Meeting Report

The COVID-19 Serology Studies Workshop: Recommendations and Challenges

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The development, validation, and appropriate application of serological assays to detect antibodies to SARS-CoV-2 are essential to determining seroprevalence of this virus in the United States and globally and in guiding government leadership and the private sector on back-to-work policies. An interagency working group of the US Department of Health and Human Services convened a virtual workshop to identify knowledge gaps and key outstanding scientific issues and to develop strategies to fill them. Key outcomes of the workshop included recommendations for (1) advancing serology assays as a tool to better understand SARS-CoV-2 infection and (2) conducting crucial serology field studies to advance an understanding of immunity to SARS-CoV-2, leading to protection and duration of protection, including the correlation between serological test results and risk of reinfection.

Introduction

A virtual workshop entitled COVID-19 Serology Studies was convened on May 7, 2020 under the auspices of an interagency working group of the United States Department of Health and Human Services to better understand the opportunities and limitations of SARS-CoV-2 serological diagnostics. A total of >300 scientists and clinicians participated in the workshop (Table S1). The purpose of the workshop was to inform plans of the working group and others to accelerate the evaluation of serologic assays and to generate the scientific evidence needed to guide decisions by government leadership and the private sector on back-to-work policies. The goals of this workshop were to (1) review ongoing SARS-CoV-2 serosurvey studies, (2) review current knowledge on SARS-CoV-2 serological assay performance, (3) identify scientific gaps and other outstanding issues, and (4) develop recommendations for conducting future SARS-CoV-2 serological studies. The workshop included presentations on relevant topics by scientists from academia, medical institutions, industry, and federal government agencies. Serological assays can detect antibodies to SARS-CoV-2, the virus that causes COVID-19; however, these assays are not designed to diagnose or rule out active infection.

The workshop was launched with an overview of multiple ongoing SARS-CoV-2 seroprevalence studies that are contributing to our understanding of the level of seroconversion in different populations and communities (Table 1). In addition, results from these studies can be used to improve modeling and forecasting and could potentially be used to target public health interventions.

Due to multiple factors, including individuals not seeking medical care when ill, variable testing availability and practices, incomplete case reporting to public health authorities, and asymptomatic infections, officially reported cases represent the “tip of the iceberg” when compared to the true SARS-CoV-2 infection incidence in the United States. As antibody prevalence reflects cumulative infection incidence, seroprevalence studies can serve a critical role in closing this gap. They can be divided into various categories, including large geographic studies, community-level studies, and studies in special populations, with each category providing complementary information (Centers for Disease Control and Prevention, 2020).

Large geographic seroprevalence studies, which typically use residual sera from clinical laboratory testing or samples derived from blood banks, can estimate antibody prevalence in populations over a large geographic area. Large numbers of samples can be obtained, and if multiple geographic areas or time points are studied, antibody prevalence can be compared among geographic areas or trended longitudinally. An important disadvantage of the surveys that use residual samples is that the information obtained with the sample is limited to that routinely associated with the sample, such as age and gender.

Community-level seroprevalence studies are performed on a smaller local level, and in contrast to larger seroprevalence studies that use residual blood bank samples or clinical samples, participants can be interviewed, and serological results can be correlated with participant characteristics, reported symptom history, and exposure risk levels. This information can provide insights into factors that determine clinical outcome, transmission dynamics, and the nature of the immune responses.

Seroprevalence studies in special populations, such as healthcare workers, first responders, immunosuppressed persons, or pregnant women, have the same advantages of community-level
Serological surveys in special populations are underway or planned. These include large-scale serological surveys of first responders and healthcare workers in New York City and Detroit that will assess SARS-CoV-2 antibody prevalence in relation to symptom history, occupational or other exposures to persons with COVID-19, and use of personal protective equipment. Another special population that will be studied will follow convalescent plasma donors longitudinally for 18 months to characterize and understand the time course of the SARS-CoV-2 immune responses.

Combining results from multiple serological studies is essential to increasing data strength and characterizing a greater portion of the United States impacted by COVID-19, underscoring the need for harmonization of these surveys in order to overcome variation in survey design, population, assays used, and statistical methods applied. Public release of serosurvey data to increase general situational awareness and allow secondary analyses by other groups is important; however, it is critical to set expectations and define the types of questions that can be answered through these types of studies. Coordination among different agencies or organizations performing serological surveys is essential to reduce duplicative efforts, improve data sharing, and further facilitate the design of complementary study types. Serosurvey results are crucial to improving outbreak

