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

Amy B. Karger[1*], James D. Brien[2], Jayne M. Christen[3], Santosh Dhakal[4], Troy J. Kemp[3], Sabra L. Klein[4], Ligia A. Pinto[3], Lakshmanane Premkumar[5], John D. Roback[6], Raquel A. Binder[7], Karl W. Boehme[8], Suresh Boppana[9,10], Carlos Cordon-Cardo[11], James M. Crawford[12], John L. Daiss[13], Alan P. Dupuis II[14], Ana M. Espino[15], Adolfo Firpo-Betancourt[11], Catherine Forconi[7], J. Craig Forrest[8], Roxie C. Girardin[14], Douglas A. Granger[16], Steve W. Granger[16], Natalie S. Haddad[17], Christopher D. Heaney[18], Danielle T. Hunt[14], Joshua L. Kennedy[19,20], Christopher L. King[21], Florian Krammer[22], Kate Kruczynski[18], Joshua LaBaer[23], F. Eun-Hyung Lee[17], William T. Lee[14,24], Shan-Lu Liu[25], Gerard Lozanski[26], Todd Lucas[27], Damodara Rao Mendu[11], Ann M. Moormann[7], Vel Murugan[23], Nkemakonam C. Okoye[12], Petraleigh Pantoja[28,#a], Anne F. Payne[14], Jin Park[23], Swetha Pinninti[9], Amelia K. Pinto[2], Nora Pisanic[18], Ji Qiu[23], Carlos A. Sariol[28,29], Viviana Simon[22], Lusheng Song[23], Tara L. Steffen[2], E. Taylor Stone[2], Linda M. Styer[14,24], Mehul S. Suthar[30], Stefani N. Thomas[1], Bharat Thyagarajan[1], Ania Wajnberg[31], Jennifer L. Yates[14,24], Kimia Sobhani[32].

[1]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota; [2]Department of Molecular Microbiology & Immunology, Saint Louis University, Saint Louis, Missouri; [3]Frederick National Laboratory for Cancer Research, Frederick, Maryland; [4]W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; [5]Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC; [6]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, Georgia; [7]Department of Medicine, University of Massachusetts Chan Medical School, Worcester, Massachusetts; [8]Department of Microbiology & Immunology, University of Arkansas for Medical Sciences, Little Rock, Arkansas; [9]Department of Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama; [10]Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama; [11]Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, New York; [12]Department of Pathology and Laboratory Medicine, Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Hempstead, New York; [13]MicroB-plex, Inc., Atlanta, Georgia; [14]Wadsworth Center, New York State Department of Health, Albany, New York; [15]Department of Microbiology and Medical Zoology, University of Puerto Rico-Medical Sciences Campus, San Juan, Puerto Rico; [16]Salimetrics, LLC, Carlsbad, California; [17]Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Department of Medicine, Emory University, Atlanta, Georgia; [18]Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; [19]Departments of Pediatrics and Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas; [20]Arkansas Children's Research Institute, Little Rock, Arkansas; [21]Department of Pathology, Case Western Reserve School of Medicine, Cleveland, Ohio; [22]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York; [23]Virginia G Piper Center for Personalized Diagnostics, Arizona State University Biodesign Institute, Tempe, Arizona; [24]Department of Biomedical Sciences, School of Public Health, University at Albany, Albany, New York; [25]Center for Retrovirus Research, Department of Veterinary Biosciences, Department of Microbial Infection and Immunity, Viruses and Emerging Pathogens Program, Infectious Disease Institute, The Ohio State University, Columbus, Ohio; [26]Department of Pathology, The Ohio State University Medical Center, Columbus, Ohio;

[27]Division of Public Health and Department of Epidemiology, College of Human Medicine, Michigan State University, East Lansing, Michigan; [28]Unit of Comparative Medicine, University of Puerto Rico-Medical Sciences Campus, San Juan, Puerto Rico; [29]Department of Internal Medicine, University of Puerto Rico-Medical Sciences Campus, San Juan, Puerto Rico; [30]Center for Childhood Infections and Vaccines of Children's Healthcare Atlanta, Department of Pediatrics, Department of Microbiology and Immunology, Emory Vaccine Center, Yerkes National Primate Research Center, Emory University School of Medicine, Atlanta, Georgia; [31]Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York; [32]Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, California.

