Understanding SARS-CoV-2 IgG Immunity Thresholds and the Process of Standardization

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Introduction

COVID-19 (coronavirus disease 2019) is the illness resulting from infection with SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) virus.[1-5] The virus spreads readily from person to person or possibly from environmental exposure.[6] Evidence supports spread by both asymptomatic and symptomatic individuals.[7] Antibodies appear approximately 1–3 weeks post-symptom onset in most patients and are produced in both symptomatic and asymptomatic infection.[8,9] Unlike typical seroconversion profiles, near-simultaneous production of both IgM and IgG has been observed in symptomatic patients with confirmed SARS-CoV-2. Antibody titers vary significantly between patients, though tend to be higher with severe vs. mild disease.[10,11]

Antibodies produced to structural proteins of the virus include spike (S) protein antibody and nucleocapsid (N) protein antibody. Data show both immunoglobulin M (IgM) and IgG antibodies and frequently IgA for these structural proteins appear with seroconversion. IgM and IgA disappear after a short time, but IgG remains detectable in most patients for a longer period of time. Spike protein is a transmembrane glycoprotein consisting of two regions: S1 and S2. S1 mediates recognition and binding of the viral receptor (ACE2) on host cells, and S2 facilitates viral fusion and entry.[12,13] S1 contains the receptor binding domain (RBD) that binds directly to ACE2 and is highly immunogenic. The S1 RBD in SARS-CoV-2 contains both unique and conserved sequences compared to other beta-coronaviruses. Available vaccines (and many in development) utilize the spike protein to generate neutralizing antibodies, with most mapping to epitopes within the RBD (but also within the S1-NTD and S2). Both in-vivo and in-vitro data indicate neutralizing antibodies can be highly protective, although the level of antibody may be an important correlate.[14-23]

Despite unprecedented advances in our understanding of COVID-19 and in providing effective vaccines, some questions remain. These include a better understanding of the immune correlates of protection in infected, re-infected, vaccinated individuals, donor convalescent plasma, and the length of time that immunity persists. For the various studies reported, including vaccine studies, antibody levels in patient sera were determined using various assays and cutoffs. This limits the ability of researchers to confidently compare results across studies. Standardization of assays is a way to allow comparison of results across the different assays and can be accomplished with reference materials that are well characterized. Recently, the United States (U.S.) Centers for Disease Control and Prevention (CDC) (Atlanta, GA, U.S.) highlighted the need for standardized SARS-CoV-2 quantitative IgG and neutralization assays.[24]

Siemens Healthineers has taken a four-step approach to implementing standardization and proving efficacy for SARS-CoV-2 serology assays.

Step 1: Select the right target

Step 2: Demonstrate the target picks up neutralizing antibodies

Step 3: Develop standards that can be used across manufacturers

Step 4: Demonstrate antibody thresholds that confer immunity

Glossary of Terms

Immunity: If a person is immune to a pathogen (such as a virus), they can be exposed to it without becoming infected (or may experience more mild disease/faster resolution).

COVID-19: Coronavirus disease 2019.

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; virus responsible for COVID-19.

Quantitative antibody (Ab) test: Defines the level (amount) of Ab present. If an immunity threshold is determined, use of a quantitative test (example antiHBsAg at 10 mIU/mL) supports rapid assessment for protection/vulnerability.

- As Ab levels can wane over time (some very rapidly and others very slowly), quantitative assessment can be very useful.
- True quantitation requires an accepted international standard utilized by all manufacturers for a shared (common) threshold value. In the absence of such a standard, tests are technically semi-quantitative, meaning the value is only relevant to that assay. However, "quantitative" is often applied to both quantitative and semi-quantitative tests.

Qualitative antibody test: A "yes" or "no" answer whether Ab is present independent of how much by identifying a value to distinguish a "positive" from a "negative" population. Some qualitative tests can be semi-quantitative if linearity in the reporting range is established relative to the internal standard used.