### Table 1. Ongoing and Planned SARS-CoV-2 Serosurvey Studies Presented at Workshop

| Study                                      | Population                                                                 | Conducted by                                                                 |
|--------------------------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| REDS Epidemiology, Surveillance, and Preparedness of the Novel SARS-CoV-2 Epidemic (RESPONSE) study | US adult blood donors                                                      | Vitalant Research Institute, Recipient Epidemiology and Donor Evaluation Study IV Pediatric (REDS-IV-P) Program, and collaborators |
| National SARS-CoV-2 Seroincidence Studies in Blood Donors | US adult blood donors                                                      | CDC and collaborators                                                         |
| Community-Level Seroprevalence Studies     |                                                                           |                                                                              |
| Georgia/Metro Atlanta Pilot Serosurvey     | households within two large Georgia counties, all ages                    | CDC                                                                          |
| Household Influenza Vaccine Evaluation (HIVE) study | US households with at least one child                                      | University of Michigan and collaborators                                      |
| CDC Prospective Pandemic Cohort Studies    | US households and individuals                                              | CDC and collaborators                                                         |
| Seroprevalence Studies in Special Populations |                                                                           |                                                                              |
| Serological surveys of first responders and healthcare workers | first responders and healthcare workers New York University Langone |                                                                              |
| CDC prospective pandemic cohort studies    | pregnant women, healthcare workers, and older adults                      | CDC and collaborators                                                         |
| National SARS-CoV-2 seroincidence studies in blood donors | Convalescent plasma donors                                                 | CDC and collaborators                                                         |

Current ongoing large seroprevalence studies will inform knowledge of SARS-CoV-2 prevalence in various geographic areas as well as aim to characterize the immune responses. The Recipient Epidemiology and Donor Evaluation Study IV Pediatric (REDS-IV-P) Program is conducting the “REDS Epidemiology, Surveillance, and Preparedness of the Novel SARS-CoV-2 Epidemic” (RESPONSE) study (https://redsivp.com/covid-19/). This study aims to determine SARS-CoV-2 infection dynamics, evaluate candidate assays by analysis of adult blood donor antibody testing in the same areas before and after periods of community transmission, and characterize the immune responses. A bio-repository will be established using samples collected longitudinally from blood donors with SARS-CoV-2 infection over 12 months (Vitalant Research Institute, 2020a). In addition, planned national SARS-CoV-2 seroprevalence studies among blood donors will test samples obtained over 18 months in 25 blood center regions in the United States. Seroincidence will be estimated by comparing seroprevalences at different time points (Vitalant Research Institute, 2020b).

The Centers for Disease Control and Prevention (CDC) has performed community-level SARS-CoV-2 seroprevalence studies by randomly sampling households distributed among census block-based clusters in two large counties in the Atlanta metropolitan area (Georgia Department of Public Health, 2020). All ages were eligible for voluntary enrollment in this study, which included interview and blood sampling. Data analysis is ongoing, and additional study sites are being considered. Existing networks also are being leveraged to gather community-based data on SARS-CoV-2 seroprevalence, transmission, and immune responses. These networks include the Household Influenza Vaccine Evaluation (HIVE) study (University of Michigan School of Public Health, 2020), a cohort established in 2009 of households containing at least one child. Multiple cohorts making up the CDC’s pandemic influenza infrastructure are also being leveraged to gather community-based data on COVID-19 seroprevalence, transmission, and immune responses.

Seroprevalence studies but may focus on groups that may be at higher risk of infection or may have differing clinical outcomes than the population at large. In addition, cohorts can be followed over time to investigate factors such as how serological status correlates with infection rates to shed light on the nature of protective immunity.

Ongoing SARS-CoV-2 Serosurvey Studies

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modeling and forecasting and informing mitigation and public health interventions. In addition, the ability to perform high-throughput, standardized, and reproducible serological studies is a crucial aspect of vaccine development.

**Current SARS-CoV-2 Serological Assay Performance**

As of May 17, 2020, twelve SARS-CoV-2 commercial laboratory serological tests or serological test kits have been authorized under an emergency use authorization (EUA) by the US Food and Drug Administration (FDA) for detection of antibodies to SARS-CoV-2. Notably, these tests are not to be used as the sole basis for COVID-19 diagnosis. Several EUA-authorized serological immunoassay types include those that utilize central laboratory instruments for high throughput, ELISA microtiter plates for moderate throughput, and rapid lateral flow formats that may be used to differentiate between immunoglobulin M (IgM) and IgG. The FDA issued updated guidance on May 11, 2020 (US Food and Drug Administration, 2020), under which the agency’s expectations that commercial serology test manufacturers will submit EUA requests within 10 business days from the date they notified the FDA of their validation testing. (The authors note that the FDA Guidance issued on March 16, 2020 and May 4, 2020, was updated on May 11, 2020; US Food and Drug Administration, 2020. The policy in the May 11, 2020 document was not changed but includes a new section that references the availability, on the FDA’s website, of templates for commercial manufacturers and laboratories intended to facilitate EUA submissions for molecular, antigen, and serology tests.) The FDA also has provided templates for EUA submission for serology test manufacturers, kit developers, and laboratories to help facilitate the preparation and submission of an EUA. The FDA’s guidance also describes independent testing of SARS-CoV-2 serology immunoassays by the National Cancer Institute (NCI) SARS-CoV-2 Serology Program at the US National Institutes of Health (NIH).

