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RESEARCH ARTICLE

The Serological Sciences Network (SeroNet) for COVID-19: Depth and Breadth of Serology Assays and Plans for Assay Harmonization

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ABSTRACT In October 2020, the National Cancer Institute (NCI) Serological Sciences Network (SeroNet) was established to study the immune response to COVID-19, and "to develop, validate, improve, and implement serological testing and associated technologies" (https://www.cancer.gov/research/key-initiatives/covid-19/coronavirus-research-initiatives/serological-sciences-network). SeroNet is comprised of 25 participating research institutions partnering with the Frederick National Laboratory for Cancer Research (FNLCR) and the SeroNet Coordinating Center. Since its inception, SeroNet has supported collaborative development and sharing of COVID-19 serological assay procedures and has set forth plans for assay harmonization. To facilitate collaboration and procedure sharing, a detailed survey was sent to collate comprehensive assay details and performance metrics on COVID-19 serological assays within SeroNet. In addition, FNLCR established a protocol to calibrate SeroNet serological assays to reference standards, such as the U.S. severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serology standard reference material and first WHO international standard (IS) for anti-SARS-CoV-2 immunoglobulin (20/136), to facilitate harmonization of assay reporting units and cross-comparison of study data. SeroNet institutions reported development of a total of 27 enzyme-linked immunosorbent assay (ELISA) methods, 13 multiplex assays, and 9 neutralization assays and use of 12 different commercial serological methods. FNLCR developed a standardized protocol for SeroNet institutions to calibrate these diverse serological assays to reference standards. In conclusion, SeroNet institutions have established a diverse array of COVID-19 serological assays to study the immune response to SARS-CoV-2 and vaccines. Calibration of SeroNet serological assays to harmonize results reporting will facilitate future pooled data analyses and study cross-comparisons.

IMPORTANCE SeroNet institutions have developed or implemented 61 diverse COVID-19 serological assays and are collaboratively working to harmonize these assays using reference materials to establish standardized reporting units. This will facilitate clinical interpretation of serology results and cross-comparison of research data.

KEYWORDS COVID-19, SeroNet, assay harmonization, serology

The National Cancer Institute (NCI) Serological Sciences Network for COVID-19 (SeroNet) was launched on 8 October 2020 as a collaborative initiative to expand research on immune responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SeroNet is comprised of investigators from 25 U.S. biomedical research institutions, working in partnership with the Frederick National Laboratory for Cancer Research (FNLCR) and the SeroNet Coordinating Center, which is managed by FNLCR (1). Of the 25 participating research institutions, 8 are designated as Serological Sciences Centers of Excellence (funded by U54 grants), 13 are funded with U01 grants to carry out specific research projects related to COVID-19 immunity, and 4 institutions are funded by subcontracts and are designated as Serological Sciences Network Capacity Building Centers (1).

One of the primary goals of this partnership is "to develop, validate, improve, and implement serological testing and associated technologies" (1). To this end, SeroNet formed a working group, the Serology Assays, Samples, and Materials Operations Group (abbreviated as Serology Assay Ops), in December 2020 to allow for coordinated development and collaborative sharing of serology assay procedures and to establish processes for harmonizing and standardizing methodologies using reference materials across institutions. Establishing harmonized and standardized SARS-CoV-2 serological

assays can allow cross-comparison and pooling of research study results and facilitate clinical interpretation of results for patient care.