Nucleocapsid (N) protein: The structural protein associated with the viral RNA inside of the cell. N protein is the most abundant viral protein and is highly immunogenic. Antibodies to N protein result from infection in almost all patients.

Spike (S) protein: A transmembrane glycoprotein consisting of two domains: S1 and S2.

S1: Mediates recognition and binding of the viral receptor (ACE2) on host cells.

S2: Facilitates viral fusion and entry.

S1/S2: Full-length S protein

S1 receptor-binding domain (S1 RBD): S protein is highly immunogenic and Abs to the S1 RBD and other S protein epitopes result from infection in almost all patients.

Binding vs. neutralizing antibodies: All Abs formed to parts (epitopes or antigens) of SARS-CoV-2 are binding, but only a subset also exhibit virus neutralizing activity.

Neutralizing antibody: The ability of an Ab to inhibit/prohibit infection, often by interfering with virus binding to the host cell.

Plaque reduction neutralization tests (PRNT): Manual microtiter method evaluation of the ability of specific Abs to interfere with viral attachment or infection, and frequently (but not always) are a surrogate for protective immunity. Neutralization techniques characteristically assess for percent inhibition (e.g., 50% or 90%) associated with specific Ab titers or values. Method requires a biosafety level-3 (BSL-3) laboratory and may take several days to complete.

Pseudovirus neutralization tests (pVNT): Alternatives to PRNT that use recombinant pseudoviruses (like vesicular stomatitis virus, VSV) that incorporate the S protein of SARS-CoV-2 and assess for inhibition of binding the viral receptor.[12,13] These approaches are faster than PRNT but still take a couple of days to complete. They can be conducted in a less stringent BSL-2 setting.

Selecting the right target

Humans produce antibodies against both the N protein and S protein as well as other proteins during an immune response to SARS-CoV-2. The key questions manufacturers must answer when developing a SARS-CoV-2 serology assay are: (1) are the antibodies the test measures neutralizing antibodies? and (2) do those antibodies protect from reinfection? For SARS-CoV-2, humans need to neutralize or block the binding of the virus to the ACE2 receptor (Figure 1).

Antibodies to S1 RBD have been shown to account for about 90% of the neutralizing activity in patient sera,[25] and multiple studies using patient sera have shown correlations between various anti-S and anti-S1 RBD IgG assays and neutralizing antibody titers.[26-33] In addition, correlations have been found between disease severity and various anti-S and anti-S1 RBD IgG assays, and between disease severity and neutralizing antibody titers.[31-33] Multiple vaccines that are available or in development target or include the S1 RBD, and antibodies to this region in vaccinated serum have demonstrated neutralizing activity.[34-40] Anti-N protein antibody assay values have correlated with neutralization[31,41] but to a lesser extent than anti-S1-related antibody assays.[31] Siemens Healthineers selected the S1 RBD as the target of neutralizing antibodies from the beginning, based on its neutralizing capabilities.

Demonstrating that the test detects neutralizing antibodies

Plaque-reduction neutralization tests (PRNT) are the gold-standard methodology to measure the neutralizing antibody titers for a virus. $PRNT_{50}$ is the standard neutralization endpoint used for PRNT, which represents the maximum serum dilution that results in ≥50% reduction in plaque count. $PRNT_{90}$ is also used but considered more rigorous criteria. The challenge with PRNT assays is they require a Biosafety Level (BSL) 3 facility, are cumbersome manual assays with manual readouts, and can take up to three days to generate a result. On top of that there is biological variability in viral strains and cell lines, variable cell densities, and viral loads used. Siemens Healthineers has examined whether serology could be a suitable substitute for PRNT and the protective level of antibody required for immunity.

The correlation of neutralization titer using a PRNT was evaluated by testing samples from 75 subjects with a clinical diagnosis of COVID-19 based on a positive SARS-CoV-2 PCR method. The Atellica® IM SARS-CoV-2 IgG (sCOVG) Assay*† results generated on the Atellica IM Analyzer provided a Pearson correlation coefficient of 0.81, demonstrating a strong relationship between the Atellica IM sCOVG Assay index values and $PRNT_{50}$ neutralization titers, as shown in Figure 2.

