Testing for SARS-CoV-2 (COVID-19): A General Review

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ABSTRACT

The rampant COVID-19 pandemic has strained the testing capabilities of healthcare centers across the country. Several nucleic acid and serologic assays are available or currently being developed to meet the growing demand for large-scale testing. This review summarizes the developments of commonly used testing methods and their strategic use in clinical diagnosis and epidemiologic surveillance. This review will cover the basic virology of SARS-CoV-2, nucleic acid amplification testing, serology, antigen testing, as well as newer testing methods such as CRISPR-based assays.

KEYWORDS: COVID-19, RT-PCR, serology testing

INTRODUCTION AND VIROLOGY OF SARS-COV-2

In December of 2019, an unknown pneumonia outbreak started in the Wuhan province, later determined to be caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. Later named Coronavirus Disease-19 (COVID-19), widespread human-to-human transmission led to over 21.7 million confirmed cases and 775,937 deaths among over 200 countries as of August 17, 2020.[1] The novel disease has and continues to spread rapidly throughout many countries including the United States.

Coronaviruses are separated into four main sub-groups: alpha, beta, gamma, and delta. Only seven alpha and beta coronaviruses are known to infect humans. These are positive-sense single-stranded RNA viruses. Four of the most common types (229E, NL63, OC43, and HKU1) are endemic globally and usually cause mild to moderate upper-respiratory tract illness, accounting for 10–30% of all such infections in adults. Three other coronavirus strains, known as severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2, are associated with epidemiological outbreaks and have a much higher mortality rate.

SARS-CoV-2 is estimated to have a basic reproduction number (R0) of about 2.5, with a global estimate displaying a 1.2%–13.9% case fatality rate (CFR) at the time of writing.[1] In comparison, SARS-CoV has an R0 value of 3 with a CFR of 15% and MERS-CoV has an R0 value of 1 with a CFR of 35%.[2, 3]

These three coronavirus strains have some distinguishing characteristics which account for their increased virulence compared to the endemic coronavirus strains. The spike glycoprotein (S protein) of MERS-CoV binds to cell surface receptor dipeptidyl peptidase 4 (DPP4), while the S protein of SARS-CoV and SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) on their host cells.[4,5] Notably, SARS-CoV-2 displays a 10- to 20-fold greater binding affinity compared to SARS-CoV – a characteristic that is explained by some unique genetic inserts in its spike glycoprotein.[6]

NUCLEIC ACID AMPLIFICATION TESTS (NAATS) OF SARS-COV-2

Overview

Similar to many RNA virus detection assays, SARS-CoV-2 NAATs use reverse transcription-polymerase chain reaction (RT-PCR) to detect viral genomes with high sensitivity and rapid turnaround times. NAATs target conserved regions located in the open reading frame-1ab (ORF1ab) gene as well as the genes of envelope (E), spike (S), and nucleocapsid (N) proteins.[7] NAATs are widely available and remain the primary methods of diagnosing COVID-19 disease.

Specimen Types

Several specimen types have received Emergency Use Authorization (EUA) from the Food and Drug Administration (FDA). The most frequently used are the nasopharyngeal (NP) swab, nasal swab, and oral pharyngeal (OP) swab. Recently, saliva-based PCR testing also received FDA EUA approval.

NP swabs are samples collected along the posterior wall of the nasopharynx and are the most appropriate sample type due to the location of the virus within the upper respiratory tract. However, its use is limited due to the requirement of special training in collection technique. Nasal swabs, on the other hand, can be self-collected by the patient, eliminating the need for contact with a healthcare provider. Nevertheless, tests with these samples are subject to a slight decrease in sensitivity due to their suboptimal sample location. OP swabs are directed towards the rear of the oropharynx and are used as an alternative site when an NP or nasal swab cannot be obtained. Saliva is the easiest to obtain and has been gaining popularity in massive testing plans despite claims of such samples having a lower yield. In addition to

upper respiratory tract specimens, lower respiratory tract specimens from bronchoalveolar lavage (BAL) have provided the best yield,[8] but the bronchoscopy procedure is considered to be rather invasive.

Testing Turnaround Time

The turnaround time is the time it takes from collecting a sample to reporting a result. Many factors should be considered, such as location and method of collection, testing method being used, and the location where the test is being performed. The vast majority of the NAATs are performed in an off-site location located away from the patient due to the requirement of high complexity laboratories to perform the NAATs. The exceedingly large volume for diagnosis and screening frequently leads to increased turnaround time in many labs. Where there is a high test volume but a limited number of certified labs, one solution is to use pooled testing.

Pooled testing aims to increase the efficiency of identifying positive cases while minimizing the number of tests needed to screen a population. Pooled testing involves combining several samples into a pool and testing them all at once. If the pool result is negative, all samples included are presumed to be negative. If the pool test is positive, then each sample will be re-tested to identify individual positive samples. Pooled testing takes two steps to identify positives but is efficient when the prevalence of the virus is low because the majority of the samples will be negative. It allows for a great number of individuals to be screened using far fewer testing resources.

