Perspective

SARS-CoV-2 antibody testing for estimating COVID-19 prevalence in the population

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SUMMARY

Reliable antibody testing against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has the potential to uncover the population-wide spread of coronavirus disease 2019 (COVID-19), which is critical for making informed healthcare and economic decisions. Here we review different types of antibody tests available for SARS-CoV-2 and their application for population-scale testing. Biases because of varying test accuracy, results of ongoing large-scale serological studies, and use of antibody testing for monitoring development of herd immunity are summarized. Although current SARS-CoV-2 antibody testing efforts have generated valuable insights, the accuracy of serological tests and the selection criteria for the tested cohorts need to be evaluated carefully.

COVID-19 (coronavirus disease 2019), caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), has developed into an unprecedented global pandemic posing enormous challenges to healthcare systems. SARS-CoV-2 can be transmitted by respiratory droplets,[1] and the course of COVID-19 infection is highly variable, ranging from asymptomatic or mildly affected individuals (approximately 80%) to severe cases requiring hospitalization (approximately 10%–20%).[2–5] Typical symptoms include fever, fatigue, cough, shortness of breath, and anosmia (loss of smell), present in the majority of individuals, whereas atypical symptoms range from gastrointestinal discomfort (diarrhea, nausea) to dizziness/confusion.[3,6,7] Asymptomatic individuals may have very mild manifestation, including lung abnormalities that usually go unnoticed.[8] Although mounting weaker immune responses (lower levels of anti-viral immunoglobulin G [IgG], pro- and anti-inflammatory cytokines), asymptomatic individuals have been shown to shed virus significantly longer than those who are symptomatic.[9] The median incubation time after SARS-CoV-2 infection is 5 days and rarely longer than 12 days.[10] Infected individuals can already transmit SARS-CoV-2 before onset of symptoms (called subclinical or presymptomatic transmission), but the exact fraction of total transmission events is unclear.[11] Entirely asymptomatic individuals are also expected to have contributed substantially to the spread of SARS-CoV-2,[8,12] with social distancing and quarantine measures plausibly providing remedy.[13]

Assessing the true population-wide spread of COVID-19, including asymptomatic individuals, is critical for epidemiological management of the outbreak and relies on robust testing methodologies. Millions of molecular qRT-PCR (quantitative reverse-transcriptase PCR) tests for SARS-CoV2 have been carried out to date, enabling detection of acute infection (Table 1). In addition to qRT-PCR, other nucleic acid-based diagnostic tests,

such as digital droplet PCR, loop-mediated isothermal amplification, and CRISPR-Cas systems, have also been established.[14] Not all of these nucleic acids-based tests are quantitative and determine the copy number of viral genomes in a sample. Some of these tests provide results within minutes, can be performed at the point of care, or may even show higher accuracy than qRT-PCR under some conditions.[14] Nonetheless qRT-PCR is still the most commonly used nucleic acid-based method because of its high accuracy, amenability for high-throughput automation, availability of equipment/reagents/trained personnel, and yielding of results within a few hours.

When the infection has been cleared by the immune system, qRT-PCR testing can no longer assess exposure because SARS-CoV2 cannot be detected in most individuals 3–4 weeks after symptom onset.[18] In contrast, serological tests yield positive results after the body mounts an antibody response. This process is called seroconversion and takes about 1–2 weeks after onset of symptoms for SARS-CoV-2.[19] High antibody titers persist for several weeks, allowing detection of past infection with serological tests even after the virus has been cleared.[19]

Hence, SARS-CoV-2 infection can be detected by qRT-PCR (for acute infection) or serology (typically past infection). Diagnosis of COVID-19 includes clinical criteria in addition to a positive qRT-PCR test. Because not all infected individuals are diagnosed during the acute phase by qRT-PCR (because they did not develop symptoms or because of limited testing capacities), antibody testing is also key to assess the infection fatality rate (IFR).[20,21] The case fatality rate (CFR) reports the percentage of deaths from all cases diagnosed with a disease, and for COVID-19, this percentage varies greatly between countries.[22] This variation may be due to population structure (relating to risk factors such as age or obesity) or could stem from countries'

