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REVIEW ARTICLE

SARS-CoV-2 Serologic Assay Needs for the Next Phase of the US COVID-19 Pandemic Response

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Background. There is a need for validated and standardized severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) quantitative immunoglobulin G (IgG) and neutralization assays that can be used to understand the immunology and pathogenesis of SARS-CoV-2 infection and support the coronavirus disease 2019 (COVID-19) pandemic response.

Methods. Literature searches were conducted to identify English language publications from peer-reviewed journals and preprints from January 2020 through November 6, 2020. Relevant publications were reviewed for mention of IgG or neutralization assays for SARS-CoV-2, or both, and the methods of reporting assay results.

Results. Quantitative SARS-CoV-2 IgG results have been reported from a limited number of studies; most studies used in-house laboratory-developed tests in limited settings, and only two semiquantitative tests have received US Food and Drug Administration (FDA) Emergency Use Authorization (EUA). As of November 6, 2020, there is only one SARS-CoV-2 neutralization assay with FDA EUA. Relatively few studies have attempted correlation of quantitative IgG titers with neutralization results to estimate surrogates of protection. The number of individuals tested is small compared with the magnitude of the pandemic, and persons tested are not representative of disproportionately affected populations. Methods of reporting quantitative results are not standardized to enable comparisons and meta-analyses.

Conclusions. Lack of standardized SARS-CoV-2 quantitative IgG and neutralization assays precludes comparison of results from published studies. Interassay and interlaboratory validation and standardization of assays will support efforts to better understand antibody kinetics and longevity of humoral immune responses postillness, surrogates of immune protection, and vaccine immunogenicity and efficacy. Public–private partnerships could facilitate realization of these advances in the United States and worldwide.

Keywords. immunity; pandemic; quantitative assays; SARS-CoV-2; serology.

In response to the coronavirus disease 2019 (COVID-19) pandemic, a number of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acid amplification tests, antigen-based tests, and serologic assays have been developed and used extensively worldwide for diagnostic, screening, and surveillance purposes. Since the start of the pandemic, the US Food and Drug Administration (FDA) has issued Emergency Use Authorizations (EUA) for nearly 200 assays to support the COVID-19 response in the United States [1]. The overwhelming majority of these FDA-authorized assays are nucleic acid amplification tests in the form of real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays for testing respiratory specimens (nasal and nasopharyngeal swabs). Recently, the FDA issued EUAs for rapid testing platforms that detect viral antigens. Since April 2020, serologic assays to detect antibodies produced against SARS-CoV-2 have become widely available in the United States.

In order to apply SARS-CoV-2 serologic testing strategies to inform public health interventions and individual patient management, serologic correlates of protection against SARS-CoV-2, in terms of antibody type and concentration, and duration of immunity conferred must be clearly established. These outcomes of individual and public health significance must be determined in association with clinical and epidemiologic data on various outcomes, such as decreased transmission, decreased duration and severity of illness, improved outcomes, prevention of re-infection, and, when available, the efficacy of vaccine candidates to protect against or decrease severity of primary infection. For broad understanding and consensus building of correlates of protection, these studies would need to include participants who are demographically diverse in terms of age group, sex, and race/ethnicity, as well as populations disproportionately affected by COVID-19.

Historically, for other bacterial and viral pathogens, such data have been derived from vaccine studies for vaccine-preventable diseases [2, 3], from studies of natural history of

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infection for diseases that are not vaccine preventable, and from animal models. As our knowledge of the natural history of SARS-CoV-2 and associated illness is currently evolving and vaccine candidates remain in development, we must base our assessment of serological correlates of protection on peer-reviewed reports of infection and reinfection, convalescent plasma therapy trials, and vaccine candidate studies. There is limited value in lessons learned from the severe acute respiratory syndrome (SARS) outbreak in 2003 [4, 5] and the ongoing outbreak of Middle East Respiratory Virus (MERS) [6]; for example, the timing and longevity of immunoglobulin G (IgG) and neutralizing antibodies to these viruses have been variable (ranging from months to years), SARS has not re-appeared, MERS infections are of low incidence, and there are currently no vaccines for either of these related coronaviruses.

