serologic test. The major types of serologic tests include rapid serology/diagnostic tests (RDTs), enzyme-linked immunosorbent assay (ELISA), neutralization assay, and chemiluminescent immunoassay. RDTs, aka. Lateral flow immunoassay (LFI) is currently the majority of approved antibody tests in the US [8].

COVID-19 serology testing functions by mixing blood in a viral-antigen infused testing platform. The presence or absence of antibodies in the individual's serum will be indicated by observing if viral antigens have bound.

For COVID-19, antigens that are commonly used for the detection of antibodies include spike protein and nucleocapsid. For spike proteins, their unique shape makes them easily recognizable. Both S1 and S2 could be used as antigen recognition (See Figure 2.) [9]. The RBD in the S1 region is specifically targeted in serological testing. Nucleocapsid may also be suitable for antibody detection due to its abundance after infection and high level of immunogenicity.

Figure 2. SARS-CoV spike protein schematic. S1 consists of the receptor-binding domain. S2 consists of putative fusion peptide in blue, heptad repeat HR1 in orange, and HR2 in brown. TM stands for the transmembrane domain (purple). The arrows indicate cleavage sites [7].

1.4 Types of antibodies

There are a total of five classes of antibodies, also known as immunoglobins (Ig). IgM and IgG are two classes that are more specifically active in response to viral infections. IgM is produced earlier and reaches a detectable level about a week after the SARS-CoV-2 infection; it lasts for a short period and gradually replaces IgGs. In comparison, IgG lasts longer and is more specific. When IgM production has reached its peak, IgG level becomes detectable and continues to increase until it peaks at about week 2 [10].

2. Types of Serologic Tests

To monitor the spread of COVID-19 among populations, a myriad of tests is developed and used by laboratories and clinics that report to public health agencies promptly. Unlike viral tests that often utilize RT-PCR to detect current viral infection via viral genome [11], antibody tests usually detect the presence or quantity of specific antibodies produced by our immune system in response to SARS-CoV-2 or its vaccine. These immunoassays can be generally categorized into either laboratory-based or cassette-based. Laboratory-based assays are mostly quantitative or semi-quantitative and require traditional blood samples [12]. Examples of lab-based tests are neutralization assay, enzyme-linked immunosorbent assay (ELISA), and chemiluminescence immunoassay (CLIA).

2.1 Lab-Based Assays

2.1.1 Neutralization assay

Neutralization assay cultures live viral cells and test if the subject's antibodies can neutralize viral activity in vitro. By convention, the level of neutralizing antibodies is detected by plaque reduction neutralization test (PRNT). A constant amount of virus is mixed with diluted serum samples, and the mixture is plated onto the cell line appropriate to the specific virus. After a few days, plaques of infected cells are formed and stained for visualization. The neutralization percentage can then be calculated as the number of plaques in one plate divided by the original number of virions [13]. It is powerful in its ability to measure functional and protective virus-specific neutralizing antibodies, so it has been widely accepted as the gold standard for serological tests and measures of immune protection [14]. However, it is a time-consuming process that takes days to obtain results, rendered impractical for large-scale serodiagnosis. Although neutralization assays have not been FDA-authorized for emergency use, continued development in COVID-19 vaccines has brought up the need to estimate each vaccine’s protection against reinfection in vaccinated people. For large-scale vaccine evaluation, Muruato et al. have reported a fluorescence-based high-throughput neutralization assay for SARS-CoV-2 neutralizing antibodies with comparable results to the standard PRNT. Their assay designed a fluorescence-tagged reporter SARS-CoV-2 virus, so the neutralization percentage can be estimated under the microscope after culturing the reporter virus with serum sample for 16 hours, greatly shortening the assay turnaround time [15].