Table 1. Testing approaches for SARS-CoV-2 and types of antibody assays

Type of assay	Readout and limitations	Current scale	References
Digital surveys	subjective report on symptoms, can help to identify new infection hotspots on the population scale	millions	e.g., Menni et al.[15]
qRT-PCR (quantitative reverse-transcriptase PCR)	detection of acute infections, cannot detect SARS-CoV-2 after the infection has been cleared (3–4 weeks after onset of symptoms in most individuals)	hundreds of thousands to millions	e.g., Johns Hopkins University[16]
Sequencing of SARS-CoV-2 strains	allows tracking of the origin of strains and phylogenetic relationships and could be used to associate mutations with pathogenicity	thousands	e.g., Lu et al.[17]
Antibody tests	enables detection after infection has been cleared; limited use for detecting acute infection because antibodies are only produced 1–2 weeks after onset of symptoms	thousands to tens of thousands (see Table 2)	e.g., Kobokovich et al.,[40]18 Gronvall et al.,[43]19 National COVID Testing Scientific Advisory Panel et al.[49]20
LFA (lateral flow assay)	also called rapid diagnostic test (RDT), fast point of care (result within minutes) at cost of accuracy		
ELISA (enzyme-linked immunosorbent assay) and CLIA (chemiluminescent immunoassay)	lab tests requiring dedicated equipment and personnel, taking hours and yielding higher accuracy than LFAs		
neutralization assay	more complex biological lab test, the only method to functionally test antibodies for their ability to hinder infection of cells by SARS-CoV-2		

different selection criteria of potential cases for qRT-PCR testing. In contrast, the IFR also captures individuals who were not diagnosed during the acute phase of the infection and thus provides information potentially less biased by symptoms or selection criteria for qRT-PCR testing. Nonetheless, IFR calculation leveraging serological data still leaves room for error because some cases may still be missed by serology, and not all disease cases will have been accounted for accurately. Knowledge of the IFR is critical to assess the severity of COVID-19 and to gain insights into its transmission.

ANTIBODY RESPONSES AGAINST SARS-CoV-2 AS A BASIS FOR SEROLOGICAL TESTING

The human adaptive immune system typically mounts a distinct response against SARS-CoV-2, including production of specific IgM, IgG, and IgA antibodies.[19] In general, detectable amounts of IgM appear after approximately 5 days after infection and level off at approximately 10 days, whereas IgG production is at first delayed but surpasses IgM concentration after approximately 10 days. This pattern reflects the canonical role of IgM as a first-line antibody response with low affinity but high avidity transition-

ing to production of high-affinity IgG.[19] However, beyond this general trend, timing of seroconversion can vary substantially between SARS-CoV-2-infected individuals, leading to possible biases in determining the accuracy of diagnostic serology tests during the first 2–3 weeks after infection. In some studies, simultaneous rather than consecutive detection of IgM and IgG has been reported.[23,24] Depending on the severity of infection, the magnitude of antibody responses can vary,[9] which can ultimately also affect the results of serology studies.

Serological tests for SARS-CoV-2 frequently test for IgM and IgG antibody isotypes to cover early (IgM) as well progressing (IgG) immune responses. Given that SARS-CoV-2 infects the nasopharynx and lungs, mucosal IgA likely also contributes to containing infection. However, most research has focused on blood IgG/IgM, possibly because of the high infectivity of mucosal saliva/sputum samples of individuals with COVID-19 (blood, on the other hand, appears to be free of infectious SARS-CoV-2 even in individuals with acute disease[25]). Previous research regarding the role of IgA in other CoV infections, such as the 2003 SARS-CoV,[26] and results of IgA in blood samples of individuals with SARS-CoV-2[27,28] point toward substantial diagnostic potential of IgA. Although antibody responses against

SARS-CoV-2 are generally considered to be protective and clear infection, concerns about potential detrimental effects of antibody-dependent enhancement have been raised that warrant careful consideration in vaccine development and application.[29,30]

Regarding antigens, serological tests for SARS-CoV-2 typically detect antibodies against single antigens, such as the spike glycoprotein or the nucleocapsid protein, yielding robust results.[31] The genome of SARS-CoV-2 encodes approximately 30 proteins in total,[32] four of which encode structural proteins forming the virion, with the spike (S), membrane (M), envelope (E) proteins representing the viral envelope and the nucleocapsid (N) protein binding to the RNA genome. Testing for multiple SARS-CoV-2 antigens in parallel may improve diagnostic power,[28] although proteins other than S tend to show higher conservation between different CoVs (see below), potentially providing less discriminatory power. For example, the nucleocapsid protein is more conserved between SARS-CoV-1 and SARS-CoV-2 than the S glycoprotein.[32] Non-structural proteins (NSPs) can also show substantial conservation[33] and yield little discriminatory power in a high-resolution SARS-CoV-2 immunoassay.[28] Current antibody tests do not allow discrimination between SARS-CoV2 strains having accumulated different mutations, and only genome sequencing can resolve phylogenetic relationships (Table 1).

