The following considerations originate from the WUSM Student COVID-19 Recovery Think Tank. They are based on data reviewed up to April 20, 2020 and thus subject to change. These considerations do not necessarily reflect the intuitional policies or opinions of Washington University School of Medicine.

Considerations on Serological Testing (Part I of II)

As recommended by the CDC, the current standard for diagnosing COVID-19 and determining clearance of infection has been through negative PCR-based tests looking at viral RNA (1) (2). Serological testing has been proposed as an alternative to identify individuals with COVID-19 in both Europe and the United States (3) (4) (5), and there are many efforts towards developing valid tests. What are some of the scientific considerations in adopting a serological test for COVID-19? We offer some initial cases to consider as well as a detailed background description.

Serological testing to demonstrate short-term immunity

There is much news on use of serological testing as "antibody certificates" to allow people to return to work. A seropositive result is expected to signify that the body has mounted an acute immune response against the virus. Initial studies, looking mostly at COVID-19 patients who were not in acute distress, suggest a large majority of patients develop a serologic response (6) (7). There have been few reports regarding reinfection in COVID-19 patients, which can be interpreted to suggest a low reinfection rate. One initial report noted a few cases (8), but importantly, these patients remained asymptomatic with a negative PCR test at discharge but a positive PCR test afterwards, which could suggest a false negative result at discharge. There have been reports of a "second wave" of coronavirus infections in China, but the article describes this as a fear that foreign travelers could serve as more sources of initial infection rather than reinfection (9). Taken together, these preliminary findings do not exclude a role of short-term immunity after a patient acquires COVID-19.

Serological testing for diagnosis or monitoring disease course

We do not propose serological testing to be used for initial diagnosis of COVID-19 or for monitoring clinical course of disease. Serological conversion is expected to occur once the body has mounted an immune system response to fight off the infection, which suggests it would occur late in the disease course.

Serological testing to suggest a patient is no longer infectious

A positive serological test does not necessarily imply that a person is no longer infectious to others. One small study looking at nine COVID-19 patients measured time-to-serological conversion and estimated infectivity of the patients by both viral culture and PCR (6). Estimates of infectivity by viral culture and PCR are known to vary (as covered further in the background section), but the general hope is that by the time a patient develops antibodies (IgG) against the infection, they will shortly clear all live virus. However, when considering several other viruses, serological testing may not be able to

distinguish between active or convalescent phase infections, which may explain why it has not been used to guide clinical management. As such, we suggest caution in using serological testing as a sole test to determine if someone is no longer infectious.

Serological testing to demonstrate long-term immunity

There are many factors contributing to the host immune response against viral infections, as reviewed in (10). In general, mucosal infections (respiratory or GI tract) seem to invoke less of a memory response, leading to more recurrent infections (11). Repeated exposures are not expected to affect immunological memory status, but, as seen with booster shots, it might select for a higher affinity antibody (12). For coronavirus specifically, a small study looking at a model strain of coronavirus found that antibody titers were detectable one year later, and though they did not prevent reinfection, the reinfection had a shorter duration of cold (mean 5-6 days of symptoms as compared to 20 days on initial infection) (13). Although little data is available for SARS-CoV2, looking at other viruses, a study looking at serological testing of H1N1 in healthcare workers of Thailand found that ~40% were positive a year after the infection (14). Similar serological testing rates one year after infection can be seen in Ebola (15) (16).

A prospective study looking at humoral immunity in 56 SARS patients found that neutralizing antibody titers began to fall around 16 months after seroconversion, and by 24 months, the titer was undetectable in ~11.5% of patients (17). In a separate prospective cohort, titers were undetectable in 50% of patients at 3 yr (18). Another longitudinal cohort of 23 patients, at 6 yr post-infection, titers were undetectable in 21 of 23 patients (~91%) (19). For MERS, one small cohort study found that at one year post-infection, most patients who had severe disease maintained an immune response, whereas those with mild disease did not have detectable titers (20). Interestingly, a screen in Qatar conducted on over 4,800 samples (many of whom were healthy donors) found anti-MERS antibodies in about 6% of samples (21).