While there are 85 serological assays approved by the FDA for emergency use (2), the quick development of assays has led to the lack of harmonized cutoffs and reporting units. Furthermore, there are no consensus guidelines on reporting standards or clarity on the clinical interpretation and relevance of results. This has created a complex landscape for interpreting both research and clinical serological assay results. For example, several studies have reported on heterogeneity in serological assay performance that would have a significant impact on research study conclusions and clinical interpretations related to longitudinal serosurveillance (3–6). Specifically, certain assays demonstrate reduced sensitivity over time after an initial SARS-CoV-2 infection diagnosis. Muecksch et al. reported that the Abbott SARS-CoV-2 anti-nucleocapsid IgG assay dropped from a peak sensitivity of 98% at 21 to 40 days post-PCR diagnosis to around 70% when patients were tested ≥81 days postdiagnosis, whereas the Roche Elecsys SARS-CoV-2 anti-nucleocapsid total antibody assay and Siemens SARS-CoV-2 anti-receptor-binding domain (anti-RBD) total antibody assay both maintained high sensitivity (95 to 100%) on the same set of serial samples (3). Narowski et al. also found a significant decline in the longitudinal sensitivity of their lab-developed nucleocapsid assay in a study of health care workers (6). Perez-Saez et al. similarly demonstrated that the rates of seroreversion at least 8 months after the initial infection differed greatly depending on the serological assay used (4). While the seroreversion rate of the EuroImmun semiquantitative anti-S1 IgG enzyme-linked immunosorbent assay (ELISA) was 26%, the rates were significantly lower for the Roche anti-nucleocapsid total antibody assay (1.2%) and the Roche semiquantitative anti-RBD total antibody assay (0%) (4). Additionally, numerous studies rely on neutralization assays as gold standard methods for determining the functional relevance of ligand-binding methods, but comparison studies have demonstrated variability in results for live-virus neutralization, pseudovirus neutralization, and surrogate neutralization assays (e.g., ACE2 inhibition assays) (7–9), raising the importance of assay harmonization and standardization across laboratories.

Therefore, SeroNet aims to address these knowledge gaps in SARS-CoV-2 serological assay research by establishing collaborative initiatives to characterize, compare, and harmonize SARS-CoV-2 serological assays. This paper describes the depth and breadth of serological assays developed and implemented within the SeroNet consortium and outlines a proposed process to establish assay traceability to the U.S. SARS-CoV-2 serology standard reference material and to the WHO international standard (IS; 20/136) for these diverse assays, with the ultimate goal of establishing harmonized reporting standards calibrated to the international standard. Availability of both national and international standards is crucial to provide easy accessibility to end users and due to the limited volume of international standard available; all national standards should be calibrated to the international standard to provide harmonized traceability. These collaborative efforts will facilitate cross-comparison of results and provide clarity for their clinical interpretation, including in response to circulating SARS-CoV-2 variants.

RESULTS

SeroNet serology assay data. Of the 25 institutions involved with SeroNet, 23 reported performing between one and seven serology assays and provided descriptive and performance data. Serology assay data were also obtained from the Frederick National Laboratory for Cancer Research (FNLCR) and National Institute of Standards and Technology (NIST), both of which collaborate with SeroNet. Collectively, SeroNet institutions reported development of 27 in-house ELISA methods (Table 1) (6, 10–26). The majority of ELISA methods were developed for testing of serum and/or plasma, with additional methods available for testing dried blood spots (DBS), saliva/oral fluid, and breast milk. Two methods have been granted FDA emergency use authorization (EUA), 3 methods are pending FDA EUA, 4 methods are validated for high-complexity

TABLE 1 Laboratory-developed singleplex ELISAs[a]

