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Commentary

COVID-19: Understanding the science of antibody testing and lessons from the HIV epidemic

Stanley H. Weiss [a,b,*], Gary P. Wormser [c]

[a] Department of Medicine, Rutgers New Jersey Medical School, Newark, NJ, U.S.A.
[b] Department of Biostatistics and Epidemiology, Rutgers School of Public Health, Piscataway, NJ, U.S.A.
[c] Division of Infectious Diseases, Department of Medicine, New York Medical College, Valhalla, NY, U.S.A.

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ABSTRACT

Potential pitfalls in the development, deployment and interpretation of antibody tests for COVID-19 are discussed. Lessons learned from the experience with the introduction of antibody tests for HIV are highlighted. Each test will need to be separately vetted for performance and clinical implementation based upon rigorous clinical trial data. The issues we highlight will also be similarly important for vaccine and therapeutic drug efficacy trials.

The social distancing imposed to reduce the spread of SARS-CoV-2 has disrupted the economy and our personal lives. It has been proposed that performing laboratory tests for SARS-CoV-2 antibody can assist in determining who is eligible to return to the workforce. Other proposed uses include helping to guide the relaxation of physical/social distancing and revision of lockdown orders. Antibody testing has the potential to more accurately determine the total number of people who have been infected with SARS-CoV-2, compared with reported cases. There are, however, a number of potential pitfalls in the deployment and interpretation of antibody tests, and in the vetting of proposed tests. It is important to differentiate two key proposed uses: identify past infection to track the pandemic, and identify possible immune protection that developed from either natural infection or from a future vaccine.

Persons who have recovered from SARS-CoV-2 infection develop antibodies to the virus. It is hoped that the presence of antibodies will be protective against reacquiring infection and might be used therapeutically. However, development of antibodies *per se* does not establish that immunity to reinfection exists (NIH, 2020). Indeed, development of antibodies to SARS-CoV-2 might have undesirable consequences. Antibody-dependent enhancement (ADE) of viral entry has been observed for coronavirus and other viruses (Buchbinder et al., 2008). With dengue, ADE can worsen the course of infection,(Jiang et al., 2020) a phenomenon which necessitated changes to the recommendations on the use of a dengue vaccine. ADE may, or may not, be pertinent to SARS-CoV-2 vaccine development.

Clinical trials to develop a vaccine to prevent SARS-CoV-2infections must be designed to evaluate both efficacy and harm. The initial planning by NIH of large, blinded, randomized, multi-center HIV-1 vaccine trials was focused just on efficacy (Katzelnick et al., 2017). The deployed large vaccine trials, however, included 2-tailed hypothesis testing, since harm was acknowledged as possible, which necessitated an expansion in the number of clinical sites compared to the requirements of just an efficacy trial. An HIV vaccine trial was stopped in 2007 by its data safety monitoring board when a statistically significant difference was found in the rates of HIV acquisition (NIAID News Release, 2020). Unfortunately, on unblinding it became clear that those who received the HIV vaccine were at increased risk for HIV infection (NIAID News Release, 2020). An HIV vaccine trial of 5407 HIV negative volunteers from 14 sites (begun back in 2006) was stopped in 2020, as it showed no efficacy (Vermund et al., 1992). These efforts demonstrate how long and difficult the road to vaccine development can sometimes be, despite being given high priority.

Recent attention has focused on the possible use of antibody testing as a tool to classify immune status to assist in returning of persons to the work place and in the determination of whether an individual might be released from quarantine. A key assumption is that the circulating virus will not mutate in a way to permit reinfection. Of course, it should be noted that the appearance of antibodies *per se* in a recently infected person does not mean *prima facie* that this person is no longer infectious. By analogy, studies have shown

* Corresponding author. Tel.: +1-973-972-4623; fax: +1-973-972-6556.
E-mail address: weiss@rutgers.edu (S.H. Weiss).

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that in some persons, whose nasopharyngeal swab testing has become negative for SARS-CoV-2 RNA by RT-PCR, still test positive on lung secretions. Press conference reports from the South Korea CDC claim reversions from negative to positive on swabs and in blood. There is a need for quantitative tests for RNA, as such reversions might simply reflect variability of results near the lower detection limits of the test, or erroneous results. Interpretations must be cautious, since the detection of viral RNA *per se* does not mean that infectious viral particles are present; re-infection or reactivation should not be presumed.

In regard to developing a laboratory test for the presence of antibodies to SARS-CoV-2, the risks associated with incorrect test results, or with an incorrect interpretation of a test result, differ depending upon the proposed uses. Antibody testing can be devised to selectively detect IgG, IgM or IgA antibody responses alone or in combination. The level and/or type of humoral response that detects exposure to SARS-CoV-2 is likely different from test results that would indicate immunity to reinfection. The sensitivity and specificity of antibody testing are two-sides of a coin, and higher values for one of these measures typically reduces the other (Wan et al., 2020). To illustrate these concerns, several case scenarios are discussed.