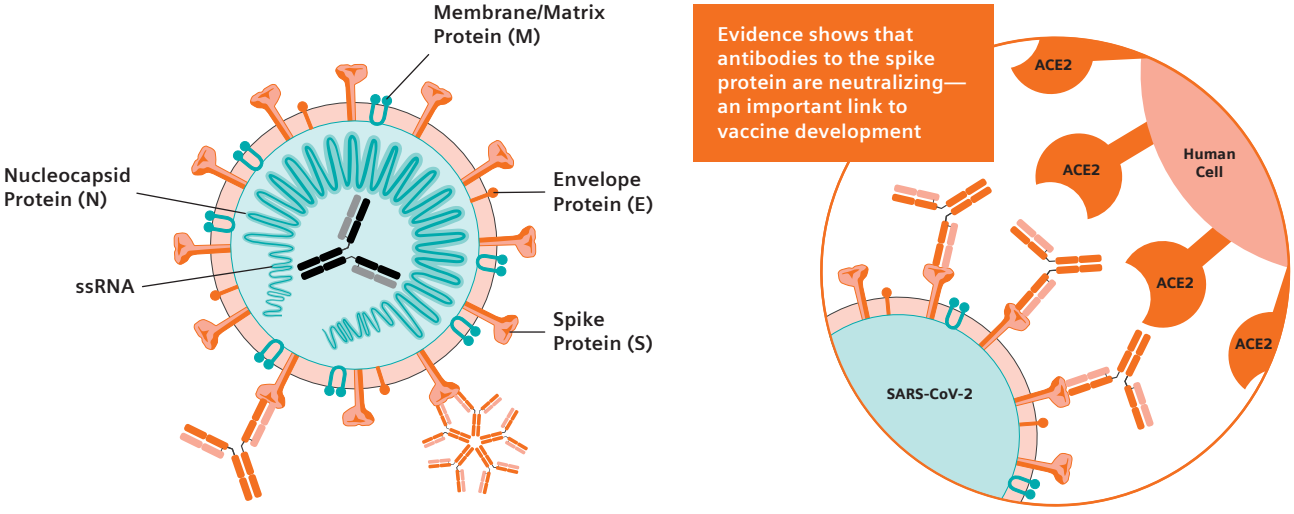

Figure 1. Importance of antigen selection to detect neutralizing antibodies.

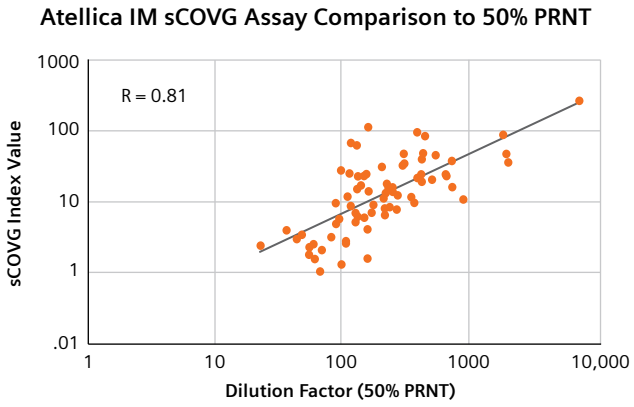

Figure 2. Correlation of Siemens Healthineers Atellica IM sCOVG Assay index results with $PRNT_{50}$ neutralization titers demonstrates a strong relationship.

This study was further supported in a published Austrian study in February 2021 from the University of Innsbruck for the ADVIA Centaur SARS-CoV-2 assay,[43] as shown in Figure 3. (The Atellica IM sCOVG and ADVIA Centaur sCOVG assays have the same cutoff values).