This performance evaluation utilizes an assay validation panel of well-characterized positive and negative serum samples, and the results from this testing are provided to the FDA. The FDA will make NCI results available once the FDA has reviewed the results and determined if any further actions are appropriate for a particular test kit prior to publication. If commercial manufacturers that are marketing serology tests fail to submit an EUA within 10 business days, the FDA is sharing this information publicly. Workshop presenters noted that the medical diagnostic industry is also developing much-needed qualitative and quantitative serology immunoassays.

Multiple US government agencies, including the NIH, the FDA, and the CDC provide key reagents to commercial kit manufacturers and laboratories to advance the development of serology assays and accelerate the development and validation of their tests. Some of these reagents are accessible through BEI Resources (https://www.beiresources.org/) and from the World Reference Center for Emerging Viruses and Arboviruses (https://www.utmb.edu/wrceva).

Additionally, well-characterized clinical specimen reference panels consisting of SARS-CoV-2 antibody positive and negative serum or plasma samples are being developed by the Biomedical Advance Research and Development Authority (BARDA) and the NIH, as well as the World Health Organization International Standards for COVID-19 Serology Reagents from the National Institute for Biological Standards and Control (NIBSC). These panels will be available shortly from BEI Resources and the NIBSC (https://www.nibsc.org/).

The workshop participants addressed the topic of antibody-mediated immunity to human coronavirus (hCoV) infection with specific focus on the kinetics of the immune response to infection, correlates of protection, and association with disease severity. A recent analysis (Huang et al., 2020) of more than 300 peer-reviewed articles showed that (1) median time to detection was similar across different antibodies for SARS-CoV-1 (12.0 days; interquartile range [IQR] 8.0–15.2 days) and SARS-CoV-2 (11.0 days; IQR 7.3–14.0 days) but longer for MERS-CoV (16.0 days; IQR 13.0–19.0 days); (2) SARS-CoV-1 and MERS-CoV IgG waned over time, but it was still detectable for 1–3 years; and (3) antibody responses were greater and detectable longer after more severe illness. The findings also demonstrated that human challenge studies with hCoV indicate that serum and mucosal immune responses (serum IgG, IgA, neutralizing titer, mucosal IgA) provide possible correlates of protection from infection and disease, and repeat human challenge experiments suggest individuals can be infected with the same hCoV 1 year after first challenge, although with decreased severity. This analysis also found that seroprevalence with the four major endemic hCoV strains shows that the median age at first infection with any strain was 4.8 years (95% confidence interval [CI] 2.5–11.2 years) and that there was no clear trend in seroincidence with age, consistent with transient protection. The data from human challenge studies indicated that higher mucosal IgA was associated with lower viremia (Huang et al., 2020).

The development of an immunoassay to detect antibody responses to the SARS-CoV-2 spike protein, the main target for neutralizing antibodies for many coronaviruses, was presented at the workshop. The spike protein ELISA immunoassay was developed based on an influenza assay and then tested with samples from patients with severe, mild, and asymptomatic COVID-19 along with negative samples from individuals who had a broad range of other viral infections, including patients with HIV. The spike ELISA endpoint titers correlated well with virus neutralization (Amanat et al., 2020). The ELISA immunoassay was tested subsequently in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory and initially demonstrated 92% sensitivity and 100% specificity when patient samples were first tested against the receptor binding (RBD) assay, and those that were positive were then tested with the ELISA spike protein immunoassay. This two-step procedure (Stadlbauer et al., 2020) was used to screen 22,000 patients with mild disease, and convalescent plasma was obtained from those patients who were both positive in the RBD assay and the spike protein ELISA. To date, >250 patients have been treated with convalescent plasma from these positive patients with high antibody titers. An interesting observation from the study indicated that some PCR-positive patients are still positive 28 days post symptom resolution (Wajnberg et al., 2020).