In addition to SARS-CoV-2, six more CoVs are known to infect humans and are potential candidates to elicit cross-reactive antibodies that could interfere with serological tests. Of them, SARS-CoV-1, which caused the SARS outbreak in 2003 and Middle East respiratory syndrome (MERS)-CoV, represent, alongside SARS-CoV-2, recent transmission of zoonotic spillover events (from bats and camels, respectively).[34] Recent work has demonstrated that cross-reactivity between CoVs can, in principle, occur.[35] Antibodies of individuals with COVID-19 cross-react against the full-length S and nucleocapsid proteins of SARS-CoV-1 and MERS-CoV. Some antibodies directed against a segment of the SARS-CoV-2 S protein critical for infection (receptor-binding domain [RBD]) failed to bind the RBD of SARS-CoV-1 or MERS-CoV,[35] but conserved regions of the SARS-CoV-2 RBD can also be recognized by antibodies developed in the immune response against SARS-CoV-1,[36] indicating some cross-reactivity of RBD-specific antibodies between SARS-CoV-1 and SARS-CoV-2.[37–39] This cross-reactivity is affected by different degrees of conservation of sections within the RBD of SARS-CoV-1 and SARS-CoV-2, related to epitopes overlapping with the ACE2 binding site. These finding suggest that investigating antibody responses against protein segments at higher resolution can provide an additional layer of information. Considering the much lower prevalence of SARS-CoV-1 (approximately 8,000 reported cases, no new cases after 2004) and MERS-CoV (fewer than 3,000 cases since 2012)[34] opposed to the spread of SARS-CoV-2 (>80 million cases diagnosed by qRT-PCR in December 2020), cross-reactivities against SARS-CoV-1 and MERS-CoV are generally not expected to bias population-scale SARS-CoV-2 testing.

In addition to the highly pathogenic SARS-CoV-1/2 and MERS, four more CoVs (OC43, HKU1, NL63, and 229E) circulate widely in humans, causing only mild common cold-like symptoms.[34] Frequent antibody responses against all four common cold strains have been detected,[40] and a recent qRT-PCR study from Scotland reported that 10.7% of all respiratory virus infections detected between 2005 and 2017 were attributed to CoVs.[41] Protective effects toward SARS-CoV-2 of cross-reactive antibodies targeting these common CoVs are understood incompletely,[19] although recent work indicated no cross-reactivity between antibodies against the RBD of SARS-CoV-2 and NL63/229E S.[42] High accuracy reported for several current SARS-CoV-2 antibody tests[43] also suggests that potentially existing cross-reactivities can be overcome by reliable test designs. Systematic testing of cross-reactivity between SARS-CoV-2 and the less pathogenic CoV strains could help to elucidate this issue.

TYPES AND CHARACTERISTICS OF ANTIBODY TESTS AGAINST SARS-CoV-2

Methodologically, antibody testing at larger scale is typically carried out by lateral flow assays (LFAs; also called rapid diagnostic tests [RDTs]), enzyme-linked immunosorbent assays (ELISAs), or chemiluminescent immunoassays (CLIAs; Table 1). LFAs are small devices (such as home pregnancy tests) and can be performed at the point of care without additional equipment, yielding qualitative results within minutes.[44] They rely on immobilizing all necessary reagents as different zones on a polymeric strip. A blood sample is added on one side and moves by capillary force through conjugated recombinant antibodies toward a detection line.[45] Because of this simple setup, no washing steps are required, and only low sample amounts are needed. However, variations in sample volume can impair the test's precision, and the low total sample volume restricts the detection limit. Furthermore, enzymatic signal amplification is not readily achievable in LFAs (because the enzymes and substrate would inherently mix and react within the polymeric strip when substrate is added).[45]

ELISAs, on the other hand, require laboratory equipment, trained personnel, and a longer workflow but can provide quantitative results and a higher accuracy than LFAs because they allow enzymatic signal amplification. Antibodies in the sample typically bind to antigens of interest immobilized on a surface, and the amount of bound antibodies is detected by addition of an enzyme-linked detection antibody specific for the isotype of interest (e.g., IgG, IgM, or IgA). Extensive washing between these detection steps reduces background signals, making ELISAs highly accurate.[46] CLIAs are similar to ELISAs but rely on coated microparticles than immobilized antigens, also yielding high accuracy with excellent amenability for automation.

