In the Know

Current State of COVID Testing

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With the outbreak of the SARS-CoV-2 virus in the Hubei province of China in late 2019, there was an immediate need for accurate testing for a novel virus on an unprecedented scale. Diagnostics play an incredibly important role in the containment of any contagious outbreak, as the identification of infected individuals allows isolation to prevent spread of the disease as well as permits contact tracing to identify other potentially infected individuals.

Because there were no diagnostics available at the time, the characteristic ground glass opacities noted in the CT scans of infected patients were initially utilized for diagnosis. With the isolation of SARS-CoV-2 as the causative agent for this disease, a flurry of activity ensued, resulting in a number of studies with the aim of developing effective and mass-producible diagnostics for that virus. A recent review by Udugama, Chan et al. highlighted some of the technologies and the performance characteristics of the diagnostics developed for the surveillance of SARS-CoV-2 (*ACS Nano* 2020;14:3822-35).

Selectivity vs. specificity in testing

Within a binary test framework (i.e., positive or negative test result, Figure 1), there are four possible outcomes of that test: a true positive (TP), where the test accurately identifies a positive result (test correctly detects disease in an individual with that disease); true negative (TN), where a test correctly identifies a negative test result (test shows no disease in an uninfected individual); false positive (FP), where a test produces a positive result for what is in actuality a negative (test incorrectly shows a positive result for an uninfected individual); false negative (FN), where the test shows a negative result for what is an actual positive (test affords a negative result for a disease in an infected individual).

Two particular metrics utilized to gauge the effectiveness of a binary result testing method are *sensitivity*, sometimes referred to as the true positive rate (a test's ability to accurately measure the actual positive test results), and *specificity*, also called the true negative rate (a test's ability to accurately identify negative results). Sensitivity and specificity are defined using the values for the four test outcomes in this binary result system. A very good explanation of these concepts is addressed in Wikipedia (https://en.wikipedia.org/wiki/Sensitivity_and_specificity).

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		Condition		
		Positive	Negative	
Test Outcome	Positive	TP	FP	Positive Predictive Value TP / (TP + FP)
	Negative	FN	TN	Negative Predictive Value TN / (TN + FN)
		Sensitivity TP/(TP + FN)	Specificity TN / (TN + FP)	

Figure 1: Binary Test Framework

Legend: TP – true positive; TN – true negative; FP – false positive; FN – false negative.

Sensitivity is the number of true positives divided by all of the actual positives (i.e., the true positives plus the false negatives, as shown in Figure 1). *Specificity* is the number of true negatives divided by all of the actual negatives (i.e., the true negatives plus the false positives).

To interpret a test outcome requires knowing the positive and negative predictive values. The positive predictive value tells you whether you should believe a positive result, and the negative predictive value tells you whether you should believe a negative result. The positive predictive value is the number of true positives divided by the total number of *positive tests* (i.e., the true positives plus the false positives). The negative predictive value is the number of true negatives divided by the total number of negative tests (i.e., the true negatives plus the false negatives).

One critical piece is missing: the prevalence of COVID in the population. Sensitivity and specificity are determined by the assay itself, because in developing the assay it is possible to know exactly the condition of each sample being tested. However, for positive and negative prediction, you need to know what fraction of tested patients actually have COVID. In other words, what is the prevalence? The positive and negative predictive values are completely different if the disease is very rare (e.g., only 0.1% of the population being tested has COVID) or very common (e.g., 90% of the population has COVID).

There are many online calculators for positive and negative predictive values. Alternatively, you can download a spreadsheet created by the authors asamonitor.pub/spreadsheet.

RT-PCR COVID testing

The genome for SARS-CoV-2 is a positive sense single-strand RNA approximately 30k nucleotide long that encodes 27 different proteins. Among the more relevant genes for polymerase chain reaction (PCR) analyses are the ones that encode the following proteins: RNA dependent RNA polymerase (*RdRp*), small envelope protein (*E*), matrix protein (*M*), nucleocapsid protein (*N*), and the spike surface glycoprotein (*S*).

There are three regions of fairly conserved sequences in SARS-related viral genomes: 1) *RdRP*, which exists in the open reading frame ORF1ab region, 2) *E*, and 3) *N*. As a consequence, many of the genetic tests developed as diagnostics for SARS-CoV-2 have focused on these genes.