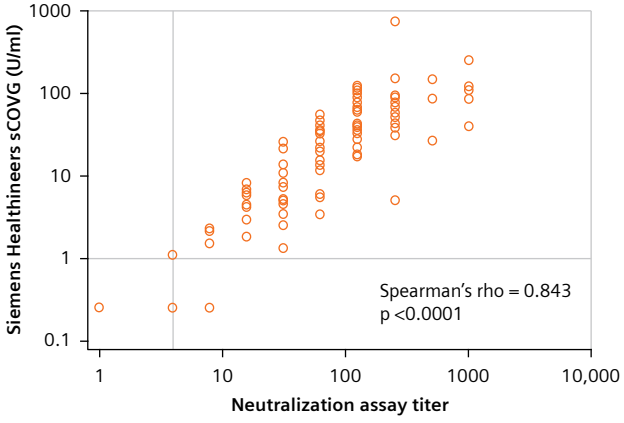

Figure 3. Clinical validation of the quantitative Siemens Healthineers ADVIA Centaur sCOVG assay demonstrates good correlation of results with virus neutralization titers.[43]

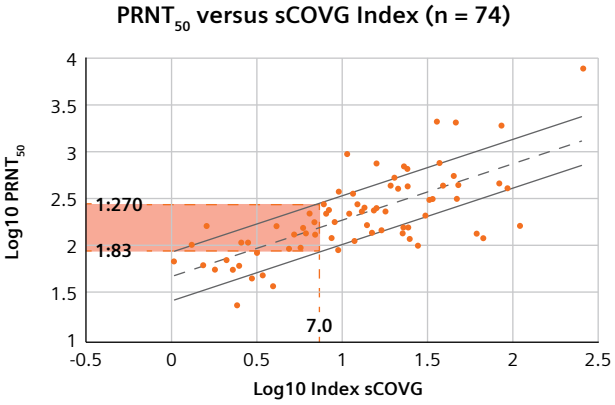

Figure 4. $PRNT_{50}$ versus Atellica IM sCOVG index values, demonstrating the 99% prediction interval at sCOVG Index of 7.0.

Table 1. $PRNT_{50}$ versus Atellica IM sCOVG Index for sCOVG Index of 7.0.

sCOVG Index	$PRNT_{50}$ titer (99% Prediction Interval)	PPV
7.0	>1:80 (1:83–1:270)	100%

Figure 4 shows the relationship between the index value of 74 reactive samples for SARS-CoV-2 antibody in the Atellica IM sCOVG Assay as a function of the $PRNT_{50}$ neutralization titer. In order to predict what index value associated with different levels of neutralizing antibodies, we determined the 99% prediction interval of $PRNT_{50}$ for different sCOVG index values. For a sample with sCOVG assay Index of 7.0, the $PRNT_{50}$ value would be above 1:80 with a 99% prediction interval of 1:83–1:270 (Figure 4, Table 1). A 1:80 $PRNT_{50}$ is a common benchmark for significant neutralization titers in convalescent plasma.[44] Of the 74 samples tested, all samples (100%) with index values above 7.0 Index produce $PRNT_{50}$ titers greater than 1:80 dilution.

**This test has not been FDA cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for detecting the presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner. Product availability may vary from country to country and is subject to varying regulatory requirements.*

†Claims for correlation to neutralizing antibody titer have not been reviewed by the FDA and are not available in the U.S.

Develop standards that can be used across manufacturers

The European Commission's Joint Research Centre (JRC) and World Health Organization (WHO) have been working on reference material for standardization. The First WHO International Standard for anti-SARS-CoV-2 Immunoglobulin (human) is the WHO reference material for SARS-CoV-2 serology tests, and it is referenced as the WHO 20/136. It 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, Siemens Healthineers believes it is not suitable for harmonization or standardization, since it only contains a single value, is not associated with neutralizing antibodies, and does not distinguish between N protein and S protein. If manufacturers claim standardization to the WHO it does not mean all assays are comparable. Instead it indicates there is alignment with a single measurement and reflects traceability.