Sample type(s)	Antigen(s)	Isotype	Result type	Assay sensitivity and specificity	Center/institution	Reference(s)	Regulatory status
Serum, plasma, dried plasma	RBD	IgG (IgA/IgM being eval)	Qualitative	Day 0–7 after infection: sensitivity, 73.01%. Day 8–14 after infection: sensitivity, 100%. Day >15 after infection: sensitivity, 100%; specificity (n = 388 samples collected prior to COVID-19 pandemic), 97.68%.	Emory University	21	FDA EUA granted
Serum, plasma	RBD and Spike	IgG, IgM, IgA	Semiquantitative	Sensitivity, 95%; specificity, 100% (n = 38 positive, n = 74 negative sera tested)	Mount Sinai	12, 19, 20	FDA EUA granted
Serum, plasma, saliva	RBD	Total Ig, with IgG, IgM, IgA titers	Qualitative	Overall sensitivity, 82.5%; overall specificity, 100% (n = 300). At >14 days from symptom onset, sensitivity, 100%, and specificity, 100% (n = 261).	University of Minnesota	18, 22	Assays validated in a high-complexity-testing CLIA laboratory
Serum, plasma	RBD	IgG, IgM	Qualitative	Sensitivity, 91% for RBD IgG 15–21 days post-onset of symptoms, 100% >21 days post-onset of symptoms, 90% for RBD IgM 15–21 days post-onset of symptoms, and 100% >21 days post-onset of symptoms; specificity, 99.75% for RBD IgG and 100% for RBD IgM	Stanford University	10	Assays validated in a high-complexity-testing CLIA laboratory
Serum, plasma	RBD-ACE2	Total IgG that blocks RBD-ACE2 binding	Semiquantitative	NA; used as a follow-up assay in seropositive specimens	Stanford University	10	Assay validated in a high-complexity-testing CLIA laboratory
Serum, plasma	RBD	IgG, IgM + IgG	Quantitative (IgG); qualitative (IgM + IgG)	Sensitivity, 98% (n = 181); specificity, 98.9% (n = 181).	University of Puerto Rico	25, 53	Assay validated in a high-complexity-testing CLIA laboratory
Serum, plasma	Spike	IgG	Quantitative	Sensitivity, 98.3% (n = 60); specificity, 99.3% (n = 150)	Frederick National Laboratory	NR	RUO
Serum, plasma	Spike	IgM	Quantitative	Sensitivity, 93.8% (n = 30); specificity, 97.6% (n = 80)	Frederick National Laboratory	NR	RUO
Serum, plasma	Nucleocapsid	IgG	Quantitative	Sensitivity, 97% (n = 34); specificity, 100% (n = 99)	Frederick National Laboratory	NR	RUO
Serum, plasma	Nucleocapsid	IgM	Quantitative	NR	Frederick National Laboratory	NR	RUO
Serum, plasma, saliva	RBD	Total Ig	Qualitative	Sensitivity, 95% (n = 259; 9 or more days after symptom onset), specificity, 96% (n = 535)	University of North Carolina	6, 16	FDA EUA pending
Serum, plasma, saliva	Spike NTD	Total Ig	Qualitative	Sensitivity, 92% (n = 259; 9 or more days after symptom onset), specificity, 94% (n = 535)	University of North Carolina	6	FDA EUA pending
Serum	Spike, RBD	IgG	Semiquantitative	NR	CVVR/BIDMC/ Harvard	11	RUO
Serum, plasma, breast milk	RBD	IgG, IgA, IgM	Semiquantitative	NR	CVVR/BIDMC/ Harvard	14, 23	RUO
Serum, plasma	Spike	IgG	Quantitative	Sensitivity, 100%; specificity, 98.8%	Tulane University	NR	RUO
Serum, plasma	RBD	IgG	Quantitative	NR	Tulane University	NR	RUO
Serum, plasma	Nucleocapsid	IgG	Quantitative	NR	Tulane University	NR	RUO

(Continued on next page)

TABLE 1 (Continued)

Sample type(s)	Antigen(s)	Isotype	Result type	Assay sensitivity and specificity	Center/institution	Reference(s)	Regulatory status
Plasma, serum	Spike, RBD	IgM, IgG, IgA	Semiquantitative	Spike: IgG, sensitivity, 96.6%, and specificity, 96.7%); IgA, sensitivity, 99.3%, and specificity, 90%; IgM, sensitivity, 97.9%, and specificity, 100%. RBD: IgG, sensitivity, 97.3%, and specificity, 100%; IgA, sensitivity, 99.3%, and specificity, 96.7%; IgM, sensitivity, 97.9%, and specificity, 96.7%. IgG data based on 126 convalescent plasma donors and 30 prepandemic samples; IgM/IgA data based on 20 hospitalized donors and 30 prepandemic samples.	Johns Hopkins University	15	RUO
Serum, plasma	Spike (ECD), RBD	IgG	Semiquantitative	NR	University of Texas-Austin	17	RUO
Serum, plasma	RBD	IgG	Qualitative	Sensitivity, 100% ($n = 155$); specificity, 96.5% ($n = 133$)	Arizona State University	NR	RUO
Serum, DBS	RBD	IgG, IgM	Qualitative	Sensitivity, 97% ($n = 39$); specificity, 100% ($n = 37$)	University of Arkansas for Medical Sciences	54	RUO
Serum, DBS	RBD, spike, nucleocapsid	IgG, IgM	Qualitative	Sensitivity, 97% ($n = 39$); specificity, 100% ($n = 37$)	University of Arkansas for Medical Sciences	13	RUO
Serum, plasma, breast milk	RBD, spike, nucleocapsid	IgG, IgM, IgA	Quantitative (IgG); Qualitative (IgM, IgA)	Sensitivity, 97% ($n = 114$); specificity, 99%	University of Alabama—Birmingham	NR	RUO
Serum, plasma	RBD, nucleocapsid, spike trimer	IgG, IgA	Quantitative	RBD: sensitivity, 70.9% for IgG and 74.4% for IgA; specificity, 100% for both IgG and IgA. Nucleocapsid: sensitivity, 81.4% for IgG and 77.9% for IgA; specificity, 98.5% for IgG and 100% for IgA). Spike trimer: sensitivity, 67.4% for both IgG and IgA; specificity, 98.5% for IgG and 100% for IgA. Data based on PCR-confirmed COVID-19 hospitalized patients ($n = 86$) and negative prepandemic samples ($n = 65$).	University of Massachusetts Chan Medical School	26	RUO
Serum, Plasma	Nucleocapsid	IgG	Qualitative	Sensitivity, 100% ($n = 44$); specificity, 99.5% ($n = 202$)	The Ohio State University	24	FDA EUA pending
Serum	Nucleocapsid	IgG	Qualitative	NR	The Ohio State University	NR	RUO
Oral fluid	Nucleocapsid	IgG	Qualitative	Sensitivity, 92% ($n = 24$); specificity, 98% ($n = 85$)	Salimetrics	NR	RUO