Compared with cellular immune assays, laboratory assays designed to measure humoral immune response based on production of immunoglobulins against SARS-CoV-2 are logistically more feasible to implement across populations and to deploy at scale through commercial and reference laboratories. Additionally, serologic assays are amenable to standardization through quality control programs (ie, regulatory and compliance channels). The currently available assays with FDA EUA in the United States measure serum IgG, combined IgG and immunoglobulin M (IgM), or total antibody (IgM, IgG, and immunoglobulin A). These assays have been developed in several general categories: rapid tests, enzyme-linked immunosorbent assays (ELISAs), and chemiluminescent assays, all of which could also be scaled to be high-throughput serological assays (HTSAs), and lateral flow assays (LFAs) [7, 8]. For ELISA and chemiluminescent assays, results are calibrated using a signal cutoff value based on average signal intensity of positive and negative controls, thus setting a seropositivity threshold. SARS-CoV-2 serologic assay results are provided as binary (positive or negative) with an indeterminate value that is usually categorized as negative [1]. The targets for SARS-CoV-2 serologic assays include the nucleocapsid (N) protein, the spike (S) protein, the S1 region of the spike protein, and the receptor-binding domain (RBD) of the S protein. While these assays are increasingly applied in seroprevalence studies and in clinical testing to assess past infection with SARS-CoV-2, there are limitations in the interpretation and application of qualitative antibody tests for clinical and public health decision-making.

Neutralization assays against SARS-CoV-2 are a functional assessment of the ability of serum antibodies to prevent cell binding, entry, or other effects of the virus on the cell in vitro. In general, antibodies against the RBD or the S1 subunit are considered neutralizing [8]. Neutralization assays, such as the plaque-reduction neutralization test (PRNT) or microneutralization (MN) methods, have traditionally used live virus and are considered gold standards. However, these assays require Biosafety Level 3 (BSL-3) precautions and are generally labor- and resource-intensive to perform. BSL-3 sparing assays, such as pseudovirus neutralization, have been developed to overcome some of these challenges and have the potential for high throughput [9, 10]. These assays are available in research laboratories globally, with only 1 with FDA EUA as of November 6, 2020, and 1 available for commercial use in the United States [11]. However, the potential for these assays to be produced and used at high throughput in the commercial sector remains uncertain.

As of November 6, 2020, there are no FDA EUA quantitative IgG assays available through commercial laboratories in the United States. There do not appear to be any quantitative assays in commercial use in China, Europe, or Asia. Two assays for semiquantitative measurement of IgG in human serum and plasma have recently received FDA EUA [1, 12]. In addition, several studies have used the signal-to-cutoff ratio from positive and negative controls as a semiquantitative method. Although this approach provides an estimate of IgG levels, these tests generally lack the sensitivity and range of IgG quantitation needed to determine longevity and decay of IgG levels and correlation with neutralization assay titers.

METHODS

Literature searches (using PubMed and Google Scholar and the following search terms: IgG assays, neutralization assays, SARS-CoV-2, human) were conducted to identify English language publications from peer-reviewed journals, news items, and preprints from internet sites such as medRxiv [13] from January 2020 through November 10, 2020. Relevant publications were reviewed for mention of IgG, or neutralization assays for SARS-CoV-2, or both, and the methods of reporting assay results. Tables were populated for country where the laboratory test was performed, the setting of the study (ie, clinical care surveillance or screening, convalescent plasma related, or vaccine candidate trials), number of individuals tested, in-house or commercial assay, type of assay (ie, ELISA, chemiluminescent, virus neutralization, microneutralization, pseudovirus neutralization, other), and quantitation metric used to represent IgG and neutralization titers.