Siemens Healthineers tested the new WHO material with the Atellica IM sCOVG Assay*‡ and the Atellica IM SARS-CoV-2 Total (COV2T) Assay*‡ (Figures 5–6, Tables 2–3). This demonstrated our traceability to the WHO material, and enabled a calculation of the assay index value to the WHO units. For example, the cut-off of 1.00 Index on the Atellica IM sCOVG Assay would have a WHO BAU/mL value of 21.8.

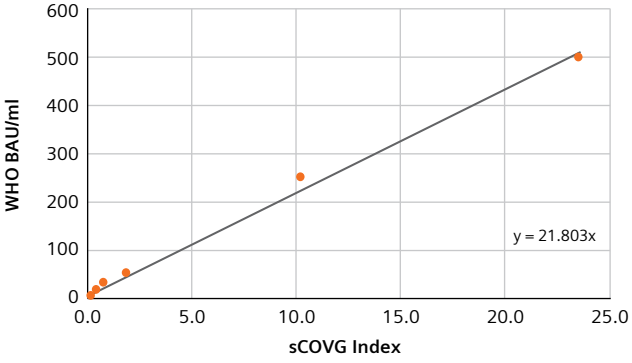

Figure 5. Atellica IM sCOVG Assay Traceability to WHO 20/136 (BAU/mL).

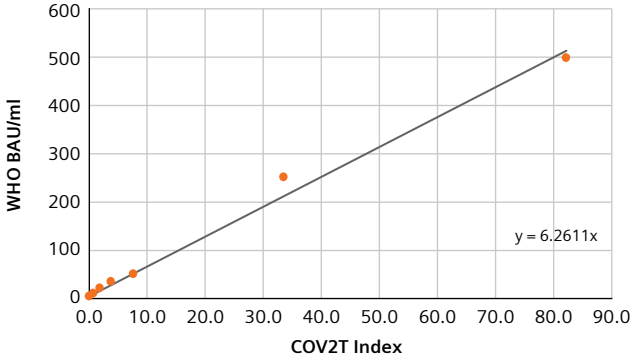

Figure 6. Atellica IM COV2T Assay Traceability to WHO 20/136 (BAU/mL).

Table 2. Atellica IM/ADVIA Centaur sCOVG Assay Traceability to WHO 20/136 (BAU/mL).

	sCOVG
Reporting Units	Index
Cut Off	1.00 Index
Slope (y-WHO: x-assay)	21.8
R	0.996
WHO BAU/mL at 1.00 Index Cut Off	21.8

Results were established using the Atellica IM sCOVG Assay, which has the same reagent formulations as the ADVIA Centaur sCOVG assay.

Table 3. Atellica IM/ADVIA Centaur COV2T Assay Traceability to WHO 20/136 (BAU/mL).

	COV2T
Reporting Units	Index
Cut Off	1.00 Index
Slope (y-WHO: x-assay)	6.26
R	0.997
WHO BAU/mL at 1.00 Index Cut Off	6.26

Results were established using the Atellica IM COV2T Assay, which has the same reagent formulations as the ADVIA Centaur COV2T assay.

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‡Claims for correlation to the 1st WHO International Standard Anti-SARS-CoV-2 Immunoglobulin (Human)(NIBSC 20/136) have not been reviewed by the FDA and are not available in the U.S. Product claims may vary from country to country and are subject to varying regulatory requirements.

The JRC has also developed standards for SARS-CoV-2. EURM-017 is quality control sera for immunoassay-based in vitro diagnostic devices and virus neutralization assays.[45] It consists of a pool of serum samples obtained from different plasma donors who had a SARS-CoV-2 infection 10 to 16 weeks before the plasma collection. EURM-017 was characterized using authentic and surrogate virus neutralizations assays, and various serology assays. Siemens Healthineers Atellica IM COV2T Assay* and Atellica IM sCOVG Assay* results for EURM-017 are shown in Table 4.

Table 4. Siemens Healthineers Atellica IM COV2T and sCOVG assay results for EURM-017 quality control sera.