Reverse transcriptase PCR (RT-PCR) may be done using either a one- or two-step process; in the one-step assay, both the reverse transcription and PCR amplification are combined into a single step. These are performed sequentially in the two-step process. The single-step protocol can provide fairly reliable and reproducible results in a high throughput setting; optimizing the simultaneous amplification and reverse transcription steps can prove challenging. The sequentially performed two-step reaction is more sensitive but requires more time and additional parameter optimization.

Antibody COVID testing

In a procedure described by Zhang et al., immunoglobulins G and M (IgG and IgM) were detected in human sera from patients with COVID-19 using an enzyme-linked immunosorbent assay (ELISA) (*Emerg Microbes Infect* 2020;9:386-9). In their procedure, the surface of 96-well plates that have been coated with recombinant proteins are treated with diluted human serum for one hour, after which the plate is rinsed. Next, the well is treated with anti-human IgG that has been functionalized with horseradish peroxidase; subsequently, the plate is rinsed and treated with a reducing agent that reacts with the peroxidase, producing a quantifiable color change. The analogous IgM test by the same researchers has a similar protocol, but instead relies upon an anti-human IgM coated plate.

Evaluation of rapid (point-of-care) testing

Researchers at the University of Bonn recently described a study in which a rapid point-of-care test for COVID-19 was compared to the considered gold standard of diagnosis – quantitative PCR (qPCR) (*Public Health* 2020;182:170-2). The dual IgG/IgM point-of-care diagnostic evaluated in this study is qualitative, showing a past or present COVID-19 infection. It only requires two drops of blood for analysis and is completed within 20 minutes. The point-of-care testing chip has detection bands coated with murine anti-human IgG and IgM antibodies. After the addition of two drops of blood and some reagent solution, positive results are obtained for IgG and IgM after 15 and 20 minutes, respectively.

In their study, a total of 39 randomly selected individuals at a COVID-19 screening center were tested simultaneously via point-of-care and qPCR diagnostics. In addition, analysis was performed on 10 individuals with confirmed prior cases COVID-19.

A total of 22 subjects of the 49 included in the study were confirmed to be COVID-positive by repeated qPCR; however, the point-of-care diagnostic identified only eight of these positive individuals for a sensitivity of 36.4%. For the remaining 27 COVID-negative subjects, 24 were properly identified using the point-of-care diagnostic, yielding a specificity of 88.9%. The authors

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conclude that the point-of-care diagnostic evaluated should not be relied upon for community screening as part of public health measures given its low sensitivity.

Antibody testing and patient characteristics

In a recent *Nature Medicine* article, researchers from across China presented data from 285 patients for the acute antibody responses to SARS-CoV-2 infection (*Nat Med* 2020;26:845-8). All subjects showed antiviral IgG seropositivity within 19 days from the onset of symptoms. Seroconversion for IgG and IgM could occur simultaneously or asynchronously, with both IgG and IgM levels reaching a plateau within six days post-seroconversion.

A subset of 63 patients confirmed with COVID-19 were followed up until discharge with longitudinal serum samples being collected at three-day intervals. During follow-up, the overall seroconversion rate was 96.8% in these subjects. Additionally, two related patients (mother and daughter) maintained IgG- and IgM-negativity throughout hospitalization.

Another subset of 26 initially seronegative patients experienced seroconversion while under observation; all of this subgroup attained seroconversion of IgG or IgM within 20 days (median-13 days) post-symptom onset. Combined IgG and IgM seroconversion could occur in one of three manners: simultaneously (n=9), IgM earlier (n=7), or IgG earlier (n=10). Based on their findings, the authors conclude that serological testing may aid in the diagnosis of both asymptomatic infections and suspected cases having negative RT-PCR results.