Neutralization assays involve a more complex workflow detecting inhibition of viral infection of cultured target cells (Table 1). Neutralizing antibodies block any step (typically entry) before the first virally encoded synthetic event, whereas impeding the spread of infection in a culture by blocking release would not represent neutralization.[47] Therefore, neutralization assays are the only method capable of assessing an individual's generation of neutralizing antibodies (NAbs), a key requirement for protection against reinfection (although non-antibody-mediated cellular immunity and non-NAb-mediated immunity also have

distinct roles in the anti SARS-CoV-2 immune response[48]). In typical neutralization assays, serum samples are serially diluted and mixed with artificially produced SARS-CoV-2. These dilutions are then used to infect cell cultures. If antibodies in the blood sample possess neutralizing capacity, lysis of the cultured cells is reduced, which can be quantified as the number of plaques resulting from different dilutions. Downsides of this approach are rather low throughput, a requirement for high safety precautions (because the experiments require working with infectious SARS-CoV-2), and a rather long incubation period until results are available (it takes several days for lysis of cells to become visible as plaques).[46] Several variations and alternatives exist for the plaque-reduction neutralization assay (PRNTs) recently reviewed by Khoury et al.[49] These include replacing live SARS-CoV-2 with replication-defective pseudoviruses in which the SARS-CoV-2 S protein is incorporated into the surface of vesicular stomatitis virus or lentiviruses (single-cycle virus neutralization assays). Pseudoviruses can also facilitate detection of infection because fluorescent reporters genes can be included that will be expressed when infecting host cells. Multi-cycle virus neutralization assays rely on replicating pseudoviruses (or native SARS-CoV-2) and follow viral replication over longer periods. Use of pseudoviruses mitigates some downsides of PRNTs relating to biological safety precautions and duration of the workflow, although S folding and presentation may vary from its native state on SARS-CoV-2. The replication dynamics of pseudoviruses may also deviate from native SARS-CoV-2.[49]

Molecular diagnostics for SARS-CoV-2 can also rely on use of antibodies specific to viral antigens. Rather than detecting the presence of immune responses in the blood, these tests detect the presence of viruses in specimens (yielding information similar to qRT-PCR testing). For such rapid antigen testing approaches, the detection antibodies need to be selected carefully because cross-reactivity, especially to seasonal common cold CoVs, could bias results. A panel of monoclonal antibodies highly specific for SARS-CoV-2 could improve accuracy.

Researchers at Johns Hopkins University have compiled a valuable overview of commercially available SARS-CoV-2 antibody tests, including the two most important criteria for examining serological test specificity and sensitivity (as self-reported by the manufacturers),[43] and researchers at Massachusetts General Hospital provide a continuously updated SARS-CoV-2 infographic that covers all categories of COVID-19 diagnostic tests.[14]

Sensitivity is a metric for the percentage of infected individuals that is identified correctly by a diagnostic test and is equivalent to the true positive rate. Actual positive samples missed by a test are false negatives. Specificity refers to the percentage of uninfected individuals who are identified correctly by a diagnostic test, equivalent to the true negative rate. A positive test result of an actually uninfected individual is a false positive. The sensitivity and specificity of antibody tests can be affected by technical aspects, such as the mode of signal amplification, as well as biological factors, such as cross-reactivity against antigens displaying similar epitopes and the duration and magnitude of antibody responses. These factors can lead to random noise and systematic bias; if errors are entirely random, then repeating the test can lower the error rates, but for systematic errors (such as samples contamination or cross-reactivity), repeating the same sample would consistently reproduce incorrect results. A systematic error caused by contamination can be corrected by collecting new samples, whereas cross-reactivity is inherent to the testing method and needs to be resolved on a technical level. Systematic errors of antibody tests caused by cross-reactivities may be mitigated by testing for multiple SARS-CoV-2 antigens in parallel. Assessing the biases of different tests requires in-depth analysis of repeated measurements of many individuals. To build a reliable combination of tests with improved error rate, different tests would need to be run on the same samples to assess the tests' consistency. After this evaluation experiment, the most reliable tests with complementary accuracies would be combined for population-scale testing. High-throughput assays that allow testing for multiple antigens include peptide arrays (spotting multiple antigens on a glass slide that can be measured in parallel) and VirScan (based on immunoprecipitation of phage-displayed oligo libraries).[50] Although yielding valuable insights into antibody responses against SARS-CoV-2 and high precision,[28,51] the large-scale applicability of these approaches remains to be demonstrated and may incur greater costs than established ELISAs and CLIAs.