Assay	Class of Antibodies	Antigen	Results
Siemens Healthineers SARS-CoV-2 Total (COV2T)	Total	S1 RBD	27 Index
Siemens Healthineers SARS-CoV-2 IgG (sCOVG)	IgG	S1 RBD	14 Index

In June 2020, Siemens Healthineers recognized that the industry needed a novel standardization process for standardizing SARS-CoV-2 assays by anchoring it to a neutralization endpoint. Different manufacturers are targeting different antigens (antibody targets); N protein, S protein, S1/S2 [full-length S], S1 RBD and reportable index units of semi-quantitative/quantitative assays are not actual concentrations so results cannot be compared across manufacturers. As a result, each manufacturer has standardized its assays with internal standards that are not linked to a common reference material. In addition to the availability of NIBSC and JRC materials, Siemens Healthineers has investigated a novel approach to define which concentration confers neutralization for different manufacturers' antigen targets. To this end, Siemens Healthineers has worked with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to publish concentrations of antibodies specific to each viral protein (S1, S2, S1/S2 [full-length S], S1 RBD, and N protein) that confer neutralization of the virus. For example, if a manufacturer is targeting the N protein, they will use the IFCC standard for N protein, and if a manufacturer is targeting the S protein, they will use the IFCC standard for S protein. Once validated, any manufacturer can adopt this method for their standardization process. Results would then be a true comparable quantitation in mg/mL, and any manufacturer reporting in mg/mL will be standardized to the antibody that the assay detects using the IFCC reference material.

As part of this work, Siemens Healthineers signed a Research Collaboration Agreement (RCA) with the U.S. CDC in August 2020. There are two separate CDC collaborations. The first is RCA I, in which the objective is to demonstrate neutralization of antigen-specific antibodies to SARS-CoV-2. This work is in progress, as is the ongoing development work with SARS-CoV-2 variants. The second is RCA II, the objective of which is to determine the antibody threshold that confers immunity. This collaboration will involve work with vaccine companies and is pending sample acquisition including sample acquisition from vaccinated individuals.

Siemens Healthineers collaborated with the JRC in parallel with the CDC, and in October 2020 started the work necessary to establish standardization endpoints based on neutralization. Five anti-SARS-CoV-2 antibody species (S1 RBD, N protein, S1, S2, and the S1/S2 [full-length S]) present in EURM-017 were affinity purified, quantified (Table 5), and $PRNT_{50}$ titer values (Table 6) determined. The amount of purified antibody required for $PRNT_{50}$ was the lowest for S1-related antibodies compared to S2 and N protein-related antibodies, indicating that S1-related purifications were enriched in neutralizing antibodies, as shown in Table 6.[46] Greater neutralizing activity was found for S1 purified antibodies compared to S1 RBD purified antibodies. This was demonstrated by the higher dilution required for $PRNT_{50}$ and the lower neutralizing concentration for S1 purified antibodies (Table 6). This indicated that neutralizing antibodies were associated with S1 sites other than those purified with S1 RBD. We do not know if the additional neutralizing activity of S1 purified antibodies was dependent on the presence of the S1 RBD site. Notably, it has been reported that sera containing neutralizing antibodies specific to S1 failed to neutralize after depletion of antibodies to S1 RBD.[23] This supports the findings reported above that S1 RBD is responsible for most of the neutralizing activity elicited by SARS-CoV-2,[25] and reflected in Figure 2 which demonstrates a strong relationship between the Atellica IM sCOVG Assay index values and $PRNT_{50}$ titer.

Table 5. Antibody protein yield from each of the five polyclonal antibody affinity purifications.

Antibody specificity	Protein concentration (µg/mL)	Total antibody (µg)	IFCC EURM-017 (µg/mL)[a]
S1 RBD	211	174	17.4
NP	769	725	72.5
S1	190	177	17.7
S2	317	297	29.7
S1/S2 (full-length S)	354	341	34.1

a. Concentration in 10 mL starting volume of EURM-017.