This activity was reviewed by the Centers for Disease Control and Prevention (CDC) and was conducted in a manner consistent with applicable federal law and CDC policy (45 C.F.R. part 46, 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq). This study did not include factors necessitating patient consent.

RESULTS

Applications for Standardized SARS-CoV-2 Quantitative Serological Assays

Based on review of the literature, applications for standardized quantitative IgG and neutralization assays include estimation of antibody longevity and decay in asymptomatic, symptomatic, ill, and recovered patients, evaluation and manufacturing of monoclonal antibody therapies, threshold estimations for correlates of protection (plotting of quantitative IgG values against neutralization titers), and vaccine immunogenicity and efficacy studies. Testing of donated convalescent plasma for antibodies would be considered a manufacturing step to determine IgG and neutralization titers before release of plasma for infusion therapy, offering another application for standardizing assays among laboratories involved in these trials.

Current Status of SARS-CoV-2 IgG Assays

Several published reports have described modifications of existing IgG serological assays to provide a quantitative or semiquantitative measurement of anti-SARS-CoV-2 IgG antibody (Table 1). The assay applications for these studies have included assessment of antibody levels in patients with clinical illness and evaluation of IgG antibody kinetics (decay) over time, with 5 reports describing studies performed as part of convalescent plasma donor therapy and 5 reports on early phase trials of vaccine candidates. There was a lack of standardization of the process of calibrating and reporting quantitative IgG results.

Current Status of SARS-CoV-2 Neutralization Assays

In reviewing current published reports of SARS-CoV-2 neutralization assays and the metrics used to report results (Table 2), there appears to be reasonable consistency across the sparse literature on this topic regarding the metrics of reporting of inhibition titers (either as 50% or 80% inhibitions) and serial dilutions.

Correlating IgG levels, Neutralization Titers, and Immunity

This important step in determining serological correlates of protection for SARS-CoV-2 has been initiated by a limited number of groups.

Serum samples from 3 confirmed patients from France (multiple serial serum samples) indicated that the plaque-reduction neutralization titers ($PRNT_{50}$) correlated in a linear fashion with IgG OD_{450} values against S, S1 subunit, RBD, and N protein for 1 patient with mild and 1 patient with severe disease [20]. Jackson et al. reported a strong correlation between binding and neutralization assays and between the live virus and pseudovirus neutralization assays [25]. In another small study, the OD levels in an IgG ELISA were reported to be statistically higher in severe/critical cases. Most of these patients, including those with mild nonpneumonic illness, developed detectable neutralizing antibodies, provided the serum samples were collected beyond 28 days after illness. There was modest correlation of IgG ELISA OD between 1 and 2.5, with microneutralization titers between 1:10 and 1:80 ($R = 0.67$) and PRN90 titers between 1:10 and 1:320 ($R = 0.73$) [15].

In a study of 187 sera from 107 patients from the Netherlands [8], investigators reported a wide diversity in immunoglobulin assay performance in different scenarios and in correlation of assay results with virus-neutralizing antibodies. One ELISA assay that detected total immunoglobulins against RBD performed best for assessing functional antibodies in different stages and severities of disease. This assay also demonstrated potential to set a cutoff indicating the presence of protective antibodies when correlated with plaque-reduction neutralization assays.

A study of 175 patients from China who recovered from clinically mild disease [31] noted that neutralizing antibodies as measured by pseudovirus neutralization varied substantially and included 10 patients in whom neutralizing antibodies were below the limit of detection.

A study of 12 plasma donors from New Mexico [23] noted variable neutralizing antibody titers among the donors with a median titer that was low; however, there was a strong correlation by linear regression analyses of neutralizing antibody titers and IgG levels ($rho = 0.938$; $P < .0001$).