Research performed by clinicians from Chongqing Medical University, including the clinical features and immune responses of RT-PCR-confirmed asymptomatic COVID-19 infected individuals in the Wanzhou District of China, were reported recently (*Nat Med* June 2020). The asymptomatic individuals included in this study displayed no clinical symptoms during and two weeks prior to hospitalization. These asymptomatic patients were found to have viral shedding for a median period of 19 days, which was significantly longer than that noted for symptomatic patients. The median signal to cutoff ratio (S/CO) of 3.4 noted for SARS-CoV-2-specific IgG levels in asymptomatic individuals was significantly lower when compared to the symptomatic individuals in the acute phase, who had a median S/CO ratio of 20.5. The authors noted that 93.3% and 81.1% of asymptomatic individuals in the early convalescent phase had reductions in IgG and neutralizing antibody levels, respectively; the same values were 96.8% and 62.2% for symptomatic patients. In the early convalescent phase of the disease, IgG seronegativity was noted in 40% of asymptomatic individuals and 12.9% of the symptomatic. Asymptomatic subjects also displayed lower levels of 18 pro- and anti-inflammatory cytokines, suggesting that they had weaker immune responses to SARS-CoV-2 infection. The authors note that reductions in IgG and neutralizing antibody levels during the early convalescent phase may have significant implications for immunity strategies and serological surveys.

The authors cite prior research on decreased neutralizing antibodies in recovering COVID-19 patients when speculating that the use of prolonged public health interventions such as widespread testing, social distancing, and isolation of "high-risk" groups may be necessary for disease containment. They further suggest that the completion of additional longitudinal serology studies of COVID-19 patients is desperately needed to assess timelines for antibody-mediated immunity. Noting the low anti-SARS-CoV-2 IgG in asymptomatic patients who are also more likely to become seronegative, they further make the urgent case for additional serological surveys to assess actual COVID-19 infection rates.

Figure 2: US States - Case Mortality vs. Testing as of 2020-07-11

Figure 3: Countries - Case Mortality vs. Testing as of 2020-07-11

Testing-case fatality rate

Conventional wisdom early in the outbreak of SARS-CoV-2 held that the initially high fatality rate would decrease over time as the number of non-life-threatening cases of COVID-19 were identified via expanded testing. As of the writing of this article, this has clearly not materialized. In fact, as one can readily see in the data for individual U.S. states (Figure 2), some of the states with the largest percentage of residents tested have the highest case mortality rates (e.g., New York and New Jersey).

The data for individual countries are a bit more muddled (Figure 3). The two countries with the highest rates of testing do have fairly low case fatality rates; however, the countries are Bahrain and Luxembourg, both small countries. The countries with the highest case fatality rates of approximately 15%, Belgium, Great Britain, and Italy, all had roughly 10% of their populations tested.

Different diagnostic needs for clinical and surveillance settings

Researchers at the University of Colorado, in a recent pre-print published online at medRxiv, concluded that the requirements for diagnostics surveillance purposes differ significantly from those being utilized for clinical purposes, and consequently, that test sensitivity, for surveillance purposes, is secondary to frequency and turnaround time in COVID-19 testing (*medRxiv* June 2020).

The authors state that because symptomatic individuals are targeted in clinical diagnoses, the need for high accuracy and sensitivity supersedes cost and diagnosis time concerns. Since those individuals are symptomatic, they can be isolated until such time as their results are obtained, preventing additional infections. For asymptomatic individuals, the delay of test results, even of a single day, can result in a number of potential infectious exposures, thus undermining surveillance efforts. The authors note that the speed of reporting is more important than sensitivity, especially when one considers an infectious agent with the transmissibility documented for SARS-CoV-2. As a result of these differing requirements, the authors suggest the development and approval of faster and cheaper diagnostics for surveillance purposes that are not held to the same rigorous sensitivity standards as those for clinical purposes, so as to not make the development process excessively onerous.

In their study, certain models suggest that different modes of surveillance may subject some individuals to unnecessary quarantine days. As an example, they cite the infrequent use of a sensitive test that will identify those with a low viral load, including those in the beginning of the infection (who should be isolated to limit viral transmission), as well as individuals who are recovering who may have detectible levels but are nonetheless beneath the threshold for transmission (whose isolation has no impact on viral spread and needlessly adds to financial burdens). The authors state that more effective quarantine decisions could be made if the use of serology, repeat testing (24 or 48 hours apart), or some other test were implemented, so as to distinguish low viral load patients who are on the upslope of infection (i.e., more likely to transmit disease) from those in the recovery phase. ■

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