Many manufacturers currently self-report more than 95% sensitivity/specificity for LFAs and close to 100% for ELISAs.[43] A systematic comparison of LFAs from nine manufacturers, however, yielded generally lower sensitivities/specificities with similar performance of devices from different manufacturers.[52] ELISAs showed greater robustness, suggesting that lab tests still surpass the reliability of point-of-care LFAs. Similar comparison efforts for COVID-19 testing are available[53] or ongoing[54,55] and will help to assess the true performance of current testing kits, pointing toward possible improvements required for reliable population-scale testing.[56]

SENSITIVITY, SPECIFICITY, AND PREVALENCE AFFECT POPULATION-SCALE SEROLOGICAL TESTING

Antibody testing of representative fractions of the population can inform on the true spread of COVID-19, including asymptomatic individuals, who may represent 20%–80% of infections.[2–4] As a general trend, antibody tests tend to reveal higher population-wide SARS-CoV-2 prevalence than what is reported by qRT-PCR tests.[20]

An antibody test with 95% sensitivity and 95% specificity may appear intuitively to be highly reliable for executing such testing, but its real-world applicability depends on the true prevalence of infection in the population.[57] Generally, high specificity of a test is critical at a low prevalence in the population (approximately <10%), otherwise the false positives may outweigh the true positives. High sensitivity is critical at high prevalence in the population, otherwise the false negatives may outweigh the true negatives (Figure 1A). Positive and negative predictive value (PPV and NPV, respectively) are metrics representing test performance at a certain prevalence in the population (reviewed in detail by Gronvall et al.[46]).

Figure 1. The effect of sensitivity and specificity on antibody testing results is linked to the true prevalence in the population
(A) Schematic overview of the effect of test sensitivity/specificity at different prevalence in the population, illustrating false positives and false negatives. Red icons represent true positive individuals and gray icons true negatives.
(B) Effect of test sensitivity and specificity on the reported prevalence by the test as a function of true prevalence in the population.

For example, testing a population with 1% prevalence of a disease with a test of 100% sensitivity and 99% specificity would report almost 2% positives (Figure 1B). Under the same conditions, a test with 95% specificity would report almost 6% positives, an overwhelming overestimation of disease prevalence. At a high prevalence of 50% in the population, the reported prevalence by two tests of 99% and 95% specificity and 100% sensitivity would report 50.5% and 52.5% positives, which represents only a slight overestimation.

In contrast, at a prevalence of 90% in the population (for antibody responses possibly only achievable by vaccination), a test with 100% specificity and 90% sensitivity would report an 81% prevalence, which may lead to underestimation of the effect of a vaccination. However, the same test (with 100% specificity and 90% sensitivity) would report only a minor difference at a true prevalence of 5% (reporting a value of 4.5%).

Hence, the sensitivity of a test does not strongly affect results at a low prevalence in the population (approximately <10%), whereas specificity does not dramatically affect results at high prevalence (Figure 1). This notion has direct implications for selecting suitable testing methods such as LFAs or ELISAs,[58] depending on the expected prevalence in the population. Beyond technical aspects of the accuracy of antibody testing, biological factors also affect the reported population-scale prevalence. The above considerations rely on the assumption that every infected individual mounts a detectable antibody response after infection (seroconversion). A complete lack of seroconversion or rapid loss of detectable antibody responses would lead to underestimation of the total prevalence. In a recent study,[59] seroconversion was reported in nearly all (621 of 624) individuals with confirmed SARS-CoV-2 infection. Seroconversion in individuals with suspected SARS-CoV-2 infection showed a lower rate (37% positive) and could be due to lack of actual infection or seroconversion. A small-scale study[60] has reported lack of seroconversion in some individuals likely exposed to SARS-CoV-2, whereas T cell responses (non-antibody-mediated cellular immunity) were readily detected. However, it is unclear how frequent such lack of seroconversion occurs and to which extend population-scale profiling may be affected.

In addition to the lack of seroconversion after SARS-CoV-2 infection, the temporal dynamics of antibody responses and cross-reactivity (as discussed in the previous section) can affect the outcome of population-scale testing. Quickly waning antibody responses after infection could also lead to underestimation of population-wide prevalence, especially when an extended period of time has passed after infection until serological testing.

