Serosurveillance for SARS-CoV-2 incidence using global blood donor populations

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Coronavirus 2019, or COVID-19, is an acute respiratory tract infection responsible for over 13 million diagnosed cases and **570 thousand** deaths globally as of July 13, 2020(1, 2). During the early stages of the epidemic few clinically ill people were tested for active infection by SARS-CoV-2, the infectious cause of COVID-19. Furthermore, a large percentage of people infected with SARS-CoV-2 present either no symptoms or relatively mild disease (3). These facts make it difficult to estimate infection rates and prevalence as the epidemic evolves globally. This information is important for calculating absolute risks of COVID-19 disease and deaths, predicting the spread of the virus in communities based on levels of presumptive immunity following infection, and development and monitoring the impact of implementation and relaxation of epidemic mitigation policies. Measures to mitigate transmission have substantially slowed the course of the epidemic in most countries, but also slowed the development of herd immunity. As infection rates increase many questions remain as to the level of protection presumed by herd immunity, particularly in asymptomatic and mildly infected individuals who develop less robust immune responses and may be at higher risk for reinfection. (4-6)

The majority of individuals mount an effective immune response during infection, leading to viral eradication and the production of specific antibodies against SARS-CoV-2 that are detectable 10 to 21 days after infection. (7, 8) Although the early focus of diagnostic test development and scale up was on molecular assays for viral RNA in oral-pharyngeal swab samples, over the past 6 months there have been many advances in development and applications of serological assays in order to conduct serosurveys to understand the level of Ab reactivity in different populations, improve epidemic modeling and forecasting informing public health policy and intervention strategies.(9, 10) Table 1 summaries the diverse applications of SARS-CoV-2 serological assays, as well as the still significant limitations in testing technologies and incomplete understanding of their performance characteristics and utility in different use cases. In the US approximately 25 serological assays have received FDA Emergency Use Authorizations (EUAs). These assays target different viral proteins (S1, S1/S2, RBD, NC) and have different configurations allowing detection of total Ig, IgG, IgM or IgA. (11) Many factors influence test performance, including cross-reactivity with other coronaviruses (12), and assay configuration and platform (laboratory based vs point-of-care lateral flow) and immunoglobulin target.

Sensitive and specific antibody tests can and are critically needed to identify and survey population infections. Such surveys of serological incidence are powerful and effective tools for monitoring the development and progression of the epidemic and in determining the increase in herd immunity (13). It is expected that individuals with strong antibody responses will be immune to reinfection, protecting both themselves from reinfection and reducing further viral spread by raising herd immunity. However, for many viruses, including other human coronaviruses that cause common colds, antibody responses can wane over time (14) leaving individuals susceptible to reinfection. Therefore, it is critical to understand immune persistence by following a cohort of individuals such as those volunteering to donate CCP to characterize their antibody levels over time.

There are many approaches to serosurveillance that involve testing of at-risk populations, such as health care workers and first responders, community recruitment strategies based on rapid POC testing or home collected dried blood spot (DBS) sampling or testing residual clinical laboratory samples. Blood donor based serosurveillance is increasingly recognized as a powerful and cost-effective strategy to monitor infectious diseases including evolving emerging infectious diseases (EIDs) epidemics. Past studies have provided insights into how to execute and interpret blood donor serosurveillance studies for a large number of viruses for which routine donor screening is performed, including HIV, HCV, HBV, HTLV, and WNV. During a large DENV-4 epidemic in Rio de Janeiro, Brazil in 2012 we calculated the seroincidence of DENV in the blood donor population over the course of the outbreak to be 6.2-6.8% (15). During the large 2013-2014 CHIKV epidemic we calculated the seroincidence of CHIKV in the Puerto Rican blood donor population to be 23.5% (16).

The use of blood donor samples allows the sampling of asymptomatic and recovered cases of COVID-19 28 days following COVID-19 symptom resolution. The earliest blood donor-based serosurveillance studies were reported from Denmark and the Netherlands (17) (refs,). The Danish study reported in this issue of CID, the first report of blood donor based serosurvey, employed rapid, low cost and convenient point-of-care testing of donor blood samples (adjusting for assay sensitivity and specificity), extrapolated the donor based results to the general Danish population, and derived infection to case and mortality ratios. Strengths of the study are its broad national population representativeness and use of the donor population to capitalize on the capacity to conduct ongoing serial cross-sectional analyses to track outbreak and correlate with implementation and relaxation of pandemic mitigation measures. However, the study findings are limited by the use of a POC assay with poor sensitivity (82.58% (75.68-88.20)) and lack of confirmatory testing. This highlights the importance of selection and evaluation of appropriate sensitive and specific assays and supplemental assays to confirm SARS-CoV-2 seroreactivity in order to effectively perform large-scale donor serosurveys.

