SARS-CoV-2 Serology Testing in the Setting of Vaccination

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Serology testing for SARS-CoV-2 will be beneficial and potentially even necessary in assessment of vaccine effectiveness, which will play a key role in promoting public health. Siemens Healthineers supports measuring SARS-CoV-2 IgG antibodies in relation to vaccine use for (1) establishing a threshold for protection or immunity, (2) confirming an initial neutralizing antibody response approximately 4 weeks post -vaccination, especially in those at risk for an insufficient immune response, and (3) tracking of antibody levels at approximately 6 months and periodically thereafter following vaccination to guide booster/revaccination efforts. An automated and scalable serology assay used for patient care in the context of vaccination should include key technical features for effective use: measurement of spike receptor-binding domain (S1-RBD)-neutralizing IgG antibodies, very high (≥99.5%) specificity, and quantitative results.

Background

In clinical practice, quantitative antibody testing for assessing the need to vaccinate/boost is common, especially in cases such as hepatitis B vaccination, where the neutralizing surface antigen-antibody threshold associated with immunity is known.[1] In population-based studies, SARS-CoV-2 antibody testing has been shown to identify a significant percentage of the population with an immune response to the virus but undiagnosed for COVID-19.[2-6] Commercially available clinical laboratory serology testing suitable for clinical practice is not expensive and can often be high-throughput, with fast turnaround and broad population access. Currently assays with very high (≥99.5%) specificity, particularly important under conditions of low disease prevalence, will be essential to vaccination campaigns, both to identify vulnerable populations as well as assess for a successful response in large populations.[7,8]

As learned during this pandemic for other types of SARS-CoV-2 testing, such as PCR, availability at a large and accessible scale is key to ensuring that the needs of the population can be met. While proof of antibody-associated immunity in SARS-CoV-2 is emerging from the vaccine trials and other datasets, extensive data to date already support a role for neutralizing antibody in protecting from (or mitigating) infection.[9-17]

Studies from natural infections indicate significant diversity in the levels and duration of neutralizing antibody responses, with declining levels over time leading to vulnerability to reinfection.[15,18-26] Long COVID (or Post COVID) syndrome has been increasingly recognized, with about one-third of patients who were initially asymptomatic reported having COVID-19 symptoms weeks after diagnosis.[27] Consequently, serology testing is essential to identify those that may have previously been infected and to distinguish successful from suboptimal vaccine responses and detect antibody declines after natural infection.[28-30] The factors influencing likelihood of a robust neutralizing antibody response are poorly defined but have been linked to immunocompetency, age, and disease severity.[30-32]

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Existing data indicates that detectable levels of circulating neutralizing antibody are necessary for protection, though the role of memory B-cells and/or T-cells is still under investigation.

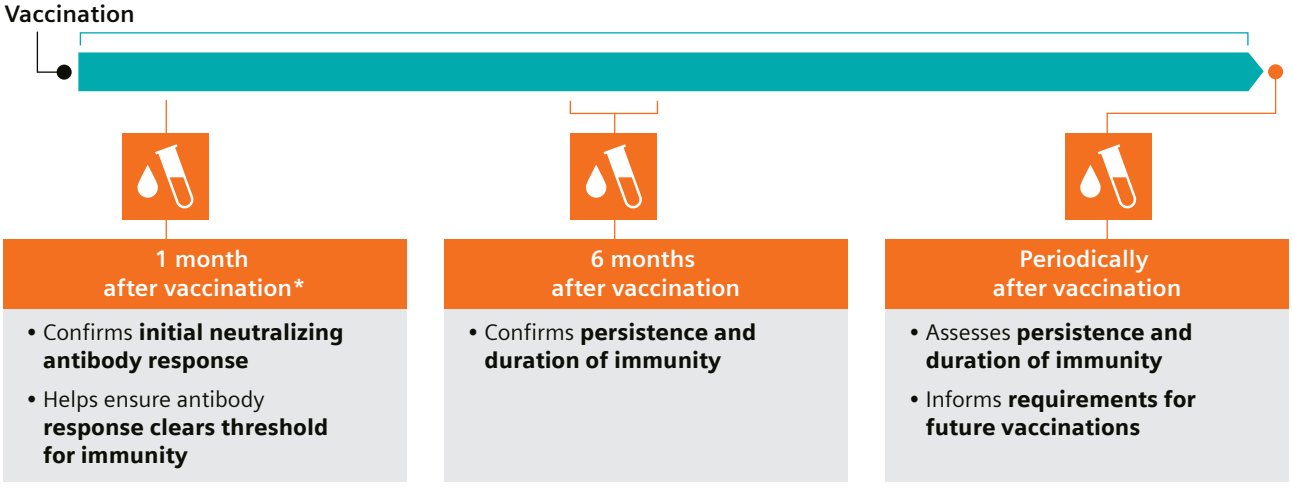
Data from vaccine Phase II/III trials indicate that the mRNA vaccines are up to 95% effective in preventing COVID-19, and the risk of severe illness has been reported to be lowered by more than 90% in the mRNA vaccine clinical trials.[33,34] Certain patient populations were excluded from these vaccine trials, and hence the efficacy and safety of a SARS-CoV-2 vaccine has not been established across all patient populations. To circumvent vaccine shortages, certain countries have chosen to extend the vaccine dosing interval, including the UK,[35] Denmark, Norway, France, and Canada.[36] The data regarding the safety and efficacy of the vaccines with alternative dosing schedules is currently lacking.[37]