PROSPECTS AND LIMITATIONS OF ONGOING COVID-19 ANTIBODY TESTING

Although numerous large-scale qRT-PCR testing efforts for SARS-CoV-2 are being conducted globally, resulting in millions of tests being carried out,[16] relatively fewer completed antibody testing studies have been reported as of December 2020. This fact may be due to the limited availability of antibody testing

kits as well as uncertainty regarding their reliability during early stages of the pandemic. With commercial product development making a multitude of testing kits available[43] and priorities shifting from immediate containment toward gaining insights into the epidemiology of COVID-19 and SARS-CoV-2 infection,[20] many antibody testing studies have been started globally. Although a few serology studies of individuals with COVID-19 have already been published in peer-reviewed journals,[23,61–63] several studies investigating population-wide spread, including asymptomatic individuals, are still ongoing and only available as preprints, press releases, or newspaper articles. Hence, details regarding the exact antibody tests and selection criteria are only partially available. Several of these studies are summarized in Table 2, reporting SARS-CoV-2 prevalence to be approximately between 1.5% and 20%.

This more than 10-fold range for the estimated spread of COVID-19 may stem not only from true differences in prevalence between regions but also from the studies' selection criteria as well as testing accuracy. Some studies have focused on risk populations, whereas others aimed to randomly selected individuals. For example, a Swedish study reporting approximately 20% positive individuals by antibody testing[65] had focused on hospital workers, who inherently were at a higher risk of being in contact with individuals with COVID-19. Similarly, a German study reporting 14.1% positives was conducted in one of the most affected municipalities (a "super-spreading event" because of carnival festivities in mid-February 2020).[64] Test results from New York City, one of the COVID-19 hotspots in the United States, reported 12.3% positive individuals by antibody testing.[75,76] A testing effort in Chelsea near Boston even reported 32% positive antibody tests of random volunteers.[72] In addition to a relatively small sample size of approximately 200 participants (Table 2), it also appears plausible that individuals who believe they may have been infected were more likely to volunteer for testing and that these numbers may represent an overestimation. However, a study in Idaho (a region affected relatively mildly by COVID-19 according to qRT-PCR data), asking for volunteers suspecting that they had contracted COVID-19, reported only 2.0% positive antibody tests.[70] These large differences are probably due to a true difference in prevalence between these locations and a possible bias of tests with different characteristics.

Systematically designed studies were also carried out, aiming to randomly test a representative fraction of the population. For example, a Swiss study derived a representative random sample of the population from annual health examination surveys.[62] The ratio of positive antibody tests increased over 5 weeks from 4.8% to 10.8%, following the trend of the estimated prevalence from qRT-PCR in this region during the testing period. Although the selection criteria appear to be random, individuals from the same household were overrepresented because contacted participants were asked to bring them along for testing. Hence, there may be a cluster effect of households affecting these results. A Finnish study has taken a different approach for random antibody testing by leveraging blood samples taken in hospitals from individuals who had undergone laboratory tests for various reasons other than infection, reporting 3.4% positive antibody tests.[68] Although, in

this study, two different tests were used, potentially yielding high accuracy, in general, as outlined above, testing reports of low prevalence may be overestimated because of limited specificity of antibody tests. An ongoing large-scale study in Spain could shed light on this issue, comparing the results of rapid LFAs with immunoassays with high accuracy.[69,77] A large-scale study of 30,576 individuals from Iceland also employed six different antibody tests, scoring positive when two specific tests passed.[63]

In the United States, several random testing efforts have been carried out, with a study in Indianapolis reporting 2.8% positive tests for SARS-CoV-2 in a mixed sample of randomly selected individuals and volunteers.[73] Some efforts, such as this study in Indianapolis, compared antibody results with qRT-PCR testing, reporting that 1.7% tested positive in qRT-PCR for the presence of virus and another 1.1% for antibodies. Given the high specificity of the antibody test used in this study (Abbott Laboratories), these results appear to be reliable despite the low prevalence in the population (although potential inaccuracies in qRT-PCR testing could affect the overall numbers). The ratio of individuals testing positive for qRT-PCR to the individuals testing positive for antibodies also matches existing estimates of a SARS-CoV-2 clearance time of 3–4 weeks from symptom onset[18] and the known course of the epidemic in Indianapolis.

Although not all of these studies have focused on determining IFRs, most reports of the IFR of COVID-19 range from approximately 0.5%–1%.[21,78–80] Given that the IFR is affected by risk factors such as age or obesity, different population characteristics are expected to yield regionally varying IFRs. Furthermore, similar to estimating the prevalence in the population, testing accuracy and selection criteria also affect IFR calculations. Because of these uncertainties, it will be important to derive improved IFR estimates from ongoing random, large-scale antibody testing efforts.