[a]ACE2, angiotensin-converting enzyme 2; BIDMC, Beth Israel Deaconess Medical Center; CLIA, Clinical Laboratory Improvement Amendments; CVVR, Center for Virology and Vaccine Research; DBS, dried blood spots; ECD, extracellular domain; EUA, emergency use authorization; FDA, Food and Drug Administration; NA, not applicable; NR, not reported; NTD, N-terminal domain; RBD, receptor-binding domain; RUO, research use only.

testing in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory, and 18 methods are for research use only (RUO). Diagnostic sensitivity and specificity for in-house ELISA methods range from 67.4 to 100% and 90 to 100%, respectively.

Eight institutions reported development or use of multiplex or protein arrays for antibody detection (Table 2) (27–37). Sample types include serum, plasma, DBS, saliva, and bronchoalveolar lavage (BAL) fluid. Diagnostic sensitivity and specificity for multiplex and protein array methods range from 85 to 98.8% and 95.2 to 100%, respectively. Neutralization assays were developed by 9 institutions, with sample types including serum, plasma, BAL fluid, nasal wash, DBS, and breast milk (Table 3) (15, 24, 29, 38–50). Assays fall into three mechanistic categories: competitive binding assays, pseudotyped neutralization assays, and live-virus neutralization assays. The competitive binding assay measures the ability of antibodies to block interactions between the SARS-CoV-2 receptor-binding domain and human ACE2 receptor. Virus pseudotype neutralization assays, mainly HIV and vesicular stomatitis virus (VSV) based, use full-length spike incorporated in the viral particle to measure the capability of neutralizing antibodies to block viral entry into the target cells. SARS-CoV-2 live-virus plaque or focus reduction neutralization assays measure the ability of neutralizing antibodies to block the spreading infection of authentic SARS-COV-2 in cell culture. Diagnostic sensitivity and specificity for neutralization methods developed within SeroNet range from 93 to 100% and 97 to 100%, respectively. Lastly, 9 institutions report use of 12 commercial serology methods (Table 4). Commercial methods detect IgG, IgM, and/or total Ig to spike, RBD, and/or nucleocapsid antigens in serum or plasma. Of the commercial methods in use, 10 have FDA EUA, 1 is pending FDA EUA, and 1 is RUO.

Establishment of SeroNet assay traceability to the U.S. SARS-CoV- 2 serology standard and first WHO international standard for anti-SARS-CoV-2 immunoglobulin. Units for the U.S. SARS-CoV-2 serology standard were initially established by FNLCR based on measurements performed by eight laboratories (Table 5). Subsequently, FNLCR further established traceability of the U.S. SARS-CoV-2 serology standard to WHO IS 20/136 by using four FNLCR ligand binding serology assays, with assessment of neutralization tested at NIAID's Integrated Research Facility (IRF) (Table 5). The U.S. SARS-CoV-2 serology standard was made available to the public in December 2020. Thus, far, there have been 124 requests for U.S. SARS-CoV-2 standard material and 19 requests for the reference panel samples.