In the U.S., the REDS-IV-P program RESPONSE (REDS-IV-P Epidemiology, Surveillance and Preparedness of the Novel SARS-CoV-2 Epidemic) initiated SARS-CoV-2 serological testing of residual donor serum in March 2020. The study is testing 1,000 individual blood donations collected monthly from each of six metropolitan regions, including San Francisco Bay Area, Seattle, New York City, Los Angeles, Boston and Minneapolis (https://redsivp.com/covid-19/). Routinely collected donor demographics including age, gender, race-ethnicity and zip code of residence are linked to coded samples tested using a robust algorithm that includes an S1 total Ig assay, confirmed with a NC Total Ig assay and a pseudovirus based neutralization assay. The US CDC recently funded a program to expand this study from six sites for six months to a total of over 50 US metropolitan sites with monthly collections of 2,000 samples per site for 12 months, plus final testing at 18 months. This will allow a detailed analysis of the increasing penetrance of infection by the virus into communities. Data will be compiled from all regions, collated and linked to demographic data from the collection organizations. To provide estimates of infection incidence based on Ab reactivity at the general population level by city, group-specific donor estimates will be computed, then adjusted to the population group distributions using post-stratification- or calibration-type weighting (18) for an

example using this approach.) Overall and subgroup-specific estimates will be calculated and reported with uncertainty statements including computation and reporting of standard errors and/or confidence intervals. We will also partner with academic and CDC colleagues leading community SARS-CoV-2 sero-studies to correlate our blood donor-derived seroincidence data with data from those studies in order to develop algorithms to correct for initial biases. We will calculate changes in overall, geographic region, age- gender- and race-ethnicity-specific seroincidence of SARS-CoV-2 in donor populations and extrapolated general populations by month over the course of the study and relate those results to clinical cases and deaths and community serosurvey data with aggregate and stratified data posted by CDC in near real time.

Persistence of humoral responses to SARS-CoV-2 that will determine lasting individual and herd immunity, must also be accounted for to accurately estimate evolving serological incidence in cross-sectional serosurveillance studies. We have conducted numerous longitudinal follow-up studies of donors following acute infections with transfusion-transmitted viruses (e.g., HIV, HBV, HCV) and emerging infectious diseases (e.g., WNV, CHIKV, DENV, ZIKV, T. cruzi, B. microti). For acute arboviral infections these studies have characterized both the dynamics of early Ab seroconversion and the waning of Abs. We have worked closely with CDC to account for the waning of WNV IgM and ZIKV IgG and nAbs to apply linear regression modeling to derive adjusted cumulative incidence estimates from cross-sectional serosurvey data for viruses with transient infections followed by waning of serological responses (19-21). The important consequence of waning of SARS-CoV-2 Ab responses for serosurveys, such as the serial cross-sectional serosurveys of blood donors, is that declining Ab responses in the months to years following infection would result in underestimation of cumulative incidence if not accounted for in the analysis.

In conclusion, serial serosurveillance studies of SARS-CoV-2 using blood donor populations, which are now being implemented in many countries, provide a powerful adjunct to other approaches that are prospectively monitoring outbreak activity. Although serosurveillance data from asymptomatic blood donors trail viral transmission and case reporting by approximately one month, if these studies are well designed and executed and carefully analyzed and interpreted, they provide longitudinal and systematic data to inform our understanding of the epidemiology and effectiveness of responses to this unprecedented pandemic.

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Table 1. Applications and limitations of SARS-CoV-2 antibody (Ab) assays

Applications	Limitations

• Diagnosis of acute **infections** (symptomatic or high risk exposure)
  ○ Adjunct to molecular testing of swabs
• Recent infection in asymptomatic exposed populations
  ○ Health care workers (HCWs), first responders (FRs), contact tracing, "back to work" population testing
  ○ Stage infections to estimate time from infection
• Qualification for COVID-19 Convalescent Plasma (CCP) and hyperimmune immunoglobulin (H-Ig)
• Serosurveillance
  ○ HCWs & FRs; regional, state and national serosurveillance studies; blood donor serosurveillance studies
• Durability of immunity
  ○ Longitudinal studies to characterize loss of protective immunity
• Detection of reinfections
  ○ Boosting of Abs as evidence of reinfection
• Vaccine efficacy monitoring
  ○ Vaccine induced seropositivity (VISP)
  ○ Breakthrough infections documented by development of Abs to proteins not in vaccines
  ○ Boosting of spike Abs as evidence for breakthrough infections
• Blood donation screening as an incentive to recruit donors and identify CCP donors
	• False positive results
	  ○ Minimize by using specific (lab) assays and confirmation algorithms
	• Cannot diagnose early infections prior to seroconversion
	• Variability of Ab dynamics relative to symptoms, past exposure to ARVs and other factors
	  ○ Impact on serosurveys and estimating dating of infection
	• Unknown implications for protective immunity from reinfections
	  ○ Individual and herd immunity
	• Humoral (and cellular) immune responses wane
	  ○ Protection from reinfection may require vaccination or natural boosting
	• Impact of vaccines on performance of Ab assays
	  ○ Vaccine-induced seropositivity (VISP)
	  ○ Detection and consequences of vaccine breakthrough infections
	• Viral diversity and evolution
	  ○ Strains with increased or reduced infectious and pathogenic potential
	  ○ Assure sensitivity of serological (and molecular) assays for diagnostics and surveillance