Although each of these antibody testing efforts has generated valuable insights for the specific cohorts tested, caution should be applied when comparing the inferred COVID-19 prevalence between studies. Testing volunteers from risk areas with high positive qRT-PCR test numbers and population-scale random testing are important and can guide decisions such as the duration of lockdown for a specific area or nationwide measures such as opening schools and businesses.

MONITORING DEVELOPMENT OF HERD IMMUNITY BY ANTIBODY TESTING

If a sufficiently large part of the population has developed immunity against an infectious disease, then new infections cannot spread rapidly. Because of this reduced spread, individuals who have not yet developed immunity are also protected.[81] This concept is called herd immunity, and the necessary fraction of immune individuals depends on the infectivity of a disease (related to the basic reproduction number [R_0]).[82,83] For example, highly infectious measles requires more than 90% immune individuals to achieve herd immunity.[46,84] For SARS-CoV-2, the exact threshold to achieve herd immunity is unclear,[85] and estimates are complicated by differences between countries, with most estimations between 50% and 80%.[86] Currently reported

Table 2. Representative SARS-CoV-2 antibody testing efforts underway globally as of October 2020

Country	Individuals tested	Positive rate	Selection criteria of participants	Description	References
Germany	919	14.1%	risk populations	random individuals from the municipality of Gangelt, one of the most COVID-19-affected sites in Germany	Streeck et al.[64]
Sweden	527	~20%	risk populations	"community" study of Swedish hospital workers	Danderyds Sjukhus[65]
Austria	269	4.71%	risk populations	individuals from 27 risk municipalities with increased COVID-19 infection numbers	Statistik Austria[66]
China	17,368	3.2 to 3.8%	risk populations	individuals from hospital (non-COVID-10 individuals, healthcare workers, and relatives) and community settings (residents, hotel/factory workers) from Wuhan and adjacent cities	Xu et al.[67]
Switzerland	2,776 over 5 weeks	4.8%–10.8% over 5 weeks	Random	participants were selected from a representative sample of the general population of Geneva (derived from an annual health examination survey); shared household members are overrepresented	Stringhini et al.[62]
Finland	442	3.4%	Random	from blood samples of individuals who had undergone laboratory tests for various reasons other than infection; area of Helsinki (Uusimaa Hospital District, HUS)	Finnish Insitute for Health and Welfare[68]
Brazil	3 rounds of testing 4,141–4,500 individuals	increasing from 0.048%–0.222% (April to May)	Random	probability sample household surveys in nine large municipalities in the south of Brazil, included testing of multiple family members	Silveira et al.[61]
Iceland	30,576	estimated at 0.9%	different criteria for subgroups	includes a longitudinal assessment of antibody responses 4 months after infection	Gudbjartsson et al.[63]
Spain	60,983	~5%	Random	ongoing study of random households, current results of rapid tests, measurements by immunoassays ongoing, reporting on a reliability study of lower accuracy of rapid tests than the manufacturer's specifications	ESTUDIO ENE-COVID19: PRIMERA RONDA[69]
USA, Idaho	15,789	2.02%	semi-random	ongoing testing effort of volunteers who suspect they have contracted COVID-19; results as of June 14, 2020; non-profit initiative "Crush the Curve Idaho"; testing through the University of Washington Virology Department	Bryan et al.[70]
USA, Boston, MA	750	9.9%	semi-random	testing of volunteers, emphasis on asymptomatic individuals (residents having previously tested positive in a qRT-PCR test and/or symptoms were excluded)	City of Boston Public Health Commission[71]

Table 2. *Continued*

Country	Individuals tested	Positive rate	Selection criteria of participants	Description	References
USA, Chelsea, MA	~200	32%	semi-random	random volunteers, carried out by Massachusetts General Hospital	Corcoran[72]
USA, Indianapolis, IN	>4,600	2.8% antibody, 1.7% qRT-PCR	random to semi-random	randomly selected individuals and volunteers tested with qRT-qPCR and antibody tests, carried out by Indiana University Richard M. Fairbanks School of Public Health, ongoing,	[73]
USA, Los Angeles, CA	846	4.1%	Random	University of Southern California and Los Angeles County Department of Public Health, drive-through antibody testing of participants recruited via a market research firm's database representative of the county's population	University of Southern California and County of Los Angeles[74]
US, New York, NY	~15,000	12.3%	N/A	testing efforts by the Wadsworth Center, the public health laboratory of the New York State Department of Health	NY State COVID-19 Testing[75]

positive rates in antibody testing (Table 2) are well below estimated thresholds required to achieve herd immunity, even in the epicenters of the outbreak.[86]

