Serology for SARS-CoV-2: apprehensions, opportunities, and the path forward

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Abstract
Serological testing for SARS-CoV-2 has enormous potential to contribute to COVID-19 pandemic response efforts, however the required performance characteristics of assays will critically depend on the use case (individual-level vs. population-level), and strong governance structures are urgently needed to rationalize the use of serosurveillance data for public health decision-making.

Making data-driven decisions on how to fight the COVID-19 pandemic without completely shutting down economies will require better tools to understand transmission. The current crisis presents an opportunity to rethink how health systems generate and use surveillance data, and how to harness the power of serological tests and sero-epidemiology. The world's health systems are rushing to develop and implement testing for clinical use, evaluations of social policy, and quantification of population-level risk, which has brought into sharp focus the challenges facing surveillance programs throughout the world. There is an urgent need to monitor variations in disease transmission across populations and geographies in near *real time*. Rapid detection of active cases and contact tracing – using direct tests for presence of the virus (acute phase diagnosis) – is the cornerstone of containment strategies. For later phases of pandemic control – when the key questions involve when, where, and how to lift confinement measures, and relax social distancing constraints – serological testing to measure antibody responses to the virus becomes paramount to refine understanding of transmission intensity and population susceptibility.

Infections leave behind immunologic impressions in the form of antibodies that last for months to years and can be detected by testing blood, blood products (serum/plasma), or saliva. With SARS-CoV-2, the virus responsible for COVID-19, new antibody tests to detect exposure are rapidly becoming available and are being introduced to all levels of health care systems. There remain numerous difficulties in assessing performance characteristics of the new tests, assuring quality control, standardizing methodology and results reporting across sites, and developing the baseline data required for test interpretation. We have not yet performed the studies, nor created the data, to infer how immunity levels and duration of immunity varies following asymptomatic, mild, and severe cases, or across diverse populations with different genetic backgrounds, comorbidities, or infection histories. In this article, we distinguish the use-cases for individual- versus population-level serological testing. We emphasize the dangers of using serologic tests for individual risk assessments at this time; in contrast, we highlight the extraordinary power of population-level serological testing (i.e. serosurveillance or sero-epidemiology), even with first generation assays of moderate sensitivity/specificity.

Use cases for SARS-CoV-2 serology

At the **individual-level**, serologic tests are frequently used to support clinical diagnosis by determining recent or prior infection (to supplement PCR detection), or to determine vaccination status and requirements for boosting. In vaccine trials, individual assessments of antibody titers may be used to determine sero-status prior to enrollment, as a tool to reduce bias, simplify analyses, and minimize required sample sizes. Specific to SARS-CoV-2, a widely discussed idea in the media has been the issuance of "immune passports" – the proposed use of serology to infer immunity and thus enable a person to work on the front lines or return to daily work routines. Such an application must be predicated on an established surrogate of protection – a given antibody titer associated with clinical protection from infection – and a test with sufficient specificity to ensure people are not unintentionally put in harm's way.[1] Serology tests with relatively high but imperfect specificity may lead to substantial numbers of false-positives when used in low transmission settings, such as the current pandemic situation (i.e. when <5% of the

population has been infected). In such settings (5% seroprevalence and 95% sensitivity/specificity), as many as 50% of tested individuals could be mistakenly classified as immune, with potentially catastrophic consequences. In addition to risks of false positives due to prior test probabilities, false negatives may occur in some previously-infected persons who fail to produce antibodies specific to the antigens/epitopes in a given assay. For these individuals that don't mount a measurable antibody titer despite having been exposed to the virus, obtaining permission to return to work could be onerous.

At the **population-level**, representative cross-sectional serosurveys can provide aggregate 'snapshots' of infection history and immunity of a population. Understanding the proportion of the population infected by SARS-CoV-2 cannot be assessed based on PCR confirmed cases alone, due to variations in testing practices and the clinical spectrum of disease (e.g. asymptomatic infections). In contrast to case data, seroepidemiological datasets provide a less biased picture of risk of death (infection fatality rate), the amplitude of transmission in different populations, and can highlight disparities in infection rates without typical health-seeking behavior biases. Understanding age-specific or spatial distribution of susceptibility could guide policymakers about where to restrict contacts and to what degree (e.g. what IgG seroprevalence in children is acceptable to allow schools to open?). Population-level surveys could also help estimate the probability and timing of future waves of disease (which will critically depend upon duration of immunity),[2] measure the impact of interventions (physical distancing, vaccination), and in later stages, confirm the absence of transmission.

Here we underscore key differences between individual- and population-level use cases and emphasize that, in the absence of a perfect assay, different use cases will require tests with specific performance characteristics: while assays that "certify" an individual's immunity need to be correlated with protection and have near-perfect specificity (to limit the number of false positives, when seroprevalence is low), assays to ascertain population-level exposure would have utility as long as the sensitivity and specificity are well-defined to adjust population-level estimates. Assays used in the hospital setting as an indirect diagnostic can be validated with positive controls obtained from patients in early convalescence, whereas assays aiming to ascertain population-level exposure need to be validated against positive controls across the full spectrum, including many asymptomatic and mild infections, and recovered cases during late convalescence.

Foundational studies to enhance the utility and interpretation of SARS-CoV-2 serology. While currently available SARS-CoV-2 serological assays have insufficient performance characteristics (sensitivity/specificity) to warrant use at the individual-level, imperfect tests may nevertheless provide highly valuable tools to address population-level questions, such as the safety of relaxing stay-at-home orders or school closures, or evaluations of alternative intervention measures. To fully realize the benefits of population-level seroepidemiological studies, a number of fundamental questions must be addressed, relating to test performance, the dynamics of antibody responses in relation to infection, and the link between antibody responses and immunity. Rapidly answering these questions across different populations and epidemiologic contexts will require various study designs as outlined below.