[#a]Current affiliation: Seqirus, CSL, Cambridge, Massachusetts

*Corresponding author: Amy Karger, MD, PhD, 420 Delaware St SE, MMC 609, Minneapolis, MN 55455; tel: 612-624-2150; fax: 612-625-1121; email: karge026@umn.edu

Abstract

Background: 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." 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.

Methods: 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. 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.

Results: SeroNet institutions reported development of a total of 27 ELISA methods, 13 multiplex assays, 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.

Conclusions: SeroNet institutions have established a diverse array of COVID-19 serological assays to study the immune response to SARS-CoV-2 virus and vaccines. Calibration of SeroNet serological assays to harmonize results reporting will facilitate future pooled data analyses and study cross-comparisons.

Introduction

The National Cancer Institute (NCI) Serological Sciences Network for COVID-19, or SeroNet, was launched on October 8, 2020, as a collaborative initiative to expand research on immune responses to SARS-CoV-2. SeroNet is comprised of investigators from 25 US biomedical research institutions, working in partnership with the Frederick National Laboratory for Cancer Research (FNLCR) and the SeroNet Coordinating Center, which is managed by the 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 cut-offs 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 – 40 days post-PCR diagnosis, to around 70% when patients were tested ≥ 81 days post-diagnosis, whereas the Roche Elecsys SARS-CoV-2 anti-Nucleocapsid total antibody assay and Siemens SARS-CoV-2 anti-receptor-binding domain (RBD) total antibody assay both maintained high sensitivity (95 – 100%) on the same set of serial samples. Narowski et al. also found a significant decline in the longitudinal sensitivity of their lab-developed nucleocapsid assay in a study of healthcare workers.[6] Perez-Saez et al. similarly demonstrated that the rates of sero-reversion at least 8 months after the initial infection differed greatly depending on the serological assay used.[4] While the sero-reversion rate of the EuroImmun semiquantitative anti-S1 IgG ELISA was 26%, the rate was 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 laborartories.

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 manuscript 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 (WHO IS 20/136) for these diverse assays, with the ultimate goal of establishing harmonized reporting standards. This will facilitate cross-comparison of results and provide clarity for their clinical interpretation, including in response to circulating SARS-CoV-2 variants.

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 their institution. 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, cut-offs, 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 recommended protocol for SeroNet institutions to establish serology assay traceability to the U.S. SARS-CoV-2 Serology Standard. In short, for enzyme-linked immunosorbent assay platforms (ELISA), 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 respectively (**Figure 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 results 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. The 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 1000 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.

Results

SeroNet Serology Assay data

Of the 25 institutions involved with SeroNet, 23 institutions reported performing between one to 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 EUA approval, 3 methods are pending FDA EUA, 4 methods are validated for high-complexity testing in a CLIA-certified laboratory, and 18 methods are for research-use only (RUO). Diagnostic sensitivity and specificity for in-house ELISA methods ranged from 67.4 – 100 % and 90 – 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 – 98.8 % and 95.2 – 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 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 – 100 % and 97 – 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 are FDA EUA approved, 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 the 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. 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 results in binding antibody units (BAU) per mL traceable to the WHO standard. These efforts may more rapidly facilitate the establishment of a universal cut-off 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 (immuno-bridging), and will guide decisions regarding optimal scheduling of future vaccine doses to optimize protective efficacy for the general immunocompetent population and susceptible immunocompromised sub-populations.

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 cut-off, which will provide a foundation for broader clinical use of serologic testing, as a guide for future decisions on scheduling of COVID-19 vaccine boosters, as well as for general assessment of COVID-19 vaccine immune responses against vaccine viruses and newly evolving variants of concern.