Table 6. The concentration, antibody amount required for $PRNT_{50}$, and the dilution required for $PRNT_{50}$ for each of the five antibody species in EURM-017.

Antibody specificity	EURM-017 (µg/mL)	Antibody required for $PRNT_{50}$ (µg/mL)	Dilution representing 50% plaque reduction
S1 RBD	17.4	0.207	1017
NP	72.5	19.7	39
S1	17.7	0.103	1846
S2	29.7	0.893	355
S1/S2 (full-length S)	34.1	0.287	1234

Demonstrate antibody thresholds that confer immunity

Siemens Healthineers is collaborating with sites that have samples from vaccinated individuals with clinical data and different levels of disease severity. These studies will demonstrate how our assay performs in vaccinated individuals. We will also be conducting studies as part of the CDC RCA II (which is aimed at determining the antibody threshold that confers immunity). Because the vaccine rollout started at the beginning of 2021, any prospective study that started with mass vaccination (early 2021) will likely not report the first of longitudinal draws until May 2021. As such, any Siemens Healthineers collaborations in addition to the CDC collaboration will not have reportable/publishable data until the second half of 2021.

We do have an early indication of results from index values associated with therapeutic levels in convalescent plasma. The ADVIA Centaur SARS-CoV-2 IgG (COV2G)* assay has been authorized by the FDA[47] to be used as a test for the qualification of high titer COVID-19 convalescent plasma in the manufacture of COVID-19 convalescent plasma. The FDA EUA for the emergency use of COVID-19 convalescent plasma for the treatment of hospitalized patients, authorized in August 2020, has been revised to include the ADVIA Centaur COV2G assay—supported by studies conducted with Vitalant Research Institution through one of our collaboration partners. It was determined that an Index of ≥4.8 was the cutoff necessary to qualify convalescent donor plasma for use in donations.

Plasma from recovered or convalescent COVID-19 patients may contain sufficiently high antibody titers to be able to treat hospitalized patients early in the course of disease and those hospitalized with impaired humoral immunity. This EUA authorization and study data support use of the ADVIA Centaur COV2G assay to qualify convalescent donor plasma. We are anticipating the inclusion of the Atellica IM COV2G Assay in this EUA soon. The ADVIA Centaur COV2G and Atellica IM COV2G assays have the same cutoff values. The use of Atellica IM sCOVG and ADVIA Centaur sCOVG assays for this application is also being pursued. Through correlation of sCOVG to COV2G the equivalence value is expected to be 9.0 Index, which can be converted to 196.2 BAU/mL.

To enable an effective vaccination strategy, we advocate for the use of accessible, automated, high-throughput SARS-CoV-2 serology testing to help confirm efficacy and promote public health. Siemens Healthineers has formulated a position paper based on input from experts in the infectious disease, immunology and vaccine development fields, and the currently available body of literature.[48]

Currently, Siemens Healthineers recommends measuring SARS-CoV-2 antibodies in relation to vaccine use to: (1) establish a threshold for protection or immunity, (2) confirm an initial neutralizing antibody response (approximately 3–4 weeks after each dose), and (3) track antibody levels in months 3, 6, and 9 months and annually following vaccination. The timing of use may be revised as additional data becomes available.

Conclusion

Ultimately, the goal is for researchers and clinicians to be able to confidently establish and compare immunity thresholds across manufacturers. Assay manufacturers contribute to this goal by ensuring they are selecting the right target for SARS-CoV-2 antibody assays and demonstrating that those assays recognize neutralizing antibodies. Standardization of assays is a way to allow comparison of results across the different assays and can be accomplished with reference materials that are well characterized. Results indicate that the Atellica IM sCOVG Assay demonstrates analytical performance capable of measuring patient IgG antibody response to the S1 RBD antigen and is acceptable for quantifying an individual's immune response, including production of neutralizing antibodies, and identifying an individual's immunocompetence to the SARS-CoV-2 virus. Studies are ongoing to determine the performance of the Atellica IM sCOVG Assay in vaccination studies.

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