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

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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 Eurolimmun 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
| 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 |
| 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 at 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 (lgG); Qualitative (lgM, lgA) | 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 |

*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).
| Sample type(s) | Antigen(s) | Isotype | Result type | Assay sensitivity and specificity | Center/institution | Reference(s) | Regulatory status |
|---------------|------------|---------|-------------|----------------------------------|-------------------|--------------|------------------|
| DBS, serum    | Spike, 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, RBD, nucleocapsid | IgG | Quantitative | Sensitivity, ≧93%; specificity, 100% | Arizona State University | NR | FDA EUA pending |
| Serum | Spike, nucleocapsid, RBD | IgG, IgM, IgA | Quantitative | 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%. | 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 |

*BAL, bronchoalveolar lavage; CLIA, Clinical Laboratory Improvement Amendments; NIST, National Institute of Standards and Technology; NYS CLEP, New York State Clinical Laboratory Evaluation Program.*
| 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          | Semiquantitative | NR                                                                                               | Mount Sinai                | 38, 39        | RUO              |
| Serum, plasma, BAL fluid | Live-virus neutralization assay (FRNT)   | Quantitative | Sensitivity, 93%; specificity, 100%                                                              | Saint Louis University Emory | 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          | Quantitative | Sensitivity, 100%; specificity, 97%; PRNT$_{50}$ sensitivity, 97%; specificity, 100%             | Wadsworth                  | 29, 42        | NYS CLEY 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 University of Puerto Rico | 15, 43–47 | RUO              |
| Serum, plasma          | ACE2 competitive binding assay           | Quantitative | Sensitivity, 93.8%; specificity, 99.4%                                                            | Tulane University          | 50            | RUO              |
| Serum, plasma          | Lentivirus-based pseudovirus assay       | Quantitative | Sensitivity, 100%; specificity, 100%                                                              |                            |               |                  |

*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.*
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.

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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

| 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      | FDA EUA granted  |
| Abbott Architect | Nucleocapsid | IgG    | Qualitative   | Arizona State University | FDA EUA granted |
| Beckman Coulter Access | Spike    | IgG    | Semiquantitative | Arizona State University | FDA EUA granted |
| Beckman Coulter Access | Spike    | IgM    | Qualitative   | Feinstein/Northwell, Kaiser, The Ohio 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   | Mount Sinai       | FDA EUA granted  |
| Kantaro SeroKlir | Spike, RBD | IgG     | Semiquantitative | Mount Sinai       | FDA EUA granted  |
| Kantaro quantitative SARS-CoV-2 | Spike, RBD | IgG    | Quantitative  | University of Alabama—Birmingham, CVVR/BIDMC/Harvard, Johns Hopkins University, Stanford | FDA EUA pending RUO |
| Meso Scale Discovery | Spike, nucleocapsid | IgG, IgM | Quantitative  |          | RUO              |
| Roche Elecsys anti-SARS-CoV-2 | Nucleocapsid | Total Ig | Qualitative   | University of Minnesota, Feinstein/Northwell, Kaiser, The Ohio State University | FDA EUA granted |
| Roche Elecsys anti-SARS-CoV-2 S | RBD          | Total Ig | Semiquantitative | University of Minnesota, Feinstein/Northwell, Kaiser, The Ohio State University | FDA EUA granted |
| Siemens Atellica | Spike      | Total Ig | Semiquantitative |                      | FDA EUA granted  |

*Samples sent to Abbott Diagnostics for testing.

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

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

*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.
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 immunofinity 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 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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U01CA261276 (R.A.B., C.F., and A.M.M.), U01CA260539 (C.L.K.), U01CA260508 (L.M.S., A.P.D., R.C.G., D.T.H., W.T.L., J.L.Y., and A.F.P.), and U01CA260462 (S.B. and S.P.).

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