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protection for serological assays. The Icahn School of Medicine at Mount Sinai has filed patent applications relating to the COVID-19 serological assay ("Serology Assay") and NDV-based SARS-CoV-2 vaccines which list F.K. ("Serology Assay", vaccines), V.S. ("Serology Assay"), A.F-B. ("Serology Assay"), D.R.M. ("Serology Assay"), and C.C-C. ("Serology Assay") as co-inventors. The foundational "Serology Assay" intellectual property (IP) was licensed by the Icahn School of Medicine at Mount Sinai to commercial entities including Kantaro Biosciences, a company in which Mount Sinai has a financial interest. All remaining authors report no relevant conflicts of interest.

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FIGURES/TABLES

Figure 1: Example plate map for assay calibration set-up

Day 1	1	2	3	4	5	6	7	8	9	10	11	12
Plate 1	C_STD	C_STD	NEG	PC1	STD-C1	STD-C2	STD-C3	STD-T1	STD-T2	STD-T3	C_STD	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

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

Table 1: Laboratory-developed singleplex ELISA assays

Sample Type	Antigen	Isotype	Assay Sensitivity & Specificity	Center/Institution	References	Regulatory Status
Serum, Plasma, Dried Plasma samples	RBD	IgG (IgA/IgM being eval)	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	PMID: 32835303	FDA EUA granted
Serum, Plasma	RBD and Spike	IgG, IgM, IgA	Sensitivity 95%, Specificity 100% (n=38 positive, n=74 negative sera tested)	Mount Sinai	PMID: 32302069, PMID: 32511441, PMID: 33142304	FDA EUA granted
Serum, Plasma, Saliva	RBD	Total Ig, with IgG, IgM, IgA titers	Overall sensitivity 82.5%, overall specificity 100% (n=300); At > 14 days from symptom onset, sensitivity 100%, specificity 100% (n=261);	University of Minnesota	PMID: 32791053, PMID: 33539808	Assays validated in a high-complexity testing CLIA laboratory
Serum, Plasma	RBD	IgG, IgM	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, 100% >21 days post-onset of symptoms. Specificity: 99.75% for RBD IgG, 100% for RBD IgM	Stanford University	PMID: 33288645	Assays validated in a high-complexity testing CLIA laboratory
Serum, Plasma	RBD-ACE2	Total IgG that blocks RBD-ACE2 binding	N/A, used as a follow-up assay in seropositive specimens	Stanford University	PMID: 33288645	Assay validated in a high-complexity testing CLIA laboratory
Serum, Plasma	RBD	IgG, IgM + IgG	Sensitivity 98% (n=181), Specificity 98.9% (n=181).	University of Puerto Rico	PMID: 34696403, https://www.biorxiv.org/content/10.1101/2020.06.11.146332v2	Assay validated in a high-complexity testing CLIA laboratory
Serum, Plasma	Spike	IgG	Sensitivity 98.3% (n=60), Specificity 99.3% (n=150)	Frederick National Laboratory	NR	RUO
Serum, Plasma	Spike	IgM	Sensitivity 93.8% (n=30), Specificity 97.6% (n=80)	Frederick National Laboratory	NR	RUO
Serum, Plasma	Nucleocapsid	IgG	Sensitivity 97% (n=34), Specificity 100% (n=99)	Frederick National Laboratory	NR	RUO
Serum, Plasma	Nucleocapsid	IgM	NR	Frederick National Laboratory	NR	RUO
Serum, Plasma, Saliva	RBD	Total Ig	Sensitivity 95% (n=259; 9 or more days after symptom onset), Specificity 96% (n=535)	University of North Carolina	PMID: 32527802, 35090596	FDA EUA pending
Serum, Plasma, Saliva	Spike NTD	Total Ig	Sensitivity = 92% (n=259; 9 or more days after symptom onset), Specificity = 94% (n=535)	University of North Carolina	PMID: 35090596	FDA EUA pending
Serum	Spike, RBD	IgG	NR	CVVR/BIDMC/Harvard	PMID: 34107529	RUO
Serum, Plasma, Breast milk	RBD	IgG, IgA, IgM	NR	CVVR/BIDMC/Harvard	PMID: 33983379, PMID: 33893169	RUO
Serum, Plasma	Spike	IgG	Sensitivity 100%, Specificity 98.8%	Tulane University	NR	RUO
Serum, Plasma	RBD	IgG	NR	Tulane University	NR	RUO
Serum, Plasma	Nucleocapsid	IgG	NR	Tulane University	NR	RUO