In a large study of 370 convalescent plasma donors [7], Luchsinger et al. demonstrated that IgG assays predicted neutralizing activity in vitro and may thus serve to predict antiviral activity against SARS-CoV-2 in vivo, with RBD ELISA titers having a modest linear correlation ($r^2 = 0.42$) to neutralization titer, commensurate with the fact that the RBD is a principal target for neutralizing antibodies. As has been hypothesized, nucleocapsid ELISA titers showed no correlation with neutralization activity ($r^2 = 0.09$). Of note, lateral flow assay IgG densitometry measurements showed the poorest correlation with neutralization activity ($r^2 = 0.22$). The authors concluded that results of all quantitative serological assays correlate to some degree with neutralization activity and that high-throughput serology assay (HTSA) signal strength scores suggest presumptive ranges of neutralizing activity based on HTSA values.

Similarly, in a study of nearly 30 082 convalescent plasma donors screened at the Mount Sinai Health System in New York City [22], antispike protein–binding titers correlated significantly with neutralization of SARS-CoV-2 in vitro; neutralizing activity above background was noted for ~50% of sera in the 1:80–1:160 titer range of IgG, 90% of sera in the 1:320 range, and all sera in the 1:960–1:2880 range. The neutralizing antibodies were noted to persist up to 5 months in this study.

In a study of 109 convalescent plasma samples from 68 US patients reported by Salazar et al. [32], a strong correlation was found between both plasma anti-RBD and antispike protein IgG titers and in vitro microneutralization titer, although anti-RBD plasma IgG correlated slightly better than antispike protein IgG

Table 1. SARS-CoV-2 Immunoglobulin G "Quantitation" as Presented in Recent Studies (From January 2020 to November 6, 2020) Among Persons With SARS-CoV-2 Infection, Convalescent Plasma Donors, and Vaccine Trial Participants

Reference	Country Where Laboratory Performed Testing	No. of Individuals Presented	Target for Binding Antibodies	Assay	Details of Assay	Quantitation Metric for Serum IgG
Patients with current or recent SARS-CoV-2 infection						
Ibarrondo, NEJM 2020	USA	34 patients with mild COVID-19 illness	RBD	In-house	ELISA	Log-transformed anti-RBD in ng/mL; ELISA was modified to precisely quantify serum anti–receptor-binding domain activity in terms of equivalence to the concentration of a control anti–receptor-binding domain monoclonal IgG (CR3022, Creative Biolabs)
Juno et al. [14]	Australia	Recovered patients (n = 41)	S, RBD	In-house	ELISA	OD 450 nm; end point titers were calculated as the reciprocal serum dilution giving signal 2× background using a fitted curve (4 parameter log regression)
GeurtsvanKessel et al. [8]	The Netherlands	Recovered patients (187 sera from 107 patients)	Various targets	Commercial	Rapid antibody tests, ELISA, and chemiluminescent assays	Rapid antibody tests (positive/negative), ELISA (OD ratios), chemiluminescent (arbitrary units/mL)
Perera et al. [15]	Hong Kong SAR	Multiple sera from 24 patients	RBD	In-house	ELISA	OD_{450}; normalized results were obtained by calculating the difference between the OD of the purified recombinant protein–coated well and the PBS-coated well
Suhandynata et al. [16]	USA	54 patients	Nucleocapsid, RBD	Diazyme DZ-LITE 2019-nCoV IgG (CLIA) Assay Kit	Chemiluminescence	The light signal is measured by a photomultiplier and is reported as calculated luminescence units per mL (AU/mL); values ≥1.00 AU/mL are considered reactive, while values <1.00 AU/mL are considered nonreactive
Sun et al. [17]	China	Multiple serial samples from 38 patients	N and S	In-house	ELISA	OD_{450} values: the cutoff value for seropositivity samples was set as the mean value at optical density 450 (at a 1:50 dilution) for the 16 negative serum samples plus 3 SDs
Yong et al. [18]	Singapore	2 patients	N and RBD	In-house	ELISA	OD units
Long et al. [19]	China	285 patients with clinical illness, hospitalized	Nucleoprotein and a peptide from the spike protein	MCLIA kits supplied by Bioscience Co.	Double-antibody sandwich immunoassay	Log_2 (antibody level); antibody levels are presented as the measured chemiluminescence values divided by the cutoff (S/CO); the cutoff value of this test was defined by receiver operating characteristic curves; antibody levels in the figures were calculated as $log_2(S/CO + 1)$
Okba et al. [20]	The Netherlands (patient samples were from France)	3 patients	S, S1 subunit, N-terminal ($S1^A$) domain; RBD, N	EUROIMMUN Medizinische Labordiagnostika AG and in-house assay	ELISA	OD_{450}; the absorbance of each sample was measured at 450 nm, and we set the cutoff value at 6 SDs above the mean value for the negative cohort
Ripperger et al. [21]	USA	153 healthy controls and COVID-19 cases	RBD	In-house	ELISA	Antibody titers were quantified for RBD by quantifying AUC across a serial dilution curve
Convalescent plasma donors						