2.1.2 Enzyme-linked immunosorbent assay (ELISA)

The most commonly used anti-SARS-CoV-2 serological test is built based on ELISA, which employs a surface coated with SARS-CoV-2 antigen ready to bind to the subject’s antibodies. If the subject’s antibodies are bound to the antigens presented, enzyme-linked secondary antibodies can bind

to primary antibodies in samples [16]. The addition of substrates produces color for detection and quantification via spectrometry, and the whole process takes a few hours to be conducted in a laboratory setting. At present, FDA has granted anti-SARS-CoV-2 serological tests with Emergency Use Authorization (EUA) only, and the ELISA tests approved detect IgG, IgM, or total antibody by targeting one or more of the viral spike, nucleocapsid, and membrane proteins [17]. In a study comparing three RDTs, four ELISAs, and one CLIA, the Wantai total anti-SARS-CoV-2 ELISA had the best overall performance in patient diagnostics and population screening through statistics analyzed correlated with PRNT as the golden standard [18]. Although not all of the eight tests compared were FDA-approved for EUA, the performance of the Wantai total Ig ELISA was reasonable because it was coated with the receptor-binding domain (RBD) of SARS-CoV-2's S1 subunit, against which antibodies are strongly correlated with the ability to neutralize virus [19].

2.1.3 Chemiluminescent immunoassay (CLIA)

Similarly, CLIA uses the binding between antibodies and antigens yet applies substrates that emit light after reacting with the enzyme-linked to the secondary antibodies [16]. CLIA and fluorescent immunoassay (FIA), which uses fluorescent reaction products for the detection, are both variants of the colorimetric ELISA using different detection systems [20]. Multiple studies have found ELISA and CLIA towards anti-SARS-CoV-2 to be comparable [21, 22], and the FDA has also granted multiple CLIA tests with EUA [17]. One of the approved, DiaSorin LIAISON SARS-CoV-2 S1/S2 IgG, is a semiquantitative, high-throughput CLIA that is found to be highly sensitive and specific. Its diagnostic sensitivity was 100% compared to neutralization assay, rendering the ability to measure serum or plasma level of neutralizing antibodies [23].

2.2 Cassette-based assays

Cassette-based assays are often referred to as rapid diagnostic tests (RDTs). The system is frequently seen in pregnancy test kits and does not require special instruments and sophisticated training. These assays take whole blood samples from finger pricks [22] and produce a qualitative outcome of visible color change within 10 to 30 minutes [12].

2.2.1 Lateral flow immunochromatographic assay (LFIA)

These cassettes mostly use LFIA to detect targeted antibodies. In an LFIA, a subject's finger-prick blood sample is absorbed first by the cassette's sample pad and then, by capillary forces, moved to a conjugated pad that stores viral antigens bound to colored indicators, such as colloidal gold. If the sample contains anti-SARS-CoV-2 antibodies, the antigen-antibody complexes then migrate along the porous nitrocellulose membrane and become trapped by secondary antibodies in the indicator region (Fig. 3). Finally, the accumulation of the complexes leads to the appearance of the color [24]. This type of LFIA used for anti-SARS-CoV-2 detection is the non-competitive, direct method, which is also called the sandwich format. This method is used for anti-SARS-CoV-2, perhaps due to the antibodies' high molecular weight. On the other hand, the competitive, indirect method of LFIA, also called the inhibition format, reveals the presence of a targeted analyte when the color doesn't appear [23]. Even though FDA approves some LFIAs with EUA by showing high sensitivity and high specificity for one or more of IgG, IgM, and combined Ig [17], many studies have pointed out considerable discrepancies of testing qualities of different LFIAs [25]. Elslande et al. reported significant variation among seven RDTs, especially their IgM results of only 70% agreement [26].