Plasma, Serum	Spike, RBD	IgM, IgG, IgA	Spike: IgG (Sensitivity 96.6%, Specificity 96.7%); IgA (Sensitivity 99.3%, Specificity 90%); IgM (Sensitivity 97.9%, Specificity 100%). RBD: IgG (Sensitivity 97.3%, Specificity 100%); IgA (Sensitivity 99.3%, Specificity 96.7%); IgM (Sensitivity 97.9%, Specificity 96.7%). IgG data based on n=126 convalescent plasma donors, n=30 pre-pandemic samples; IgM/IgA data based on n=20 hospitalized, n=30 pre-pandemic samples.	Johns Hopkins University	PMID: 32764200	RUO
Serum, Plasma	Spike (ECD), RBD	IgG	NR	University of Texas-Austin	PMID: 32910806	RUO
Serum, Plasma	RBD	IgG	Sensitivity 100% (n=155), Specificity 96.5% (n=133)	Arizona State University	NR	RUO
Serum, DBS	RBD	IgG, IgM	Sensitivity 97% (n=39), Specificity 100% (n=37)	University of Arkansas for Medical Sciences	PMID: 34478478, https://www.medrxiv.org/content/10.1101/2021.08.04.21261592v3	RUO
Serum, DBS	RBD, Spike, Nucleocapsid	IgG, IgM	Sensitivity 97% (n=39), Specificity 100% (n=37)	University of Arkansas for Medical Sciences	PMID: 34478478, https://www.medrxiv.org/content/10.1101/2021.08.04.21261592v3	RUO
Serum, Plasma, Breast milk	RBD, Spike, Nucleocapsid	IgG, IgM, IgA	97% Sensitivity (n=114), Specificity 99%	University of Alabama-Birmingham	NR	RUO
Serum, Plasma	RBD, Nucleocapsid, Spike Trimer	IgG, IgA	RBD: Sensitivity (70.9% for IgG, 74.4% for IgA) and Specificity (100% for both IgG and IgA); Nucleocapsid: Sensitivity (81.4% for IgG, 77.9% for IgA) and Specificity (98.5% for IgG, 100% for IgA); Spike Trimer: Sensitivity (67.4% for both IgG and IgA) and Specificity (98.5% for IgG, 100% for IgA). Data based on PCR confirmed COVID-19 hospitalized patients (n=86) and negative pre-pandemic samples (n=65).	University of Massachusetts Chan Medical School	PMID: 32780998	RUO
Serum, Plasma	Nucleocapsid	IgG	Sensitivity 100% (n=44), Specificity 99.5% (n=202)	The Ohio State University	PMID: 33035201	FDA EUA pending
Serum	Nucleocapsid	IgG	NR	The Ohio State University	NR	RUO
Oral fluid	Nucleocapsid	IgG	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; NR: Not reported; NTD: N-terminal domain; PMID: PubMed Identifier; RBD: receptor binding domain; RUO: research use only