DISCUSSION

SeroNet has collectively established a diverse array of methodologies for measurement of SARS-CoV-2 antibodies in a variety of biological fluids. Methods include laboratory-developed ELISAs, multiplex assays, and neutralization assays, most used for research-only purposes, as well as commercial assays available for patient care or research studies. Assays have been developed to test unique sample types, including DBS, saliva/oral fluid, breast milk, nasal washes, and bronchoalveolar lavage fluid. Binding assays identify IgM, IgG, IgA, and/or total antibodies to nucleocapsid, spike, RBD, and/or N-terminal domain (NTD) antigens, and neutralization assays rely on three methods to quantify antibodies with functional neutralizing activity. Assays vary in result reporting, with qualitative, semiquantitative, and quantitative assays. This diversity of assay methods allows for robust investigation of multiple aspects of the serological response to SARS-CoV-2 infection and vaccination and for cross-comparison of assay performance across platforms and institutions within SeroNet.

With the rapid development of numerous methods for serological assessment, as exemplified by the depth and breadth of assays within SeroNet, it is critical to establish assay harmonization and standardized reporting units to facilitate cross-comparison of results across studies, as well as for streamlined meta-analyses. To this end, FNLCR has provided the U.S. SARS-CoV-2 serology standard reference material, which has traceability to the first WHO international standard for anti-SARS-CoV-2 immunoglobulin, to SeroNet sites performing serological assays, to allow establishment of standardized reporting of semiquantitative or quantitative results in binding antibody units (BAU)

TABLE 2 Laboratory-developed multiplex assays[a]

Sample type(s)	Antigen(s)	Isotype	Result type	Assay sensitivity and specificity	Center/institution	Reference(s)	Regulatory status
DBS, serum	Spike S1, nucleocapsid	IgG	Qualitative	Sensitivity, DBS, 94% for symptomatic (n = 774 samples collected >20 days after PCR^+ result) and 85% for asymptomatic (n = 115 samples collected >20 days after PCR^+ result); specificity, DBS, 99% (n = 730), and serum, 99% (n = 701)	Wadsworth	27, 28	NYS CLEP approved
Serum, plasma, DBS	Spike, nucleocapsid, RBD	Total Ig	Semiquantitative	Sensitivity, >97%; specificity, 99%	Wadsworth	29	FDA EUA granted; NYS CLEP approved
Serum, plasma, DBS	Spike, nucleocapsid, RBD	IgG, IgM, IgA	Semiquantitative	Sensitivity, >97%; specificity, 99%	Wadsworth	30	NYS CLEP approved; FDA EUA pending
Oral fluid, serum, plasma	Spike, RBD, nucleocapsid	IgG, IgM, IgA	Semiquantitative	Oral fluid IgG assay, sensitivity, 98.8% ≥15 days post-symptom onset (n = 81); specificity, 100% (n = 127)	Johns Hopkins University, supporting Michigan State University	31, 36	Oral fluid assays validated in a high-complexity-testing CLIA laboratory; serum/plasma RUO
Serum, plasma, BAL, DBS	Spike, RBD (different variants), nucleocapsid	IgG	Quantitative	Sensitivity, >97% (n = 89); specificity, 99% (n = 260)	Case Western Reserve University	32	RUO
Serum, plasma, saliva, BAL fluid	Spike, RBD, nucleocapsid	IgA	Quantitative	Sensitivity, >98%; specificity, 99%	Case Western Reserve University	32	RUO
Serum, plasma	Spike	IgG	Quantitative	Sensitivity, ≥93%; specificity, 100%	NIST	33	RUO
Serum, plasma	RBD	IgG	Semiquantitative	Sensitivity, ≥93%; specificity, 100%	NIST	33	RUO
Serum, plasma	RBD, nucleocapsid	IgG	Semiquantitative	Nucleocapsid: sensitivity, 90.3% (n = 155), and specificity, 98.0% (n = 133). RBD: sensitivity, 90.1% (n = 155), and specificity, 97.0% (n = 133).	Arizona State University	NR	FDA EUA pending
Serum	Spike, nucleocapsid, RBD	IgG, IgM, IgA	Quantitative	NR	Yale	34	RUO
Serum	Alpha, Beta, Gamma, and Delta variants (spike, RBD)	IgG, IgM, IgA	Quantitative	NR	Yale	35	RUO
Saliva	Spike, nucleocapsid, RBD	IgG	Semiquantitative	Sensitivity: nucleocapsid, 97.7%, RBD, 92.9%, and spike, 98.8%. Specificity: nucleocapsid, 95.2%, RBD, 96.4%, and spike, 97.6%. Combined nucleocapsid and spike sensitivity, 96.5%, and specificity, 98.8%.	Salimetrics	NR	RUO
Serum, plasma	Spike S1, S1-RBD, nucleocapsid, S1-NTD	IgG, IgA, IgM (combined); IgG, IgA, IgM (individual)	Quantitative	Sensitivity: combined antigens and isotypes, 99%; S1-RBD combined isotypes, 99%, and S1-RBD IgG, 99%. Specificity: combined antigens and isotypes, 99%, S1-RBD combined isotypes, 99%, and S1-RBD IgG, 99%. During the acute phase, sensitivity, 92%, and specificity 99%.	Emory/MicroB-plex	37	RUO