Table 1. Continued

Reference	Country Where Laboratory Performed Testing	No. of Individuals Presented	Target for Binding Antibodies	Assay	Details of Assay	Quantitation Metric for Serum IgG
Wajnberg et al. [22]	United States (NY)	Convalescent plasma donors and employees (n = 30 082)	S, protein	In-house	ELISA	ELISA results reported as discrete titers at 1:80, 1:160, 1:320, 1:960, or ≥1:2880
Bradfute et al. [23]	USA	Convalescent plasma donors (n = 12)	S1 subunit	In-house	ELISA	OD450 values
Salazar et al.	USA	Convalescent plasma donors (n = 109)	S, RBD	In-house	ELISA	Titer was defined as the last dilution showing an optical density greater than average negative control plus 3 SDs
Klumpp-Thomas et al. [24]	USA	68 symptomatic donors	S, RBD	In-house	ELISA	Signal intensity (absorbance, OD)
Luchsinger et al. [7]	USA	370 unique samples from convalescent plasma donors	S1 and RBD	LFA; in-house; high-throughput serologic assays: Abbott SARS-CoV-2 IgG chemiluminescent microparticle immunoassay with the Abbott Architech i2000SR (Abbott Core Laboratories), as well as the VITROS Immunodiagnostic Products	ELISA	Lateral flow assays: relative quantification of anti-SARS-CoV-2 IgG and IgM in convalescent plasma samples was performed using built-in gel analysis macros in FIJI ELISA: absorbance readings were collected at 450 nm; standard curves were constructed in Prism 8.4 (Graphpad Software Inc.) using a sigmoidal 4PL nonlinear regression (curve fit) model; HTSA: arbitrary units
Vaccine candidate trials						
Jackson et al. [25]	USA	45 individuals from phase 1 clinical trial of the mRNA-1273 SARS-CoV-2 vaccine encoding a stabilized prefusion spike trimer, S-2P	S-2P, RBD	In-house	ELISA	Reciprocal end point titers from 10^0 to 10^6
Mulligan et al. [26]	USA	38 individuals from phase 1/2 study to describe the safety and immunogenicity of a COVID-19 RNA vaccine candidate (BNT162b1)	RBD	In-house	Luminex	Data were captured as MFIs using a Luminex reader and converted to U/mL antibody concentrations using a reference standard curve with arbitrary assigned concentrations of 100 U/mL and accounting for the serum dilution factor; assay results were reported in U/mL of IgG
Folegatti et al. [27]	UK	Vaccine phase 1 volunteers (n = 44)	S, RBD	In-house	ELISA	Metric: EU; the standard pool was used in a 2-fold serial dilution to produce 10 standard points that were assigned arbitrary EUs; standardized EUs were determined from a single dilution of each sample against the standard curve, which was plotted using the 4-Parameter Logistic Model (Gen5 v3.09, BioTek)
Logunov et al. [28]	Russia	Vaccine trial (total n = 38 from phase 1 and 2 trials)	RBD	In-house	ELISA	The IgG titer was determined as the maximum dilution of serum, in which the OD450 value of the serum of the immunized participant exceeds the value of the control serum (serum of the participant before immunization) by >2 times
Sadoff et al. [29]	USA, Belgium	Phase 1/2a vaccine trial (total n = 377 and 393 in different cohorts)	S protein	In-house	ELISA	EU/mL, expressed as geometric mean titers with 95% CIs