Case Control studies.
These studies compare the results of a serologic test in PCR-confirmed cases against a group of controls, such as samples drawn from pre-pandemic serum banks representing populations with different background exposures to other coronaviruses and other infectious diseases, or contemporary samples from individuals known to be negative for SARS-CoV-2. Cases should represent the full spectrum of infection and disease expected in the target population for the assay. For example, an assay that will be used to establish seropositivity in the general population, needs to be validated against samples from individuals with mild and severe disease as well as fully asymptomatic individuals. With this design we can estimate the sensitivity and specificity of a given serologic test at different post-infection time-windows, although it does not address questions of immunity.

Longitudinal cohort studies with serial sampling of persons with confirmed infection.
These studies will elucidate the post-infection dynamics of different anti-SARS-CoV-2 antibody responses and can be critical for reconstructing historical transmission trends from cross-sectional serosurveys. Cohorts should include persons with disease across a spectrum of clinical severity and other relevant demographic or health features. If antibody profiles of uninfected individuals are available, when compared with the profile of infected individuals, these data can allow for estimation of single- or multi-antibody test performance as a function of time since infection.

Studies of household contacts of index COVID-19 patients and other high-risk individuals.
Household contacts of confirmed index infected cases represent a unique population to learn about antibody responses to SARS-CoV-2 infections as they are at high risk of acquiring infection. By comparing the serological response of contacts who go on to become infected, with those who do not, this study design helps identify which antibody responses and magnitude are best correlated with protection. Additionally, when coupled with routine surveillance for virus these studies provide information on risk factors for symptomatic versus asymptomatic infection, and whether protected (asymptomatic) seropositive individuals can still serve as vectors of transmission. Similar longitudinal studies of other high risk individuals, such as healthcare workers, may inform correlates of protection through assessing baseline antibody titers among those who go on to become infected, versus those who do not.

Serological responses to vaccines in large efficacy studies.
In the early stages of vaccine development, protection thresholds measured from case-control and cross sectional studies as described above could serve as guideposts for evaluating vaccine derived immunity. In phase 3 efficacy trials, comparison of serological responses of vaccinees who go on to become infected to those who do not would allow for identification of the antigen-specific antibody responses that best correlate with protection against infection (a third estimate of these measures, specific to vaccine-induced immunity).

Governance

Serosurveillance for SARS-CoV-2 will only be capable of contributing to 'actionable' public health information if serology measurements flow into efficient data pipelines. Scale-up of serological testing for pandemic response must therefore be accompanied by a governance model at the sub-national, national and international levels, and by an operational research agenda that evaluates the utility of assays within specific contexts. National-level governance could link the collection, oversight, and maintenance of samples and the resulting analysis to the scale at which resulting policy may be implemented. Much like a national census is translated into infrastructure appropriations, serosurveillance could be used for resource allocations (and future vaccination efforts) to target transmission hotspots.[3]

Data from carefully designed serostudies (such as detailed above) are urgently needed prior to widespread adoption or implementation of antibody testing programs. To ensure comparison across studies, there is a need for harmonization of assay protocols, sharing of reference standards, and a set of best-practices for reporting results.[4] Because seroepidemiological studies will require measurement of healthy individuals, various strategies for opportunistic sampling of individuals in community settings should be explored, as described in a proposed Global Serum Bank.[5] A host of ethical and privacy issues will need to be addressed; we suggest that serosurveillance platforms should incorporate broad consent, enabling future screening of serum collections for multiple biomarkers of public health concern beyond SARS-CoV-2 alone. The SARS-CoV-2 pandemic has highlighted the value of transparency in disease surveillance for all nations. We see a role for international coordination of national sero-epidemiology programs to facilitate standardizing methods and dissemination of results among national public health laboratories, as there are now an unprecedented number of large-scale initiatives underway.[6,7,8]

Beyond the current crisis

For many countries, SARS-CoV-2 has put additional strain on existing public health surveillance infrastructure, which may already struggle to generate reliable and comprehensive data on disease burden of priority infectious diseases. In settings where case-based surveillance and reporting are poor, integrated serosurveillance systems are particularly valuable to provide robust evidence on which to rationalize scarce resource allocations. Reference laboratory networks for vaccine-preventable diseases already support large-scale serologic testing programs, including use of high-throughput multiplexed serologic assays. International disease elimination programs for malaria and selected neglected tropical diseases have already established data pipelines with semi-automated aggregation, analysis, and mapping of surveillance data with digital dashboards. Recurring large-scale population based surveys that collect biological specimens, like the Demographic Health Survey, have led to the technical, ethical, and administrative infrastructure for maintaining national serum repositories.[9] These surveys and infrastructure could provide an efficient avenue for monitoring trends in SAR-CoV-2 transmission.

In summary, seroepidemiological studies and integrated serosurveillance platforms are urgently needed to guide and tailor SARS-CoV-2 response efforts, and will continue to be critical for mitigating post-pandemic resurgence. Coordinated serosurveillance provides opportunities to

complement or even re-convert vertical disease-specific programs into an integrated program delivery,[10] and platforms should be designed with a longer term vision beyond COVID-19, to generate capacity for ‘precision public health’ to monitor additional major diseases, and provide insights into how disease occurrence is interrelated with other health risk factors. Finally, we stress that investing now in a fundamental and operational research agenda will allow us to rapidly develop serosurveillance as a powerful tool for population-level public health; however, the complexity of using serological assays within low prevalence settings to inform individual-based risk assessments – i.e. to inform decisions regarding return to work – is dangerously premature.

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