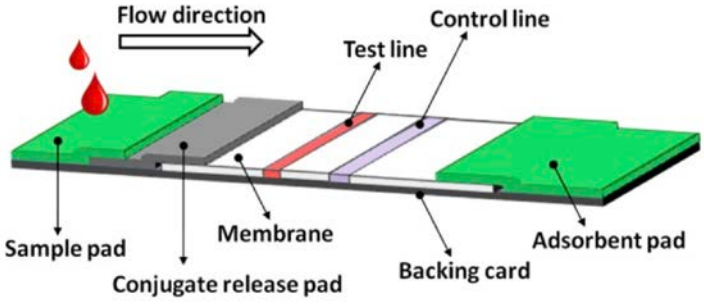

Figure 3. Common setup of an LFIA test strip [24].

2.3 Comparisons of Different Types of Serologic Tests

Among the three laboratory-based immunoassays, neutralization assay often sets the gold standard for quality control and case confirmation in many laboratories due to its complicated procedures and days of cell culture. In contrast, ELISA and CLIA are more suited for first-line screening on account of their high throughput, relatively short processing time (within hours), and simple operating procedures [27]. Generally, lab-based immunoassays are more sensitive and accurate [28] but are more costly and time-consuming than cassette-based immunoassays. Before an affordable and efficient lab-based assay is devised, this type of serological test is likely to mainly remain in the hands of laboratory clinicians for research purposes, such as looking for a cut-off point of antibodies to protect us from severe infection or re-infection [17]. Even though lab-based assays are not practical to be taken by the general public for at-home tests or quick drive-thrus, their sensitivity and specificity have allowed screening of donors with convalescent plasma containing anti-SARS-CoV-2 antibodies [29]. The convalescent plasma treatment places hope upon the anti-SARS-CoV-2 antibodies circulating in the blood of donors who have already recovered from COVID-19 infection, enabling these antibodies to neutralize the SARS-CoV-2 viruses inside receivers' bodies [30]. This treatment has successfully treated the COVID-19 patients of severe symptoms, especially before the distribution of approved vaccines, alleviating the burden of their immune system before their own antibodies can be produced. The success of this treatment has relied on the power of lab-based antibody testings to identify the valid donors with a sufficient concentration of anti-SARS-CoV-2 antibodies since a higher level of the antibodies are associated with a lower risk of death in patients receiving the transfusion of convalescent plasma [31].

Compared to laboratory-based assays, cassette-based assays are much more time-efficient by generating an outcome in less than 30 minutes. Although some cassette-based serological tests do not have the sensitivity and specificity to pinpoint the eligible convalescent donors for blood transfusion, test kits of rapid, low-cost cassette-based assays can be mass-produced and can potentially fulfill the colossal demand of antibody testing of the public. These cassettes have material implications at the point of care (POC), which requires an enlarged scope of diagnosis and to be handled outside of labs [32]. Despite the cassettes only revealing qualitative results via color change, many of the COVID-19 POC cassettes display outcomes for both IgM and IgG antibodies along with a control band [12]. Assuming the control band is not compromised and displays color, since both the IgM band and the IgG band can be positive (colored) or negative (not colored), there are a total of four combinations. Combining IgM and IgG detection raises its accuracy compared to presenting only one class of antibodies [33] and conveys separate messages about the subject's status. When IgM and IgG are both negative, the subject hasn't developed any antibodies towards the virus, so the subject either hasn't been infected, or the infection is still in the incubation period, meaning that it has only been less than a week since infection. When only IgM is positive, viral infection in the subject has usually entered the acute phase, and the subject has started to generate a humoral immune response towards the virus not long ago [33]. When IgM and IgG are both positive, the subject has probably been producing antibodies for more than a week, suggesting that the virus was contracted about two weeks ago. When only IgG is positive, the disease has likely been resolved with the virus neutralized and phagocytosed. For the last three scenarios, test administrators or test-takers themselves can quickly determine if self-quarantine is required. However, in the first scenario, the subject may be asymptomatically infected, which should better be supplemented with viral testing [34]. Considering the low prices and rapid processing of these cassette immunoassays, everyone can potentially get tested anywhere at any time. However, this can only be achieved by standardizing the cassettes, issuing clear interpretations, and integrating molecular testing.