Abbreviations: AU, arbitrary units; AUC, area under the curve; COVID-19, coronavirus disease 2019; ELISA, enzyme-linked immunosorbent assay; EU, ELISA units; FIJI.sc, "Image Processing Package"; HTSA, high-throughput serological assay; IgG, immunoglobulin G; MFI, median fluorescent intensity; N, nucleocapsid protein; OD, optical density; RBD, receptor-binding domain; S, spike protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 2. SARS-CoV-2 Neutralizing Antibody Assay Quantitation as Presented in Recent Studies (From January 2020 to October 20, 2020)

Reference	Country Where Laboratory Testing Was Performed	Neutralization Assay	Metric
Lei et al. [30]	China	Pseudovirus neutralization system combined with a cell-fusion inhibition assay using β-gal as a reporter gene	IC_{50}; which was defined as the concentration at which the β-gal activity was reduced by 50%
Okba et al. [20]	The Netherlands	PRNT	$PRNT_{50}$ titers; the serum neutralization titer is the reciprocal of the highest dilution resulting in an infection reduction of >50% (PRNT50); a titer >20 is considered positive
Jackson et al. [25]	USA	PsVNA and live wild-type SARS-CoV-2 PRNT	ID50 serial dilutions for PsVNA; PRNT80 serial dilutions for PRNT
Mulligan et al. [26]	USA	PRNT	Titers were calculated in GraphPad Prism, version 8.4.2, by generating a 4PL logistical fit of the percent neutralization at each serial serum dilution; the 50% neutralization titer was reported as the interpolated reciprocal of the dilution yielding a 50% reduction in fluorescent viral foci
Perera et al. [15]	Hong Kong SAR	MN, PRNT	MN: the highest serum dilution that completely protected the cells from CPE in half of the wells was taken as the neutralizing antibody titer; $PRNT_{90}$: antibody titers were defined as the highest serum dilution that resulted in >90% ($PRNT_{90}$ plaques)
Luchsinger et al. [7]	USA	Pseudovirus neutralization assays	The half maximal neutralizing titer (NT50 or IC50) for plasma was determined using a 4-parameter nonlinear regression in Prism 8.4 (GraphPad)
Folegatti et al. [27]	UK	Pseudovirus MN Marburg virus neutralization	Virus neutralization IC50 and serial dilutions
Juno et al. [14]	Australia	MN	The neutralizing antibody titer was calculated using the Reed- Muench method, as previously described
Wajnberg et al. [22]	USA	MN	ID50 results presented in log scale
GeurtsvanKessel et al. [8]	The Netherlands	An in-house plaque-reduction neutralization test	PRNT50 titers with doubling dilutions
Bradfute et al. [23]	USA	An in-house plaque-reduction neutralization test	PRNT80 titers
Wu et al. [31]	China	In-house pseudovirus neutralization assay	ID50 values categorized as low (ID50, <500), medium–low (ID50, 500–999), medium–high (ID50, 1000–2500), and high (ID50, >2500), and the detection limit was 40
Salazar et al. [32]	USA	In-house MN assay	Percent neutralization for each plasma sample at each dilution was determined relative to untreated, virus-only control wells
Logunov et al. [28]	Russia	In-house MN assay	TCID50 values; neutralization titer was defined as the highest serum dilution without any cytopathic effect in 2 of 3 replicable wells
Sadoff et al. [29]	USA, Belgium	In-house wild-type virus neutralization assay	Reciprocal serum dilution neutralizing 50% of the test virus dose (IC50), displayed on a log10 scale and described using GMT and 95% CIs
Ripperger et al. PRNT90 values were determined as the last dilution by which 90% neutralization occurred	USA	In-house wild-type virus neutralization assay	PRNT90 values were determined as the last dilution by which 90% neutralization occurred