Background

Serological conversion in SARS-CoV2

Three considerations to make regarding serologic conversion towards a pathogen include [a] what percentage of patients will respond, [b] how long does it take to generate a response, and [c] is the response effective. One small study in Germany looking at 9 COVID-19 patients with mild symptoms found that about 50% were seropositive after 7 days, with all 9 testing seropositive after 14 days (6). Notably, the last detected positive viral culture for any patient was day 8, but viral RNA could persist for up to 28 days, with different duration depending on if the sample was sputum, stool, or nasopharyngeal swab. A preprint study in China for 80 patients found acute serologic conversion in all patients after exposure (7). Though seroconversion heralded a decrease in viral load (viral RNA), there are still detectable amounts of viral RNA for quite some time (e.g. weeks) after seroconversion. The trend in viral RNA has been seen in larger cohorts (22), where peak viral load may be highest from two days

before onset of symptoms to one day after (23). Another cohort for 173 patients, including 32 in critical condition, reported conversions rates of any antibody, IgM, and IgG as 93.1%, 82.7%, and 64.7%, with median seroconversion time of 11, 12, and 14 days after onset of symptoms (24). One preprint study looking at 222 patients has found that greater serological titers may be associated with worse disease (25). Though unusual, antibody-dependent enhancement of viral infections has been reported in other viruses, including MERS-CoV, as proposed in this review (26). It is unclear how many people in the general public might test serologically positive (e.g. have had history of exposure but were asymptomatic). One preprint estimate in Santa Clara suggested that of the 3,330 participants tested, ~1.5% were tested positive, which, depending on sampling corrections, might reflect an upper limit of ~5% of the general public testing positive (27).

Acquiring samples and conducting the test

The current standard COVID-19 test involves collecting a sample, usually by nasopharyngeal swab, extracting viral RNA, and conducting RT-PCR. Sample collection involving disrupting the airway can increase risk of exposure to providers, especially with aerosol-generating procedures such as bronchoalveolar lavage or sputum cultures (28), though for certain viruses, nasopharyngeal swabs may carry risk as well (29).

There can also be much variability in test effectiveness when collecting nasopharyngeal swabs because technique of sample collection can make a difference (30). To illustrate this, respiratory viral panels are also drawn from nasopharyngeal swabs and have varying sensitivity and specificity (31). A single flu swab test has estimated sensitivity of 22%-80% (32) (33). As such, clinical suspicion can guide interpretation of if a negative test result is a true negative or false negative, as seen for influenza (34). Looking at SARS-CoV1, there is at least one estimate that NP swabs had a sensitivity of ~60-70% (35). Although data are limited for SARS-CoV2, it is likely that sensitivity of an individual NP swab is similar (estimates at ~70% (36) (37)). A case report found a symptomatic patient who was first diagnosed with COVID-19 on day 5 of admission, with four prior negative PCR results (38), so there can be false negatives.

In comparison, a serological test involves collecting a blood sample and testing for the presence of certain antibodies (or antigens). Drawing blood from a fingerstick or by peripheral I.V provides less risk to the provider for exposure to what is presumed to be a respiratory virus. Additionally, we expect test results to be less affected by sample quality than NP swabs (if there is antibody production, it likely circulates through all blood vessels).

Though bottlenecks for testing via NP swabs have been reported (39), it is important to note that some bottlenecks likely are present for serological testing as well. Both viral RNA and antibodies can be denatured with temperature, suggesting both need to be kept cool before analysis. Given that these are patient samples, both viral RNA detection and a serological test from human blood likely requires Biosafety level 2 or greater certification (40).

Challenges in validating serological tests

One challenge in the development of validated serological tests is cross-reactivity of antibodies to other similar viruses (as reviewed for testing of SARS-CoV1 (41)). Notably, coronavirus is expected to cause the common cold and can be found on regular respiratory viral panels (42). Cross-reactivity is not necessarily reported in the development of these assays, though it can be extrapolated from test results conducted on cohorts without COVID-19. Another challenge in validating serological tests is determining if the detected antibodies are neutralizing antibodies; after all, ELISA only detects presence of binding antibodies to the provided antigen. There is at least one study evaluating multiple test kits with varying sensitivity and specificity (43). One great resource for status of serology-test development can be found here (44)**.** Another opinion piece highlights just how many variables are necessary to consider in validating a serological test (45).

Viral DNA vs live viral culture

Before evaluating where serological testing can be used, it is important to clarify what assays are used in primary literature. To determine if a person with a viral infection is infectious towards others, ideally, we would track how much infectious virion (viral genetic material + associated capsid/packaging proteins) is present and how much of this virion is necessary to infect someone else. Two common options to estimate this are viral DNA/RNA and viral culture. Although viral RNA/DNA is relatively straightforward to identify via PCR, the presence of viral RNA or DNA does not necessarily imply the presence of infectious material (for instance, that DNA/RNA may not be associated with any viral proteins). In fact, some experimental COVID-19 vaccines are based on viral mRNA (46). As such, viral DNA/RNA is typically interpreted as history of infection, which may not have culminated in disease.