[a]BAL, bronchoalveolar lavage; CLIA, Clinical Laboratory Improvement Amendments; NIST, National Institute of Standards and Technology; NYS CLEP, New York State Clinical Laboratory Evaluation Program.

TABLE 3 Neutralization assays[a]

Sample type(s)	Antibody neutralization assay type	Result type	Assay sensitivity and specificity	Center/institution	Reference(s)	Regulatory status
Serum, plasma, BAL fluid	HIV lentiviral vector	Quantitative	Sensitivity, 100%, and specificity, 100%, using SeroNet FNLCR blinded reference panel set ($n = 110$)	The Ohio State University	24	RUO
Serum, plasma	Live-virus neutralization assay (microneutralization)	Semiquantitative	NR	Mount Sinai	38, 39	RUO
Serum, plasma, BAL fluid	Live-virus neutralization assay (FRNT)	Quantitative	Sensitivity, 93%; specificity, 100%	Saint Louis University	25, 40	RUO
Serum, plasma, BAL fluid	Live-virus neutralization assay (FRNT/FRNT-mNG/PRNT)	Quantitative	NR	Emory	41	RUO
Serum, plasma, DBS	Live-virus neutralization assay (PRNT)	Qualitative	$PRNT_{50}$: sensitivity, 100%; specificity, 97%. $PRNT_{90}$: sensitivity, 97%; specificity, 100%	Wadsworth	29, 42	NYS CLEP approved (serum and plasma)
Serum, plasma, breast milk	VSV pseudotype particle-based assay	Quantitative	NR	University of Alabama—Birmingham	NR	RUO
Serum, plasma, nasal washes	$TCID_{50}$ neutralization assay	Semiquantitative	NR	Johns Hopkins University	15, 43–47	RUO
Serum, plasma	ACE2 competitive binding assay	Quantitative	Sensitivity, 93.8%; specificity, 99.4%	University of Puerto Rico	25	RUO
Serum, plasma	Lentivirus-based pseudovirus assay for Wuhan D614G, Brazil, South Africa, and Delta variants. Assay performed in CHO/ACE2 cells.	Quantitative	Sensitivity, 100%; specificity, 100%	Tulane University	50	RUO

[a]CHO, Chinese hamster ovary; FNLCR, Frederick National Laboratory for Cancer Research; FRNT, focus reduction neutralization test; HIV, human immunodeficiency virus; mNG, mNeonGreen; $PRNT_{50}$ and $PRNT_{90}$, 50% and 90% plaque reduction neutralization test; $TCID_{50}$, 50% tissue culture infectious dose; VSV, vesicular stomatitis virus.