Considerations

Antibody-mediated immunity

Immune responses to pathogens are diverse and involve both adaptive and innate elements.[38-39] Adaptive immunity is pathogen-specific and principally mediated by B- and T-cells. Humoral immunity is driven by B-cells that produce antibody (often "helped" by T-cells that secrete specific cytokines). With many pathogens, antibodies are the principal effector of protection, particularly if they can block (neutralize) viral entry.[40] Because antibody-mediated viral neutralization is a correlate for immunity, confirmed protection in vivo associated with specific antibodies/levels must be established. A growing body of data supports the potential for neutralizing antibody to confer protection from SARS-CoV-2.[9-17,41-43] This includes both in vitro demonstrations of antibody neutralization and in vivo evidence in a range of experimental animal models challenged with live virus.

Early longitudinal studies on the duration of neutralizing antibodies following SARS-CoV-2 infection, were somewhat hampered by the short length of those studies. Several studies undertaken over longer time periods are now available, providing key insights into the long-term dynamics of antibody responses. One such study found anti-spike and anti-RBD IgG and neutralizing activity to persist in the majority of patients (90% and 60%, respectively) 9–11 months after symptom onset,[44] while others showed detectable neutralizing antibody responses lasting 8–12 months in patients with severe disease.[45-47] Data from a national laboratory and clinical trials supports a sustained positivity rate of antibodies against the SARS-CoV-2 spike protein past ten months post-PCR confirmed COVID-19 infection.[48-52] Antibody seropositivity persistence has been shown to last for up to two years in other coronaviruses, so epidemiological evidence of antibody duration provide longitudinal data to properly view population-level seropositivity rates, which can help shape public policy moving forward.[53] As vaccine-induced production of neutralizing antibodies proves effective, assessment of neutralizing antibody levels to identify/confirm a protective threshold will be vital in establishing broad population-based immunity. Modeling studies predicted a significant loss in protection from SARS-CoV-2 infection following the decay of the neutralization titer over the first 250 days post immunization.[54] Recently, evidence regarding efficacy of vaccines have been available from longitudinal trials indicating a gradual decline in vaccine efficacy through 6 months post vaccination.[55] Evidence have emerged describing antibody decay kinetics in vaccinated individuals and previously infected individuals. The vaccinated group had higher initial antibody titers but had faster decay of these levels over time compared to previously infected individuals.[56] Waning humoral immune response in vaccinated individuals was also reported 6 months following a second vaccination dose in men, immune suppressed individuals, and individuals age 65 and older.[57]



**For a 2-dose regimen, the proposed timing is after the second dose.*

Figure 1. Key timepoints for serology testing to assess initial antibody immune response and duration post-vaccination.