Table 2: Laboratory-developed multiplex assays

Sample Type	Antigen	Isotype	Assay Sensitivity & Specificity	Center/Institution	References	Regulatory Status
DBS, Serum	Spike S1, Nucleocapsid	IgG	Sensitivity: DBS 94% for symptomatic (n=774 samples collected >20 days after PCR+ result) 85% for asymptomatic (n=115 samples collected >20 days after PCR+ result), Specificity: DBS 99% (n=730), Serum 99% (n=701)	Wadsworth	PMID: 32648546 PMID: 34319133	NYS CLEP-approved
Serum, Plasma, DBS	Spike, Nucleocapsid, RBD	Total Ig	Sensitivity >97%, Specificity 99%	Wadsworth	PMID: 33104179	FDA EUA granted; NYS CLEP-approved
Serum, Plasma, DBS	Spike, Nucleocapsid, RBD	IgG, IgM, IgA	Sensitivity >97%, Specificity 99%	Wadsworth	PMID: 34151306	NYS CLEP-approved; FDA EUA pending
Oral fluid, Serum, Plasma	Spike, RBD, Nucleocapsid	IgG, IgM, IgA	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	PMID: 33067270, 34695724	Oral fluid assays validated in a high-complexity testing CLIA laboratory; Serum/plasma RUO
Serum, Plasma, BAL, DBS	Spike, RBD (different variants), Nucleocapsid	IgG	Sensitivity >97% sensitivity (n=89), Specificity 99% (n=260)	Case Western Reserve University	PMID: 33993265	RUO
Serum, Plasma, Saliva, BAL	Spike, RBD, Nucleocapsid	IgA	Sensitivity >98%, Specificity 99%	Case Western Reserve University	PMID: 33993265	RUO
Serum, Plasma	Spike	IgG	Sensitivity ≥ 93%, Specificity 100%	NIST	PMID: 33800363	RUO
Serum, Plasma	RBD	IgG	Sensitivity ≥ 93%, Specificity 100%	NIST	PMID: 33800363	RUO
Serum, Plasma	RBD, Nucleocapsid	IgG	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	NR	Yale	PMID: 33171100	RUO
Serum	Alpha, Beta, Gamma, and Delta variants (Spike, RBD)	IgG, IgM, IgA	NR	Yale	PMID: 31229590	RUO
Saliva	Spike, Nucleocapsid, RBD	IgG	Sensitivity: Nucleocapsid 97.7%, RBD 92.9%, Spike 98.8%; Specificity: Nucleocapsid 95.2%, RBD 96.4%, Spike 97.6%. (Combined Nucleocapsid & Spike sensitivity 96.5%, specificity 98.8%)	Salimetrics	NR	RUO
Serum, Plasma	Spike S1, S1-RBD, Nucleocapsid, S1-NTD	IgG, IgA, IgM (combined) IgG, IgA, IgM (individual)	Sensitivity: combined antigens and isotypes 99%; S1-RBD combined isotypes 99%, S1-RBD IgG 99%; Specificity: combined antigens and isotypes 99%, S1-RBD combined isotypes 99%, S1-RBD IgG 99%. During the acute phase, Sensitivity 92%, Specificity 99%.	Emory/MicroB-plex	PMID: 34001652	RUO

BAL: Bronchoalveolar lavage; CLIA: Clinical Laboratory Improvement Amendments; DBS: Dried blood spots; EUA: Emergency use authorization; FDA: Food and Drug Administration; NIST: National Institute of Standards and Technology; NR: Not reported; NYS CLEP: New York State Clinical Laboratory Evaluation Program; PCR: Polymerase chain reaction; PMID: PubMed Identifier; RBD: Receptor binding domain; RUO: Research use only