Furthermore, there are several caveats requiring careful consideration when leveraging antibody testing results for assessing herd immunity.[46,56] A positive result of an antibody test (even leaving testing inaccuracies aside), does not necessarily indicate protection against reinfection of an individual. The produced antibodies may not neutralize the virus, and the necessary magnitude of the immune response is also unclear (i.e., very low antibody concentrations detected by sensitive tests may not be sufficient for viral clearance of a reinfection). A recent study detected antibodies in 99% of 1,343 convalescent COVID-19 individuals,[59] and other work reported variable antibody titers between infected individuals.[87] In a study of convalescent humans, 13 of 14 individuals produced detectable amounts of NAbs in the weeks following infection,[88] and an in-depth study confirmed convergent NAb responses.[37] Testing the quality of human antibody responses longitudinally by neutralization assays on a larger scale could validate their protective potential. Animal models of COVID-19 may also provide insights into the protective nature of immune responses against SARS-CoV-2 reinfection. In recent studies, rhesus macaques were protected against reinfection, although a rather short time period of approximately 1 month had passed after initial infection, and fewer than 10 monkeys were challenged per study.[89,90]

Vaccines are key accelerators to protect individuals as well as to achieve herd immunity, and various efforts are ongoing to develop vaccines against SARS-CoV-2.[91,92] However, the protection provided by vaccines as well as immunity from natural infection depends on the duration of immune responses against SARS-CoV-2. Therefore, it will be critical to monitor recovered individuals (and vaccinated individuals when vaccines become

available) over the next years. Recent studies indicate that antibody responses against SARS-CoV-2 can become undetectable after few weeks,[24] but despite waning antibody titers, long-lived plasma cells and memory B cells may provide protection from reinfection. If immune responses against SARS-CoV-2 should wane over 2–3 years, as observed for other CoVs,[19,93–101] a large part of the population would need to be vaccinated annually or biennially to achieve herd immunity. Given the relatively low mutation rates of CoVs, it might not be necessary to develop yearly new vaccinations against SARS-CoV-2 strains (as necessary for influenza). However, considering the large animal reservoir of CoVs and their amenability to spill over to human hosts,[34] and recombine,[102] new pathogenic CoVs may emerge and require development of new vaccines.

OUTLOOK

Serological testing of individuals with COVID-19 has yielded important insights into the adaptive immune responses elicited by SARS-CoV-2. Ongoing systematic comparisons of different commercially available immunoassays suggests that ELISAs/CLIAs generally provide sufficient accuracy for population-scale testing at current prevalence (although some ELISAs/CLIAs also lack sufficient sensitivity/specificity). Point-of-care rapid LFAs could still benefit from improvements (depending on the application).[52,54,55] Some LFAs have displayed excellent specificity, although these may not have been widely applicable, given the large availability of LFAs with varying accuracy, especially early during the pandemic. Laboratory ELISAs/CLIAs currently appear to be the method of choice for monitoring the population-wide spread in a post-lockdown world. Although this approach is viable in high-income countries with the required molecular diagnostic infrastructure in place, carrying out such testing efforts in

low- and middle-income country may be challenging. Therefore, improving low-cost rapid diagnostic kits will be critical and could be achieved by testing for multiple antigens and isotypes (including IgA) in a single assay or combining different low-cost tests with independent systematic biases to reduce the overall error rates of LFAs. LFAs also have the key advantage of enabling self-testing, which is highly relevant in any country imposing restrictions on individual movement to contain case numbers. In any setting, the applied serological tests should be validated thoroughly and independently for the situation in which they will be employed.

Even leaving possible testing inaccuracies aside, estimated thresholds for herd immunity have not been achieved in any region of the world; thus, measures to prevent the spread of COVID-19 should continue. Given the relatively short time of the current outbreak, longitudinal studies will need to assess long-term antibody responses of recovered individuals. When vaccines have been deployed broadly, analogous studies will need to assess the duration of vaccine protection against SARS-CoV-2 reinfection. The first large-scale studies of thousands of random individuals have deepened our understanding of the true prevalence of COVID-19, and these efforts will narrow down the IFR as a key metric for the severity of COVID-19. A large denominator identified by serology testing (i.e., many infected people who have developed antibodies without diagnosis during the acute phase) could reveal that SARS-CoV-2 is less severe than estimated from qRT-PCR testing. These findings will increase our understanding of the transmission dynamics of SARS-CoV-2, will help to improve modeling efforts, and can therefore guide preparations for possible future outbreaks.

AUTHOR CONTRIBUTIONS

T.V., S.L., and E.S. conceived the topics and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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