3. Discussion

3.1 Comparison of serologic test with molecular test (RT-qPCR)

There are a few ways antibody testing can be valuable. To begin with, serologic tests are relatively cheap compared to molecular tests under a wide application. It is easy to produce because human antibodies are more stable than viral RNA during sample collection, preparation, transport, or storage. It can be easily implemented in clinical settings and laboratories. It is also more convenient and less painful for many people as only a few drops of blood are taken rather than nasal and respiratory samples. One of the most prominent purposes of this test is to identify individuals previously infected by SARS-CoV-2, even if they are not currently ill. It can also avoid false-negative cases that occur in other methods such as the RT-PCR method because the antibodies have a more uniform distribution. This aspect of serology tests is also used to diagnose suspected cases with negative viral RNA tests. From a global health perspective, it can serve as surveillance and epidemiologic assessment. It is capable of tracking a virus through different phases of disease. For example, a large-scale epidemiological study can be conducted at the end of COVID-19 to develop a specific IgG antibody test. By doing so, we can understand the true scale and impact of coronavirus transmission [35].

The shortcomings of serologic tests are also obvious. One of the most notable flaws is that the existence of antibodies is not a reliable indicator of immunity. It is not suggested to determine the necessity for immunization in someone who has never been vaccinated. In certain situations, seriously afflicted patients may fail to produce antibody responses, resulting in inaccurate results. Heterogeneity in antibody detection time after symptom onset and large variance in antibodies levels in different samples can also be difficult. Furthermore, there is a lack of product evaluation and regulation, which leads to excessive product designs and industry malfeasance. In addition, antibody testing is limited to specific clinical and research uses and some outbreak investigations only. Although home testing kits are accessible, CDC does not recommend it, and results cannot be properly interpreted. Moreover, international standards are non-existent, which leads to limited innovation and non-existing standardization.

3.2 Status of serologic tests

As of May 2021, the only legal and accessible tests are for emergency use only. It is issued by CDC and is for use for public health and clinical purposes. Only serologic tests that yield qualitative (ELISA) or semi-quantitative results have been granted EAUs. Quantitative tests have not been granted authorization due to the lack of international standards. The European Union states that for a serology test to market, it must achieve at least 98% specificity. The World Health Organization recommends 90% sensitivity.

Under regulation for production, there is an abundance in the number of developers and companies working on serology test kits for the pandemic. Under regulation for production, there is an abundance in the number of developers and companies working on serology test kits for the pandemic. The US government is in the midst of the first round of evaluation of serological test kits. Currently, 5 out of 27 antigen tests and 26 out of 203 antibody tests are being reviewed. Antigen testing is a more established and mature sector. In contrast, due to a lack of technology, increased demand, and people's interest in their antibody statuses, we see a rise in antibody test innovation and development. These tests are being evaluated continuously.

3.3 Limitations of serologic tests

Due to current regulations, virus-based neutralization assays are currently not authorized for emergency use, except for an ELISA-based competitive neutralization test that yields qualitative results on the total neutralizing antibodies. Plus, immunofluorescence assays (IFA), enzyme-linked immunosorbent assays (ELISA), and Western blot (WB) analysis were developed as different serological assays during this outbreak. IFA and ELISA were shown to be highly sensitive (85–100%), but lacking in specificity in other studies. In addition, false-positive results were caused by antigens that were conserved across CoV species and reacted with autoantibodies in autoimmune disorders that

resulted in false-positive results [36]. Thus, serological assays based on recombinant antigens generated from both S and N proteins have become commonplace in laboratory diagnosis. The usage of recombinant antigens has the advantage of allowing researchers to work without having to be strict biosafety regulations, and they're also better for assay uniformity. Because of its modest size and lack of glycosylation sites, the N protein is particularly straightforward to clone onto prokaryotic or eukaryotic expression plasmids [36]. According to the researchers, recombinant protein-based WB and ELISA are highly sensitive (73–100%) and have a low to moderate specificity due to its full-length S protein that is difficult to express in prokaryotes to be used in immunoassays.