Abbreviations: 4-PL, 4-parameter; β-gal, β-galactosidase; CPE, cytopathic effect; GMT, geometric mean titer; IC, inhibitory concentration; ID, inhibitory dilution; MN, microneutralization; NT, neutralizing titer; PRNT, plaque-reduction neutralization testing; PsVNA, pseudotyped lentivirus reporter single-round-of-infection neutralization assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

titer. The probability of a virus neutralization titer of ≥160 was ≥80% with anti-RBD or antispike protein titers of ≥1:1350.

Evaluating the ability of a chimpanzee adenovirus-vectored vaccine (ChAdOx1 nCoV-19) to elicit immune responses [27], Folegatti et al. found that titers from a pseudovirus neutralization assay and a SARS-CoV-2 neutralization assay correlated positively with other live neutralization assay titers and with an anti-RBD ELISA. They concluded that the correlation of neutralization assays with IgG quantitation indicates that, if confirmed, a standardized ELISA might be sufficient to predict protection, should neutralizing antibody also be shown to be protective in humans.

Ripperger et al. [21] correlated area under the curve IgG titers against RBD with neutralization assays and reported an *r* of 0.84 for 153 samples from healthy volunteers and COVID-19 patients. PRNT90 values were determined as the last dilution by which 90% neutralization occurred. Antibody titers were quantified for RBD by quantifying area under the curve (AUC) across a serial dilution curve.

In addition, several studies have published quantitative IgG levels and neutralization titers in vaccine recipients with no attempt to assess correlation of these results. An mRNA vaccine candidate has been reported to elicit robust IgG titers consistently after the second dose [25], along with neutralization titers in all 45 trial participants. Mulligan et al. [26] reported on another RNA vaccine candidate; RBD-binding IgG concentrations and SARS-CoV-2 neutralizing titers in sera increased with dose level and after a second dose. Geometric mean neutralizing titers reached 1.8- to 2.8-fold that of a panel of COVID-19 convalescent human sera. Logunov et al. [28] reported on a trial of a vaccine candidate based on a nonreplicating adenovirus 26–based vector wherein all 76 trial participants produced antibodies to the spike protein as well as neutralizing antibodies. Another vaccine candidate using the adenovirus-based vector (Ad26.COV2.S) directed against the spike protein has been shown to elicit IgG and neutralization titers in a majority of trial participants [29]; these investigators have reported ELISA results in ELISA units and neutralization assay results using wild-type virus neutralizations as IC50 values.

Apart from 1 report of 3 individuals being protected from re-infection on a fishing vessel [33], there are currently no data to estimate the level of neutralization activity that is needed to confer protective immunity based on clinical and epidemiologic studies of infection, recovery, and re-infection.

Preliminary Efforts to Standardize SARS-CoV-2 Antibody Assays

Efforts led by the US government are underway to coordinate work across federal agencies, industry, and academic partners to standardize and validate SARS-CoV-2 antibody assays and their use in seroprevalence studies [34]. The World Health Organization has recognized the importance of this standardization [35] and has explicitly endorsed the need for standardization of assays used to (1) measure antibody responses elicited by vaccination; (2) diagnose previous infection by SARS-CoV-2; and (3) determine SARS-CoV-2 antibody content in COVID-19 convalescent plasma. Furthermore, the World Health Organization has recognized the challenges posed by a lack of knowledge regarding the need for standard antigen preparations to calibrate antibody-binding assays.