Antibody targets and neutralization

Current commercially available SARS-CoV-2 antibody assays have diverse targets, including nucleocapsid (N) protein, whole spike (both S1 and S2 regions), S1, and S1-RBD.[58-80] Robust evidence in vitro and from animal model studies supports a mechanism of viral neutralization by antibodies to the spike glycoprotein, primarily through inhibition of recognition/attachment to the ACE2 host cell receptor. While several epitope-specific neutralizing spike antibodies have been identified (in both S1 and S2), most target the S1-RBD, as these antibodies can interfere with recognition and binding to ACE2.[12-15,81] Since both whole spike and S1-targeted assays include the RBD region, they can indicate, but not specifically identify, the presence of RBD-associated neutralizing antibodies. S1-RBD-specific assays are likely to prove advantageous over S1 and whole spike, especially if using a quantitative assay, as neutralizing versus binding antibodies might be expected to be enriched and therefore a better correlate to immunity. Data from a randomized efficacy trial of ChAdOx1 nCoV-19 (AZD1222) vaccine in the UK was analyzed to determine the antibody levels associated with protection against SARS-CoV-2, indicating that correlates of protection can be used to bridge to new populations using validated assays. The data can be used to extrapolate efficacy estimates for new vaccines where large efficacy trials cannot be conducted.[82] Another study examined the effectiveness and the immunogenicity of the heterologous ChAdOx1 nCoV-19/BNT162b2 combination by conducting a longitudinal survey of the anti-spike immunity conferred by each vaccine combination.[83] While not all antibodies to the RBD are equally neutralizing, the RBD is identified as the immunodominant source. Depletion analysis indicates an estimated ~90% of known neutralizing antibodies target epitopes within the RBD.[12,15,41] Recent data comparing S1-RBD antibody levels with virus neutralization titers showed a good correlation ($r=0.843$; $p<0.0001$) and an overall qualitative agreement of 98.5%.[84]
The emergence of SARS-CoV-2 variants has led to altered sensitivity to antibody-mediated immunity through a reduction in antibody neutralization by sera from individuals with a vaccine designed against the wild type virus.[85, 86] Data are needed to characterize antibody thresholds that define susceptibility to these breakthrough infections. While current data on S1-RBD vaccines may preclude the need for changes to vaccine design, the emergence of SARS-CoV-2 variants may require second generation vaccines designed with a broader set of antigenic targets to address variants.[87,88]

With the widespread availability of vaccination, differential reactivity of spike and nucleocapsid specific antibodies might be used to help differentiate previous infection from vaccination in serologic studies, particularly for vaccines that produce antibodies only against the spike protein.[89-91] Current vaccines induce antibodies to the S protein. Thus, the presence of anti-N antibodies indicates previous infection regardless of a person's vaccination status, while presence of anti-S antibodies indicates either previous infection or vaccination. The presence of antibodies to the spike protein and absence of nucleocapsid antibodies in the same specimen indicates vaccination in a person never infected or could signal prior infection in a person whose nucleocapsid antibodies have waned. Studies on duration of antibody response have indicated a longer lasting detection of antibodies against the spike protein compared to nucleocapsid antibodies.[24,92,93]

Qualitative versus quantitative reporting

Qualitative SARS-CoV-2 antibody assays have a defined cut-point based on presence/absence of immune response rather than a threshold value based on antibody level and neutralization of the virus. Therefore, they only provide a "yes" or "no" indication of a response to infection. Quantitative assays of neutralizing antibody support identification of an immune threshold, above which individuals are likely to be protected and below which they are susceptible. A number of IgG and total antibody quantitative assays for the spike protein (including the S1-RBD) are already commercially available.[94-97] Antibodies to SARS-CoV-2 can decline quickly and at different rates for different epitopes,[19,21-25] so quantitation would prove salient for rapid assessment of immunity or need to boost. Quantitative testing would be a valuable tool for establishing a protective threshold, as well as initial assessment of vaccination response and monitoring of antibody levels over time when a threshold is established. Ongoing efforts to better understand antibody kinetics, longevity of humoral immune responses, correlation of binding antibody levels to neutralizing antibodies, and serological surrogates of immune protection are dependent on wider availability of quantitative binding antibody assays that are standardized and traceable to an international standard.[98] Standardization of assays is required to allow comparison of results across the different assays and can be accomplished with reference materials that are well characterized by anchoring it to a neutralization endpoint. The European Commission's Joint Research Centre (JRC) and World Health Organization (WHO) have developed reference material for standardization.[99,100] The First WHO International Standard (WHO 20/136) is intended to be used for calibration and harmonization of serology assays and consists of pooled convalescent plasma from recovered SARS-CoV-2 positive individuals. While its intention is for calibration, it is not suitable for harmonization or standardization, since it only contains a single value, and is not associated with neutralizing antibodies.