ELISA methods can also be used to validate and test different SARS-CoV-2 antigens with S, S1, RBD, N protein [37]. In addition, it indicated that N and S1 were more specific in detecting SARS-CoV-2 antibodies among the others. Since the ELISA method included the full-length S protein and other RBD fragments, it indicated that antibodies mounted after infection target the full antigens and immunogenic fragments [37]. This idea is necessary because experiments applying recombinant proteins are more difficult to standardize. Based on this suggestion, some studies combined nucleic acid tests (NAT) and serological tests to improve the diagnostic sensitivity rate from 78.7% to 100%. However, the most recent studies have a significant bias due to the varying time lags between initial virus exposure and serological assays confirming identification [10]. In other words, data still have some limitations to use for today's research while comparing with another positive antibody testing under evaluation among different isotopes [10].

3.4 Concepts of combination use

Combinations between genetic and serologic testing results from a single group at risk can also aid in the control of COVID19 infection. For example, estimations based on a point prevalence of 4% paired with high estimates from serologic testing could indicate that the infection is likely declining in the population as a whole since the population has already established an immune response to the virus [38]. However, because the population at risk had not previously been exposed to the virus, a similar point prevalence of 4% based on molecular testing combined with low estimates of infection based on serologic testing in the population at risk can potentially mean that the infection will rise in the future [38]. Despite the limitations of the tests, the creation of hybrid assays to reduce the fraction of false-negative results is seen to be the best outcome and "clear-cut diagnostic gold standard." While people got infections under a certain situation, combinations of the genetic and serologic testing would provide an extra diagnostic value for accurate and speedy COVID-19 diagnosis in vitro diagnostics [39].

3.5 Further application of serologic test (beyond diagnostic)

Although the whole world recovered from this pandemic, some parts of the countries still face serious outbreaks today, which means the long-term immunological response to the virus among the survivors is still being researched. Early observations suggested that 3 months after the onset of symptoms, there was an immunological signal in asymptomatic or mildly symptomatic patients. However, most recent research provides data about the immune response's sustainability that IgG levels dropped by 71.1 percent on average 2 months after the onset of illness [38]. Moreover, other researchers showed IgM and IgG levels against spike RBD, S2 was stable for 5 to 7 months following the beginning of symptoms, and these neutralizing antibodies are still detectable 5 to 7 months later [38]. These data show the serious consequences of developing vaccines and immunotherapies to control the spread of the virus. Furthermore, Longer-term data of the SARSCoV2 serologic response will be required to assess the efficacy of immunological treatments in society as a whole in the future.

4. Conclusion

Antibody tests are critical for controlling COVID-19, it provides scientific guidelines to design and evaluate vaccines in the future. However, this test has many limitations, including potential false-negative results, extra specificity, and precarious availability. Serological tests have generated

substantial evidence to be a better choice between RT-PCR and other NAT in diagnosing acute infection; especially, some tests are cheaper and easier to apply in the real assays. They can identify individuals previously infected by COVID-19 that some point-of-care tests have been available quickly. However, the rate of development has outpaced the rate of careful examination, and there is still a lot of ambiguity about test accuracy. Thus, we still need more information from researchers to improve on the applications of the tests.

Based on the data we collected, we still do not know if serological assays show better as a screening test, a diagnostic tool for the greatest accuracy, or a purpose to reach the end of the pandemic. Screening and management of clinical patients with close contacts, especially those who had a negative RNA test, are potential applications of serology; plus, this test still needs to develop among the critical populations that include those citizens back to school and work from high incidence areas. To find the true prevalence and pathogenicity of SARS-CoV-2 infection, we need to create a serological survey and analyze the antibody level and spectrum of antibody epitopes among those COVID-19 patients. This will provide scientific instruction to design; evaluate vaccines and therapeutic antibodies in the future.

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