Due to the rapidly evolving landscape of published and preprint literature on SARS-CoV-2, it is possible that not all relevant reports were identified and reviewed for inclusion in this review. Similarly, visibility on efforts underway within countries, professional organizations, and laboratories is limited.

DISCUSSION

The lack of standardization and use of in-house-developed serologic assays by different laboratories preclude comparison of results from the various studies being reported in the literature. This has implications for interpreting results across studies of SARS-CoV-2-infected patients and convalescent plasma, monoclonal antibody, and vaccine candidate trials. Gaps in knowledge and recommendations to expand the use of serologic assays for SARS-CoV-2 have been recently described [36].

Regarding attempts at reporting quantitative IgG and neutralization assays, despite similar types of titers reported, there remains a need for validation and standardization of assay results due to variability in how these assays are developed and performed. Development of clear criteria regarding the appropriate use of quantitative vs qualitative serologic assays would also facilitate standardization of reporting and comparison across studies.

Correlating IgG levels (using quantitative metrics) with neutralization titers, as has been described for other viruses such as measles [37], is an essential step before describing threshold ranges of levels (with 95% CIs) of IgG that would serve as a surrogate of protection when measured in isolation with no companion neutralization assay results [2]. These ranges could then be used to predict outcomes in patients, follow patients after recovery and for re-infection, and help elucidate the response to vaccination. Importantly, these data could provide insight on development of postinfection immunity and whether, if present, it correlates with a specific serologic response in terms of antibody type and titer.

This review highlights specific advances needed in these areas: validation and standardization of quantitative IgG and neutralization assays and estimation of serologic correlates and thresholds of protection.

For quantitative IgG assays, there is a need to (a) further define various viral protein targets; (b) validate targets with respect to quantification and standardization of the source of these targets (proteins and plasmids); (c) develop international standards for the quantitation of IgG so that results from different

assays can be compared; (d) develop and deploy standardized assays at scale in sufficient numbers of persons in all age groups, races and ethnicities, with an emphasis on disproportionately affected and special populations, to allow generalizability of results; and (e) describe distinctions in viral protein targets from natural infection vs vaccination or monoclonal antibody therapy.

For neutralization assays, there is a need to (a) further define viral protein targets of neutralization activity, such as the receptor binding domain of the spike protein; (b) develop international standard metrics for reporting neutralization titers; and (c) develop and deploy BSL-3-sparing assays, such as pseudovirus neutralization assays, at scale as noted above, to allow generalizability of results.

For estimating serologic correlates of protection, there is a need to estimate thresholds for protection of neutralization titers and plot quantitative IgG values against neutralization titers. These studies would ideally be performed at scale as described above and sufficiently powered to allow for estimating thresholds in various populations. Furthermore, it would be important for studies to be representative of the general population to allow generalizability of results. Inclusion of persons who are demographically diverse (eg, age, sex, race/ethnicity) and who represent the spectrum of disease (asymptomatic to ill), along with persons from disproportionately affected populations, will permit greater generalizability.

Ideal Future State

The ideal future state would be to first develop and validate quantitative IgG assays with similar protocols. This would be followed closely by at-scale testing of populations using quantitative IgG assays to determine surrogate correlates of protection in recovering patients and possible re-infections; estimate antibody kinetics and longevity of humoral immune responses postillness (using IgG as the surrogate rather than neutralization assays); and determine vaccine efficacy (using IgG as the surrogate rather than neutralization assays or infection rates). These laboratory-based studies must be correlated with observed levels of protection against infection and re-infection in clinical and epidemiological studies.

Ongoing intragovernmental collaboration among the CDC, FDA, National Institutes of Health (NIH), and National Institutes of Standards and Technology (NIST), combined with international cooperation and development of public–private partnerships (eg, with the clinical laboratory community, convalescent serum repositories, and commercial manufacturers of serologic laboratory assays, vaccines, and monoclonal antibodies for therapeutic and prophylactic use), can foster the standardization, development, and deployment of assays at scale and thus facilitate achievement of these goals.

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