Table 3: Neutralization assays

Sample Type	Antibody Neutralization Assay Type	Assay Sensitivity & Specificity	Center/Institution	References	Regulatory Status
Serum, Plasma, BAL	HIV Lentiviral Vector	Sensitivity 100%, Specificity 100%, using SeroNet FNLCR blinded reference panel set (n=110)	The Ohio State University	PMID:33035201	RUO
Serum, Plasma	Live Virus Neutralization Assay (microneutralization)	NR	Mount Sinai	PMID: 32585083 PMID: 33115920	RUO
Serum, Plasma, BAL	Live Virus Neutralization Assay (FRNT)	Sensitivity 93%, Specificity 100%	Saint Louis University	PMID: 34100029 PMID: 33326500	RUO
Serum, Plasma, BAL	Live Virus Neutralization Assay (FRNT/FRNT-mNG/PRNT)	NR	Emory	PMID: 33215858.	RUO
Serum, Plasma, DBS	Live Virus Neutralization Assay (PRNT)	PRNT50: Sensitivity 100%, Specificity 97%; PRNT90: Sensitivity 97%, Specificity 100%	Wadsworth	PMID: 33104179 PMID: 33417696	NYS CLEP-approved (serum and plasma)
Serum, plasma, breast milk	VSV Pseudotype Particle based assay	NR	University of Alabama Birmingham	NR	RUO
Serum, Plasma, Nasal Washes	TCID50 Neutralization Assay	NR	Johns Hopkins University	PMID: 34253053 PMID: 33571169 PMID: 33571162 PMID: 33427749 PMID: 33139419 PMID: 32764200	RUO
Serum, Plasma	ACE2 Competitive Binding Assay	Sensitivity 93.8% Specificity 99.4%	University of Puerto Rico	PMID: 34100029	RUO
Serum, plasma	Lentiviral based pseudovirus assay for Wuhan D614G, Brazil, South Africa, and Delta variants. Assay performed in CHO/ACE2 cells.	Sensitivity 100%, Specificity 100%	Tulane	PMID: 33306985	RUO

ACE2: Angiotensin converting enzyme-2; BAL: Bronchoalveolar lavage; CHO: Chinese hamster ovary; DBS: Dried blood spots; EUA: Emergency use authorization; FDA: Food and Drug Administration; FNLCR: Frederick National Laboratory for Cancer Research; FRNT: Focus reduction neutralization test; HIV: Human immunodeficiency virus; mNG: mNeonGreen; NR: Not reported; NYS CLEP: New York State Clinical Laboratory Evaluation Program; PMID: PubMed Identifier; PRNT: Plaque reduction neutralization test; RUO: Research use only; TCID: Tissue culture infectious dose; VSV: Vesicular stomatitis virus

Table 4: Commercial assays

Instrument/Assay	Antigen	Isotype	Center/Institution	Regulatory Status
Abbott Alinity	Spike	IgM	Mount Sinai	FDA EUA granted
Abbott Architect	Spike, Nucleocapsid	IgG	Cedars-Sinai*	FDA EUA granted
Beckman Coulter Access	Spike	IgG	Arizona State University	FDA EUA granted
Beckman Coulter Access	Spike	IgM	Arizona State University	FDA EUA granted
DiaSorin Liaison	Spike	IgG	Feinstein/Northwell, Kaiser, The Ohio State University	FDA EUA granted
DiaSorin Liaison	Spike	IgM	Feinstein/Northwell	FDA EUA granted
Kantaro SeroKlir	Spike, RBD	IgG	Mount Sinai	FDA EUA granted
Kantaro Quantitative SARS-CoV-2	Spike, RBD	IgG	Mount Sinai	FDA EUA pending
Meso Scale Discovery	Spike, Nucleocapsid	IgG, IgM	University of Alabama - Birmingham, CVVR/BIDMC/Harvard, Johns Hopkins University, Stanford	RUO
Roche Elecsys Anti-SARS-CoV-2	Nucleocapsid	Total Ig	University of Minnesota, Feinstein/Northwell	FDA EUA granted
Roche Elecsys Anti-SARS-CoV-2 S	RBD	Total Ig	University of Minnesota, Feinstein/Northwell	FDA EUA granted
Siemens Atellica	Spike	Total Ig	Kaiser, The Ohio State University	FDA EUA granted

*Samples sent to Abbott Diagnostics for testing

BIDMC: Beth Israel Deaconess Medical Center; CVVR: Center for Virology and Vaccine Research; EUA: Emergency use authorization; FDA: Food and Drug Administration; RBD: Receptor binding domain; RUO: Research use only

Table 5: Units assigned to the U.S. SARS-CoV-2 Serology Standard

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

FNLCR: Frederick National Laboratory for Cancer Research; WHO: World Health Organization; NU/mL: Neutralizing Units per milliliter; BAU/mL: Binding Assay Units per milliliter; IU/mL: International units per milliliter