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EXPERT OPINION

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The battle of testing in COVID-19: the secrets of victory against the virus

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The risk of COVID-19 morbidity and mortality is increased in patients with cardiovascular diseases.[1] The most common cardiac complications include arrhythmia, myocardial cell injury, heart failure, and myocarditis, as well as chest pain and palpitations - some of which are also recognized complications of the post-COVID-19 syndrome. Testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is crucial to manage the Coronavirus Disease 2019 (Covid-19) pandemic. Detection of SARS-CoV-2 virus uses two main strategies; molecular tests for viral nucleic acids and immunoassays which either detect viral specific proteins or antibodies to the SARS-CoV-2 virus.[2] Tests for SARS-CoV-2 infection can be used for diagnosis, screening, or surveillance in laboratories or as point of care assays in health care facilities, workplaces, schools, universities, and at home. Diagnosis, screening, and surveillance serve different purposes and demand distinct strategies which will impact on which SARS-CoV-2 test will be used.[3] Testing embedded in health care strategies encompassing contact tracing, isolation of positive cases, non-pharmacological interventions, and public health surveillance has been a vital tool in managing the COVID-19 pandemic. The aim of this commentary is to update the cardiologist on current use of SARS-CoV-2 tests in the management of the COVID-19 pandemic.

Molecular tests

Reverse transcriptase-polymerase chain reaction

The goal standard for the diagnosis of symptomatic SARS-CoV-2 infection is reverse transcriptase-polymerase chain reaction (RT-PCR).[2] After extraction, viral RNA is reverse transcribed to a cDNA sequence. PCR primers amplify different nucleotide targets (N, S, E ORF1ab) which vary with different assays within the SARC-CoV-2 genome over 30–40 cycles. DNA copies are detected by fluorescent probes and the number of PCR cycles needed to produce a reading above background (cycle threshold Ct) is used to define a positive reaction. The Ct value is inversely related to viral load; however, what viral RNA levels are required for transmission of infection is not known. Sensitivity and specificity of RT-PCR are 81.5–92.2% and more than 98%, respectively.[4] Site of specimen collection (nasopharyngeal, sputum, stool), how well the specimen has been taken, presence or absence of symptoms, purpose of the test, timing of the sample relative to onset of symptoms, and integrity of sample transport and storage can all influence the outcome of test results. The RT-PCR assay is mainly used for the diagnosis of symptomatic COVID-19 infection and for contact tracing in individuals with a history of known exposure. This test detects nucleic acid fragments well beyond post-infectious period, identifying as contagious those who are no longer infectious.[5] The RT-PCR test is performed in centralized laboratories, requires trained personnel and specialized equipment, and turnaround times vary between 12 and 72 h. Prolonged RNA shedding, cost, inability to perform very large number of tests, and slow turnaround times are significant limitations to use of this technology for screening.[5] Point of care molecular tests incorporating RNA extraction, PCR amplification, and assay readout sealed cartridges and loop isothermal PCR amplification have had limited impact on COVID-19 diagnostics to date largely due to limited sample throughput, cost, and deployment of rapid antigen tests.[3] RT-PCR tests have also been used to detect SARS-CoV-2 in sewage and wastewater samples to provide public health information on the epidemiology of the disease and act as an early warning for re-emergence of COVID-19.[3]

Next-generation sequencing

The emergence of viral variants of concern, linked to increase rate of transmission and mortality and evidence of reduction in surrogate marker of vaccine efficacy has resulted in efforts to scale up genomic surveillance of circulating SARS-CoV-2 strains using next-generation sequencing (NGS) to detect novel viral variants and high throughput RT-PCR platforms targeting specific viral mutations or deletions to prevent future waves caused by new variants.

Immunoassays

Antigen tests

Antigen tests detect the presence of virus-specific proteins (e.g. nucleocapsids) on either nasopharyngeal or nasal swabs using a lateral flow assay.[2] Lateral flow assays work in a similar manner to pregnancy tests using immunochromatography to look for the presence/absence of a SARS-CoV-2 nucleocapsid protein. Specificity of the assay is similar to RT-PCT test; however, analytical level of detection is 100–1000 greater

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than RT-PCR resulting in moderate/low sensitivity in comparison with molecular nucleic acid amplification test. Reduced sensitivity means that only high protein concentrations are detected which serve as a surrogate marker for infectiousness. Antigen tests are currently used to screen for individuals with pre-symptomatic or asymptomatic infection to interrupt community transmission and reduce the prevalence of COVID-19 infections. Lateral flow antigen tests have a number of advantages for screening as they can be performed by untrained personnel either at home or work, give a result within 15–30 mins and can be scaled to millions of tests/day in addition to being cheaper than RT-PCR tests. Modelling studies suggest that rapid release of serial test results are more important than analytical sensitivity in preventing the spread of infection.[6] This testing strategy remains controversial and there is little evidence to show its impact on the incidence of COVID-19 infection despite widespread use.[5,7]

Serology tests

Serology tests assess the generation of antibody responses to SARS-CoV-2 proteins (either the spike protein or its components, and the nucleocapsid protein).[2] Assay format includes point of care lateral flow devices (limited analytical performance to date) and laboratory-based tests, enzyme-linked immunosorbent assays (ELISA), and chemiluminescence enzyme-linked immunoassays. The latter offer clear operational advantages over ELISA such as the ability to handle a large number of samples with greater sensitivity (77–100%) and specificity (90–100%) and quantitative test results. The latter can be correlated albeit imperfectly with viral neutralization assays (a surrogate marker for the capacity of immunoglobulins to inhibit viral replication) in an attempt to establish an immune correlation of protection following SARS-CoV-2 infection and protective antibody thresholds after vaccination. Serology tests have limited diagnostic utility; they can be helpful in those who present at least 2 weeks after symptom onset and to confirm a diagnosis of paediatric/adult multisystem inflammatory syndrome. Age, disease severity, immune antigenic target, immunoglobulin isotype, assay platform, potential for cross-reactivity with common cold coronaviruses can all influence analytical performance of antibody tests. The main role of serology testing has been to estimate COVID-19 prevalence in the population to guide public health and economic policies.[8]

Current strategies for assessing vaccines include assays to detect the nucleocapsid protein as a marker of past or recent infection and assays against the spike protein, in particular the receptor-binding domain to assess post-vaccine antibody responses. T-cell immunity to SARS-CoV-2 is likely to be an important correlate of protection against SARS-CoV-2. Current assays use enzyme-linked immunospot (ELISA) or ELISA platforms to detect interferon-γ secretion following stimulation with SARS-CoV-2 peptide pools. Attempts to define both the range of immune responses and threshold of protection following SARS-CoV-2 vaccination are an active area of research. Studies to date have demonstrated that antibody and T-cell responses are significant elevated following the first vaccine dose in those with a history of previous COVID-19 compared to infection naïve subjects, as well as an enhancement of antibody and T-cell responses following the second dose of an mRNA SARS-CoV-2 vaccination.[9,10]

Conclusion

Remaining challenges in COVID-19 diagnostics include the need for standardization of tests, application of novel assays (digital droplet PCR and CRISPR) for large scale population point of care screening and deployment of NGS, as well as high throughput RT-PCT platforms to detect and monitor novel viral variants to prevent further waves of SARS-CoV-2 infection.

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