TABLE 4 Commercial assays

Instrument/assay	Antigen(s)	Isotype	Result type	Center/institution	Regulatory status
Abbott Alinity	Spike	IgM	Semiquantitative	Mount Sinai	FDA EUA granted
Abbott Architect	Spike	IgG	Semiquantitative	Cedars-Sinai[a]	FDA EUA granted
Abbott Architect	Nucleocapsid	IgG	Qualitative	Cedars-Sinai[a]	FDA EUA granted
Beckman Coulter Access	Spike	IgG	Semiquantitative	Arizona State University	FDA EUA granted
Beckman Coulter Access	Spike	IgM	Qualitative	Arizona State University	FDA EUA granted
DiaSorin Liaison	Spike	IgG	Qualitative (Feinstein/Northwell, Kaiser); quantitative (The Ohio State University)	Feinstein/Northwell, Kaiser, The Ohio State University	FDA EUA granted
DiaSorin Liaison	Spike	IgM	Qualitative	Feinstein/Northwell	FDA EUA granted
Kantaro SeroKlir	Spike, RBD	IgG	Semiquantitative	Mount Sinai	FDA EUA granted
Kantaro quantitative SARS-CoV-2	Spike, RBD	IgG	Quantitative	Mount Sinai	FDA EUA pending
Meso Scale Discovery	Spike, nucleocapsid	IgG, IgM	Quantitative	University of Alabama—Birmingham, CVVR/BIDMC/Harvard, Johns Hopkins University, Stanford	RUO
Roche Elecsys anti-SARS-CoV-2	Nucleocapsid	Total Ig	Qualitative	University of Minnesota, Feinstein/Northwell	FDA EUA granted
Roche Elecsys anti-SARS-CoV-2 S	RBD	Total Ig	Semiquantitative	University of Minnesota, Feinstein/Northwell	FDA EUA granted
Siemens Atellica	Spike	Total Ig	Semiquantitative	Kaiser, The Ohio State University	FDA EUA granted

[a]Samples sent to Abbott Diagnostics for testing.

per milliliter traceable to the WHO standard. For qualitative assays, standardization is crucial for comparing and then harmonizing assay cutoffs for positivity that are traceable to the WHO standard. These efforts may more rapidly facilitate the establishment of a universal cutoff as a correlate of protection, which will be critical to broaden the clinical utility of serological testing for patient care, will allow vaccine trials to transition to an immunogenicity endpoint rather than morbidity or mortality endpoints (immunobridging), and will guide decisions regarding optimal scheduling of future vaccine doses to optimize protective efficacy for the general immunocompetent population and susceptible immunocompromised subpopulations.

While the first step toward harmonization is calibration of assays to a common standard, there will be remaining challenges to pooling data given differences in assay performance metrics, sample types, isotypes, result type (qualitative, semiquantitative, or quantitative), methodologies, and antigen targets. SeroNet and FNLCR continue to work collaboratively to lay the groundwork for effective serology assay data pooling; FNLCR is currently conducting a comprehensive assay comparison study using split blinded samples sent to different SeroNet laboratories to assess the success of harmonization efforts, as well as assay performance (repeatability, sensitivity, and specificity). Currently, there is a broad range of epidemiologic studies being conducted across SeroNet, as previously described, and SeroNet's future work will include a focus on integrated analysis of pooled data with standardization of reported data elements and assay harmonization (51).

In summary, SeroNet is well positioned to rapidly and collaboratively advance our understanding of the immune response to both SARS-CoV-2 infection and vaccination, with ongoing evaluation of serological responses to SARS-CoV-2 variants of concern. The collective effort of institutions involved with SeroNet, to both establish diverse and complementary serological assays and establish traceability of these diverse assays to the WHO standard, will allow for comprehensive investigation of immune responses and facilitate pooled analyses within the SeroNet consortium. This will enable achievement of the ultimate goal: establishment of a universal correlate-of-protection cutoff, which will provide a foundation for broader clinical use of serological testing, as a guide for future decisions on scheduling of COVID-19 vaccine boosters, as well as for

TABLE 5 Units assigned to the U.S. SARS-CoV-2 serology standard[a]

Units assigned by FNLCR			WHO-calibrated units				
Functional activity	Spike and nucleocapsid IgM	Spike and nucleocapsid IgG	Functional activity	Spike IgG	Nucleocapsid IgG	Spike IgM	Nucleocapsid IgM
200 NU/mL	100 BAU/mL[b]	1200 BAU/mL[b]	815 IU/mL	764 BAU/mL[c]	681 BAU/mL[c]	246 BAU/mL[c]	1037 BAU/mL[c]

[a]WHO, World Health Organization; NU, neutralizing units; IU, international units.
[b]BAU/mL, binding assay units per milliliter.
[c]BAU/mL, binding antibody units per milliliter.