Serology testing for determination of immune response to vaccines

Vaccination-related testing for neutralizing antibody can be utilized at multiple time-points. Ongoing clinical trials for authorized vaccines, and vaccines in development, are utilizing serology testing for neutralizing antibody titer as a surrogate of efficacy.[11,16,30,58,60,62,64,65,67-70,72,74-79,101] These trials are assessing neutralizing antibody immunogenicity in response to vaccine administration over time, which will be necessary to inform antibody-mediated protection. Immunobridging studies have been utilized as well to infer effectiveness of vaccines in the pediatric population. Immunogenicity assessments of the BNT162b2 COVID-19 vaccine in adolescents were performed before vaccination and 1 month after the second dose with neutralization assays and RBD-binding or S1-binding immunoassays.[102] A modeling study assessing vaccine prioritization strategies demonstrated there may be value in pairing serology testing with vaccination in areas with higher SARS-CoV-2 seroprevalence for additional reductions in cumulative incidence and mortality.[59] As current vaccines require a 2-dose regimen to broadly stimulate levels of neutralizing antibody, serology testing would measure for an effective response, approximately 4 weeks post vaccination.[63,101] Initially while facing limited vaccine supplies, some countries have opted for an extended vaccine dosing interval (11–12 weeks) to get as many people as possible partially vaccinated. Serology testing helps evaluate how such a delay affects SARS-CoV-2 antibody levels and could inform vaccine scheduling decisions in other countries. In a cohort study in the UK, adults aged >80 years with 11–12 week intervals between doses of the Pfizer-BioNTech vaccine had 3.5-fold higher peak titers of anti-SARS-CoV-2 spike antibodies compared to adults >80 years of age given a standard three-week interval between doses.[103] Studies have suggested that people who previously had COVID-19 may get a strong immune response from only one dose of these vaccines.[43,104,105] Serology testing can identify previously infected individuals and evaluate antibody response following a one or two dose regimen. France has formalized a vaccine policy for people who have had and recovered from SARS-CoV-2 infection and announced that vaccine centers will be equipped with antibody tests to test everyone before they are vaccinated.[106] Quantitative periodic antibody testing one month post-vaccination, and after approximately 6 months and periodically thereafter, would assure a sustained antibody response at sufficient levels for virus neutralization and guide booster shot prioritization (Figure 1). The timing of appropriate serology testing would be optimized and refined as needed and may differ between individuals based on the strength of their initial response to vaccination. Vaccine manufacturers have indicated a likely need for booster doses to combat variants and prolong protection against wild-type SARS-CoV-2.[107,108] Periodic antibody testing can identify at risk populations that would benefit from getting a booster dose and inform decision-making to guide prioritization strategies for booster dose administration in various populations.

A serology-defined threshold (from either natural infection or vaccination) remains a key need and this periodic testing would offer additional data on antibody response patterns to determine optimal serology testing utilization. Longer time-frame periodic quantitative testing for waning levels of protective antibody would inform the need to revaccinate/boost if SARS-CoV-2 becomes a seasonal disease.

Vaccines and efficacy in clinical trials

In phase 3 vaccine trials, protection from disease, i.e., immunity, has been demonstrated relative to the placebo group despite a finite incidence of infection in the vaccinated subjects. A vaccine could achieve statistical significance for the primary endpoint for protection from disease despite significant incidence of disease in the vaccinated group (example FDA accepted primary endpoint >50%).[109] Even with high efficacy, a proportion of those inoculated would not have protection from disease. Assessment for seroconversion failure or declining levels in the vaccinated but susceptible population is a critical parameter with implications for patient care, population management, and public policy.[2,110] Data from initial vaccine trials is limited to certain populations and exposure patterns. Additional data on antibody response and duration will be needed to help inform vaccine efficacy in larger, more-diverse populations to determine appropriate use in the context of variables such as vaccine design/manufacturer, ethnicity, level of viral load exposure, chronic diseases, and individual immune system strength.[111] All vaccines in use or development published on to date include or are based solely on the spike protein, with spike- or RBD-specific antibodies serving as a surrogate of efficacy along with elements of the cellular response. In this scenario, natural infection can be monitored by testing for antibody to the N protein. However, testing for quantitative S1-RBD antibodies would be the preferred method to assess levels relative to susceptibility following vaccination due to their correlation to neutralization and protection. Additional data on vaccine use and antibody response in already-seropositive patients is needed to determine response patterns in a more-diverse antibody population. Following the identification of multiple SARS-COV2 variant strains, additional data on the ability of antibodies developed from vaccines to infer protection against these strains would be necessary.[85,86,88]