1) For a person known to have recovered from proven SARS-CoV-2 infection (e.g. diagnosis was based on a positive RT-PCR), there will be a high probability for the antibody test to become positive. In this scenario, the predictive value of a positive test will be high (Wan et al., 2020; Weiss & Cowan, 2004). The predictive value of a negative test, however, may be limited (Wan et al., 2020). Once carefully assessed trials provide better guidance on the time course for development of various types of detectable antibody responses, the roles of particular assays can be more clearly defined.
2) Antibody testing for a currently asymptomatic person deemed to have had exposure to a person with documented SARS-CoV-2 infection or likely disease, is anticipated to be one of the most important uses. Current epidemiologic data suggest that many persons who become infected only have mild symptoms, and thus may never have been tested by RT-PCR while actively carrying the virus. If the predictive value of a positive antibody test is high, and if data are accrued consistent with the hypothesis that antibody positivity confers protection, such testing may be very useful in deciding that it is safe for that individual to return to employment at a job site – i.e. this person is likely not to be at risk for either transmitting or acquiring infection. This would be especially relevant for situations with a continuing high risk of potential exposure to SARS-CoV-2, such as for health care workers, first responders, or other workers (such as retail clerks) who have many inter-personal contacts in their job. The risk of a false positive test – signifying protection when none exists – would potentially place such a person at risk of becoming infected with SARS-CoV-2, so false positives need to be minimized.

High test specificity is especially important for those persons who are deemed to be at particular risk for morbidity or mortality from COVID-19 disease, such as persons with certain underlying diseases or those who are older. If such individuals test antibody negative and were to return to work before an effective vaccine has been widely deployed, social distancing will still need to be encouraged even at modest SARS-CoV-2 incidence rates, with nuanced policies that will vary geographically and temporally.

With neither gold nor criterion tests available,(Wan et al., 2020) and significant variability in current models as to the frequency of infection that has occurred in different population groups, it is not currently possible to accurately estimate the pre-test probabilities. As data evolve, reasonable estimates about local prevalence of SARS-CoV-2 infection will help guide the interpretation of positive results (Table 1).

Even for a test with 100% sensitivity and 99% specificity, if the true antibody prevalence were 1%, one-half of all positive results would actually be false positives. If infection prevalence is 10% but sensitivity and specificity are both 90%, again just one-half of all positive results will be true positives. The predictive value of a positive result is reduced for lower test specificity. In low prevalence areas, which is the expectation for the vast majority of U.S. regions as of May 1, 2020, the positive predictive value will be quite low.

Table 1
Displayed are the predictive values of a positive test result, assuming maximal (100%) test sensitivity. (Real world sensitivity will be less, reducing the predictive value from that shown.) A range of test specificity values are in the first column. A range of pre-test probabilities, representing the estimated population prevalence, head the remaining columns.

Specificity	Prevalence in population to be tested (the pre-test probability)					
	1 in 5 (20%)	1 in 10 (10%)	1 in 20 (5%)	1 in 100 (1%)	1 in 1000 (0.1%)	1 in 10,000 (0.01%)
90.0%	71.4%	62.6%	34.5%	9.2%	1.0%	0.1%
95.0%	83.3%	69.0%	51.3%	16.8%	2.0%	0.2%
98.0%	92.6%	84.7%	72.5%	33.6%	4.8%	0.5%
99.0%	96.2%	91.7%	84.0%	50.3%	9.1%	1.0%
99.9%	99.6%	99.1%	98.1%	91.0%	50.0%	9.1%

The FDA needs to develop clear guidelines for the assessment of SARS-CoV-2 antibody tests. There were lessons learned early on in the HIV epidemic, which helped the FDA in its assessment of proposed tests by vendors and implementation by the blood banking industry and later in clinical testing. This included:

1) Assessment of possible causes for false positivity (Wan et al., 2020; Weiss & Cowan, 2004; Weiss & Goedert, 1985; Weiss et al., 1985a). Since coronaviruses other than SARS-CoV-2 have been circulating for a long time, infection from some of these might potentially lead to positive antibody tests for SARS-CoV-2.
2) Every aspect of the manufacturing process needs to be carefully detailed by vendors so that the test can be assessed for situations whereby cross-reactivity might develop. For the early HIV tests, virus was grown in cell culture, and remnants of those cells might have existed. The first systematic assessment of an HIV antibody screening test found false positive reactivity in some samples with high HLA reactivity(Weiss & Cowan, 2004); this was specifically related to persons who had antibodies only to the HLA of the cell line in which the virus was cultured (Weiss et al., 1985a).
3) Reactivity may vary with the time since onset of infection, the type of illness that developed or with the severity of the COVID-19 infection (Wan et al., 2020; Weiss & Goedert, 1985).

In conclusion, a combination of antigen and antibody testing might be used routinely as part of vigorous contact tracing of newly identified cases with an amplified public health workforce. The first step of the U.S. government should be to ramp up efforts to produce accurate and reliable point-of-care antigen tests (Weiss et al., 1985b). Antibody tests have the potential for providing valuable information on whether an individual was previously infected with SARS-CoV-2, which is important for surveillance, and potentially for determining whether the person is immune to reinfection, which may assist in decisions about whether that individual may return to the workforce when new COVID-19 cases are still occurring. Lessons learned from the development of antibody tests for other infections, such as HIV, should be useful in designing the appropriate test validations that are required.

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