Day 1 Plate 1	1 C_STD	2 C_STD	3 NEG	4 PC1	5 STD-C1	6 STD-C2	7 STD-C3	8 STD-T1	9 STD-T2	10 STD-T3	11 C_STD	12 C_STD
A	50	50	50	50	200	200	200	200	200	200	50	50
B	100	100	150	150	400	400	400	400	400	400	100	100
C	200	200	450	450	800	800	800	800	800	800	200	200
D	400	400	1350	1350	1600	1600	1600	1600	1600	1600	400	400
			No Sample	PC2								
E	800	800	50	150	3200	3200	3200	3200	3200	3200	800	800
F	1600	1600	150	450	6400	6400	6400	6400	6400	6400	1600	1600
G	3200	3200	450	1350	12800	12800	12800	12800	12800	12800	3200	3200
H	6400	6400	1350	4050	25600	25600	25600	25600	25600	25600	6400	6400

FIG 1 Example plate map for assay calibration setup. Numbers indicate suggested serial dilutions. Serial dilutions of primary and secondary calibrators (reference materials) are plated in triplicate, and the daily internal assay standard is plated in quadruplicate. C_STD, daily internal assay standard; STD-C1, -C2, and -C3, primary calibrator (primary reference material or standard); STD-T1, -T2, and T3, secondary calibrator (secondary reference material or standard); NEG, negative control sample; PC1, positive control sample 1; PC2, positive control sample 2.

general assessment of COVID-19 vaccine immune responses against vaccine viruses and newly evolving variants of concern.

MATERIALS AND METHODS

Compilation of data on SeroNet serological assays. SeroNet institutions were queried by email between January and July 2021 and asked to complete a comprehensive serological assay survey to describe serological assays developed or implemented at the institutions. The survey requested information on assay and sample type(s), instrument platform and reagents, data output, antibody isotype(s) detected, targeted antigens and virus strain(s), assay performance, cutoffs, use of standards and quality controls, method comparison studies, regulatory status, current use/applications for assays, and publications using each assay.

Protocol for establishing traceability of serology assays to the U.S. SARS-CoV-2 serology standard and first WHO international standard for anti-SARS-CoV-2 immunoglobulin. FNLCR developed a protocol for SeroNet institutions to establish serology assay traceability to the U.S. SARS-CoV-2 Serology Standard. Through FNLCR's participation in the drafting group for the *WHO Manual for the Preparation of Reference Materials for Use as Secondary Standards in Antibody Testing*, the protocol has been made available to the public as of 11 May 2022 (see Appendix 8 of reference 52).

In short, for enzyme-linked immunosorbent assay platforms (ELISAs), the U.S. SARS-CoV-2 standard is measured on the same 96-well plate as the daily assay standard, run as serial dilutions in triplicate and quadruplicate (Fig. 1). Standard curves are constructed for both the U.S. SARS-CoV-2 Serology standard and daily assay standard. A test of parallelism and linearity between the two dose-response curves is then performed to ensure that immunoaffinity differences or matrix effects do not prevent accurate calibration with the U.S. SARS-CoV-2 serology standard. Units based on the U.S. SARS-CoV-2 serology standard can then be assigned to the assay daily standard, to harmonize assays and units for result reporting. For non-plate-based assay platforms, similar dilution-based standard curves are constructed.

Traceability of the FNLCR standard to the first WHO international standard (IS) for anti-SARS-CoV-2 immunoglobulin (20/136) was established, to allow SeroNet assays to convert U.S. serology standard units to WHO IS units. WHO IS 20/136 is a freeze-dried equivalent of 0.25 mL of pooled plasma from 11 individuals with a history of SARS-CoV-2 infection. Once reconstituted, the WHO standard has an arbitrary unitage of 1,000 binding antibody units (BAU)/mL. Eight serial dilutions of the U.S. SARS-CoV-2 serology standard and WHO IS 20/136 were run in triplicate. Parallel line analysis, which included tests for parallelism and linearity, was utilized to assign WHO IS 20/136 standard units to the U.S. SARS-CoV-2 serology standard; this will allow SeroNet institutions to convert U.S. SARS-CoV-2 serology standard units to WHO standard units for serological methods.

Patient consent statement. This work involves a descriptive summary of serological assays and assay harmonization plans and does not include factors necessitating patient consent.

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