Vaccine response in at-risk populations

Initially, additional data on duration of antibody-mediated protection is needed across populations, and in the long-term testing may be focused on populations with known risk of insufficient immune response such as the elderly.[112,113] The timing of appropriate serology testing would be optimized and refined based on the accumulation of evidence. In 2013, researchers from the CDC estimated that the prevalence of immunosuppression among adults in the United States was 2.7%.[114] This patient population has been excluded from the vaccine trials. Data for other vaccines, such as regular-dose influenza vaccine, show a reduced response in solid organ transplant recipients when compared with the general population.[115,116] Studies assessing the immune response of SARS-CoV-2 vaccinated individuals have relied on the measurement of the level of antibody response (or lack thereof) in immunocompromised individuals in comparison to healthy individuals as a measure of vaccine efficacy.[117,118] Studies in various patient populations such as solid organ transplant recipients,[119-123] dialysis,[121,124-127] hematologic malignancies,[117,128,129] multiple myeloma,[130,131] inflammatory bowel disease receiving biologic therapies,[132,133] and treatments with immunosuppressive medications,[134] have shown a varied antibody response following vaccination. Studies on duration of antibody response in these populations have been lacking. During phase I/II Pfizer-BioNTech and Moderna vaccine trials, 100% seroconversion was observed, and 90% seroconversion with the Janssen vaccine in the general population.[16,67,135] Decreased rates of vaccine-induced seroconversion have been reported among persons with a variety of immune suppressing conditions. A study of mRNA vaccination in hematologic malignancy patients showed that 46% did not seroconvert,[117] and another in chronic lymphoblastic leukemia patients showed that 60% did not seroconvert.[128] In kidney transplant recipients only 36.4% tested positive for anti-S antibodies after receiving the BNT162b2 vaccine.[123] In a study of solid organ transplant recipients, a third of patients that did not seroconvert after 2 mRNA vaccine doses were still seronegative following a third dose.[136] Quantitative serology testing would allow physicians to assess the presence, levels, and duration of antibody response following SARS-CoV-2 vaccination, and help guide the need for additional or higher doses as with other vaccines in solid organ transplant recipients.[116]
With the rollout of vaccinations to pediatric population, serology testing to assess immune response in more common immunocompromised populations, such as acute lymphoblastic leukemia and sickle cell disease, and populations on immunomodulating drugs will be needed.[137]

Recently anti-S IgG testing has been utilized to assess antibody presence in studies investigating the administration of monoclonal antibody therapy in hospitalized patients and has been shown to be helpful in assessing the potential utility of such therapies. Data from the RECOVERY trial has demonstrated that the investigational antibody combination reduces the risk of death when given to patients hospitalized with severe COVID-19 who have not mounted a natural antibody response of their own (seronegative).[138] The UK and France have recently authorized the use of monoclonal antibody therapy in at-risk immunocompromised individuals who are not sufficiently protected by vaccination.[139]

Summary

To enable an effective vaccination strategy, Siemens Healthineers advocates the use of automated SARS-CoV-2 serology testing to help confirm efficacy.

Serology assays should have the appropriate characteristics for assessment of vaccine response:

- Quantitative results
- S1-RBD-neutralizing IgG antibody detection
- Very high (≥99.5%) specificity

Serology testing can inform vaccination utilization by assessing status of protection at multiple junctures, especially in at-risk populations:

- Post-vaccination initial response after approximately 4 weeks
- Duration of vaccination response after approximately 6 months and periodically thereafter to guide decision making regarding the need for booster doses

Additionally, quantitative neutralizing-antibody testing could support determination of an antibody threshold for immunity/susceptibility to SARS-CoV-2 and provide critical data needed to understand vaccine-facilitated antibody response and duration in populations not included in initial vaccine trials. Serology is a scientifically sound, cost-effective surrogate for vaccine efficacy and able to meet high-volume testing needs. With the emergence of different variant SARS-CoV-2 strains, ensuring the effectiveness of vaccines will play a key role in promoting public health, including assessing sufficient and durable protection.

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