Viral culture involves inoculation of an isolate into cell lines *in vitro* or certain models *in vivo* (e.g. chicken eggs for influenza (47). As these represent models for transmission or growth of a virus in human patients, there may be differences between the models and true infectivity. It is unclear how an unoptimized culture condition affects the results of a viral culture assay; one possibility is that it might underestimate the ID50. Certain pathogens can only be grown under specific culture conditions, with aerobic/anaerobic cultures as one example (48). More practically for clinical management, viral cultures may take longer to complete as compared to PCR, ranging from several days to weeks. Thus, with respiratory syncytial virus (RSV) in pediatric patients as an example, even though viral culture is the "true standard" for determining an infection, viral DNA/RNA and the presence of clinical symptoms may represent sufficient evidence to guide clinical management (49).

Significance of viral DNA/RNA in asymptomatic individuals.

Because the presence of viral RNA or DNA does not necessarily imply infectivity, for either the host or to others, the significance of a positive RNA or DNA test in an asymptomatic individual is unclear. For instance, one study conducting random respiratory viral panel PCR tests on healthy adults found that 6% tested positive for at least one of the viruses despite remaining asymptomatic (50), and another one conducted on tourists in NY found similar results (51). Rates of these positive tests in asymptomatic children may be even higher (52). Notably, the significance of these results depends on how infectious the virus is and rates of asymptomatic transmission. SARS-CoV2 is expected to have high asymptomatic

transmission. Depending on the cohort, the percentage of asymptomatic carriers can be quite high; for example, 18% of the passengers on the Diamond Princess were positive but asymptomatic (53). More recently, a universal screen for all expectant patients presenting for delivery found 13.7% were asymptomatic but tested positive (54).

How is serological status used for diagnosis or management of other viral infections?

Every virus can represent a distinct clinical challenge. Nevertheless, it can be helpful to look at the role serological status plays across other viral infections and to clarify what advantages and disadvantages they have.

For many respiratory viral infections, clinical management may be based more on symptoms rather than test results. For instance, up to 42% of sepsis patients do not have a culture result, suggesting viral origin, yet if they resolve their symptoms and are otherwise in good health they can be discharged (55). Part of this decision may be that most available interventions help mitigate symptoms (56). In some cases clinical suspicion drives interpretation of test results. For example, for influenza, a negative test may be interpreted as a "false negative" if it is flu season and suspicion is high but as a "true negative" if it is not flu season and suspicion is low (57). If tests in general are not often used in management of these infections, it should not be surprising that serological testing is not commonly used in this setting.

Additionally, for respiratory syncytial virus, serological testing is not preferred for diagnosis or management because the formation of stable antibodies may mask the recurrence of an infection. This concern extends to mothers, whose anti-RSV antibodies can be found in children [uptodate]. A similar concern is seen with adenovirus, where there is a high prevalence of anti-adenovirus antibodies in the general population (possibly from prior infections) (58). Also, there can be significant cross-reactivity of antibodies to different strains of adenovirus (59). For general management of influenza, serological tests do not distinguish flu in the acute phase versus the convalescent phase of infection, but it may be very useful for research (57).

Serological tests may have a different role for chronic infections as compared to acute infections. Looking at chronic infections, such as in hepatitis B, there can be a high number of patients with quantifiable viral DNA regardless of their surface antigen, core antigen, or serological status (60). Another example of a chronic viral infection is HIV, where formal diagnostic testing is often conducted by serological or antigen testing. Viral PCR is only approved as diagnostic under certain circumstances (61), but it, along with CD4 count, are often used for clinical monitoring (62).

It is possible that serological testing plays different roles in pandemic-level infections as compared to traditional infections. For influenza H1N1, there are some retrospective data suggesting that, in ICU patients, serological testing might have been more accurate than nucleic acid tests for diagnosis of symptomatic patients (63). For measles, serological testing, viral RNA detection, or viral culture are all accepted as diagnostic tests depending on the resources available (64). Interestingly, none of these tests are used for active monitoring of measles, and instead, infection control is achieved by self-isolation for 4 days.

SARS-CoV2 belongs to the coronavirus family, which has led to several epidemic level infections in recent years. For SARS-CoV1, serological testing was thought to be sensitive, but seroconversion occurred on average at day 19 or 20, making it less useful for acute phase diagnosis (65) (66). However, serological testing could be used, in conjunction with evidence of viral clearance (PCR), to rule someone had cleared an infection (67). In this context, one estimate of serological test predictivity of viral clearance suggested it was quite high at ~98% (68). Notably, there were reports of cross-reactivity among coronavirus strains (65). Serological testing was also considered for MERS-CoV , and at one point the WHO suggested that a patient with a positive serological test but no PCR testing should be considered a probable case (69). Seroconversion rates were high (>90%) within 2 weeks of diagnosis, corresponding to within 3 weeks of symptom onset (69)

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