A recent study evaluated the Abbott Architect SARS-CoV-2 IgG assay for performance, seroprevalence, and potential utility in vaccine clinical studies (Bryan et al., 2020). This is a chemiluminescent microparticle immunoassay (CMIA) that is used for the qualitative detection of IgG antibodies to SARS-CoV-2. The
assay detects antibodies to the nucleocapsid protein (NP) of the virus and can be performed on human serum or plasma using the automated ARCHITECT iSystem immunoanalyzer with a potential throughput of >3,000 samples/day/analyzer. The study showed the Abbott IgG assay had 100% sensitivity and 99.6% specificity. The Abbott assay is capable of discriminating between natural infection (anti-NP) versus immunization (anti-S), suggesting a possible role in vaccine clinical studies (Bryan et al., 2020). The workshop participants did not recommend a specific level of performance for serologic assays.

Scientific Gaps and Outstanding Issues

Multiple ongoing seroprevalence studies are contributing to our understanding of the level of SARS-CoV-2 seroconversion in various populations and communities. However, additional data are needed to increase our understanding of the immune responses that lead to protection and duration of protection. These data include the specific antibody titers that correlate with protection from disease and viral shedding upon reinfection. T cells have been implicated in protection against symptomatic disease in other respiratory infections, and it will be important to determine their role in SARS-CoV-2 immunity (Sridhar et al., 2013). The consensus of the workshop participants was that until sufficient data to determine if a positive serological test correlates with protection become available, these tests should not be used as a standalone tool to make decisions about personal safety related to SARS-CoV-2 exposure. The participants noted that serological tests should continue to be used to conduct serosurvey studies in the population. Critical data on antibody-based immunity should become available in the next few months from convalescent human serum passive transfer studies in animals and longitudinal natural history studies in people at high risk for reinfection (e.g., healthcare workers).

Recommendations for Conducting Future SARS-CoV-2 Serological Studies

As highlighted in Box 1, workshop participants recommended a series of next steps for advancing serology assays as a tool for understanding SARS-CoV-2 infection. The workshop participants recommended that additional serological tests continue to be developed, and these should be independently evaluated and validated for sensitivity, specificity, and reproducibility, especially tests that are based on biospecimen types other than blood, serum, and plasma. Additionally, the workshop participants recommended that quantitative assays specific for IgG and IgA and high-throughput tests are crucial to support SARS-CoV-2 vaccine development. They recommended that the US government continue to support these efforts by providing panels of convalescent serum or plasma, by facilitating independent evaluation through a central laboratory (e.g., NCI) and by enabling for the broad and rapid dissemination of data on assay performance. The broad availability of well-characterized positive and negative serum panels was identified as one of the main limiting factors by diagnostic developers.

The workshop participants also delineated the crucial serology field studies essential to advance our understanding of immunity to SARS-CoV-2 (Box 2). Additional longitudinal natural history studies are needed to better understand the immune responses that may lead to protection and the duration of protection, including the correlation between serological test results and risk of reinfection. They noted that additional types of studies can contribute to a better understanding of immunity and yield results faster than longitudinal natural history studies. These include serum passive transfer studies in animals and potentially human challenge studies if they can be conducted safely and ethically (e.g., with an attenuated strain or when a rescue therapy becomes available). Results from ongoing therapeutic trials using convalescent Ig or plasma transfusion may also yield valuable information on the role and level of antibodies in protection from reinfection.

Additional field studies of transmission in various populations, especially populations in long-term care facilities, prisons, and/or childcare facilities, are needed to fill critical knowledge gaps and better understand practices that facilitate transmission of infection in high-risk communities. In order to better reconcile and understand the large amount of information being generated by numerous serological studies in the United States, the workshop participants proposed that it may be possible to harmonize data from various serological studies using a common international serum standard for serology assays and data collection parameters. In addition, they recommended that it would be highly desirable to develop an interactive serological database that can capture country-wide infection trends over time and in different geographical areas. Such a real-time tool would have several advantages, including (1) informing public health officials on how to implement mitigation strategies in different areas and measure their effects, (2) informing the design and implementation of vaccine efficacy trials, and (3) improving the precision of predictive mathematical models of the epidemic.

Conclusion

In the last 5 months, the world has witnessed the spread of an unprecedented pandemic, and intense debates are currently ongoing on its future direction and the best course of action to mitigate its devastating effects on public health and the economy. Although many important research questions remain to be addressed, significant investments in research by the US government and expanded collaborative efforts between federal agencies and partners in academia and the private sector are advancing the field at an unprecedented speed. Continued
financial support by international research agencies and organizations, coordination of research efforts, sharing of specimens and data in real time, and the development of sustained effective partnerships are essential to providing the best opportunity for controlling this pandemic.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.immuni.2020.06.012.

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Box 2. Next Steps for SARS-CoV-2 Serology Field Studies

Advance natural history studies to understand the immune responses that may lead to protection, duration of protection, and risk of SARS-CoV-2 reinfection

Implement field studies in high-risk populations to define risk factors associated with infection

Establish an interactive serological database to capture infection with trends in time and different geographic areas