Assay Sensitivity and Specificity

Despite having high analytical sensitivity and specificity values, NAATs have a few limitations. Notably, the detectability of the SARS-CoV-2 genome may vary depending on the disease stage. The current consensus is that NAAT assays are the most sensitive during the acute stage of infection. The timing of the test is critical, as testing in the early phase of the incubation period and during the later stages of infection will lead to significant false negatives. When used appropriately, these tests have a very high sensitivity, being able to detect as few as 10–100 copies of viral RNA per milliliter in a sample.[9] They also have a high specificity in that they do not cross-react with other coronaviruses. While these values vary depending on the specific test and manufacturer used, all such assays have comparable performances in terms of their accuracy.

Utility

The overall benefit of NAATs is that they amplify a small amount of viral target RNA to a detectable level. They are more sensitive than an antigen-based test and much faster and safer than performing viral culture. However, a significant drawback is that they can detect viral RNA shedding for an extended period in some patients, even after they are no longer symptomatic and presumed no longer infectious.

SARS-COV-2 TESTING – SEROLOGY TESTING

Overview

Serological tests detect antibodies present in the blood and thus can reveal any current or previous infection. Antibody tests must be specific enough to prevent cross-reaction with antibodies against other pathogens. For SARS-CoV-2, antibodies against S and N proteins are commonly tested, where the antibodies against two subunits S1 and S2 of the S protein can be tested individually or together. The antibody isotypes in SARS-CoV-2 tests are IgM, IgG, and IgA, although IgM and IgG antibodies are generally tested individually or together as total antibodies.

Antibody responses generally occur between 10 to 21 days after infection, with mild cases potentially taking upwards of four weeks. In a recently published study, COVID-19 specific IgM and IgG antibodies were first detectible 3–4 and 5-6 days post-symptom onset, respectively, with a marked increase in antibody detectability and test sensitivity 14 days post-symptom onset.[10] Therefore, such tests are not useful for early screening or initial patient visits.

It is unknown how long COVID-19 specific antibodies remain detectable and whether they correlate to any long-term protection. A recently published study suggests that most patients showed sharp declines of COVID-19 specific IgG antibodies within two to three months after infection onset.[11] A possible new area of inquiry is the study of cellular immunity. A study on medRxiv done by Staines et al. has found that a small percentage of infected patients do not develop COVID-19 antibodies at all, suggesting that the immune response in these patients could be through separate antigens or mediated through T cells.[12]

Testing Platforms

Of the few dozen serology tests currently in the market, four particular testing platforms are currently being used to analyze SARS-CoV-2: the lateral flow assay (LFA), the enzyme-linked immunosorbent assay (ELISA), the chemiluminescent assay (CLIA), and the cyclic enhanced fluorescence assay (CEFA).

LFAs prioritize speed and ease of use, offering a flexible and cost-effective method of obtaining a result. Nevertheless, limitations of LFAs include the difficulty to perform large-volume testing and multiple analyte testing. ELISA tests provide standard antibody titers; however, the tests are rather labor-intensive, if not assisted by automation. As opposed to other immunoassays, CLIAs measure photons of light to discern a result, leading to its high sensitivity and specificity. While these tests require expensive instruments and highly purified reagents, the high sensitivity permits the use of very small reagent volumes per test, keeping the

assay cost-effective.[13] The main advantage of CEFA tests lies in the cyclic amplification of the fluorescence signals to detect antibodies sensitively and specifically, and have shown promising clinical utility in evaluating the immune response in infected and convalescent patients.[14]

While current serology testing serves as an excellent indicator of prior or current infection, they do not directly assess the neutralizing capabilities of the antibodies. For this purpose, neutralizing antibody assays aim to identify antibodies that recognize the SARS-CoV-2 virus and block its host cell entry.

There are two recognized types of neutralizing antibody tests: virus neutralization tests (VNT) and pseudovirus neutralization test (pVNT). VNTs utilize SARS-CoV-2 viruses from clinical isolates and can only be performed in a Biosafety Level 3 laboratory by highly trained personnel. Alternatively, pVNTs use recombinant pseudoviruses that express the S protein of SAS-CoV-2 to construct the spikes on the viral surface.[15] A specific example is the pseudovirus luciferase assay (PVLA), where the inhibition of viral entry into cells by the neutralizing antibody correlates to the decreased luciferase signals in the cells. pVNTs are safer, simpler, and more accurate than conventional assays.[16]

Utility

Serologic testing is primarily used to detect the presence of antibodies specific to a given virus and is therefore not a good indicator of current infection, as a positive result indicates that a patient is either in the late phase of the disease or he/she may have been infected in the past. Nevertheless, using a serological test alongside a NAAT has proven effective in providing more accurate diagnoses.[17]

Serologic testing is frequently used for disease surveillance and is thus an integral part of policymaking, both on the governmental and communal level. It is also utilized in transfusion medicine (e.g. with the convalescent plasma treatment) to determine the antibody titer in the unit. Finally, serologic testing will be useful in verifying whether or not a vaccine incites the desired immune response. Distinguishing the immune response to the vaccine from that to the real infection will be challenging in individuals inoculated by inactivated virus-based vaccines, but the presence of RBD or S-protein antibodies and absence of N-protein antibodies should be sufficient to identify an immune response to the S-protein based vaccines.

Other Assays

Currently, NAAT and serologic tests are the most prevalent assays used to diagnose or screen COVID-19. But due to the continued shortage of available tests, there has been a continued push to utilize existing and novel methods for viral detection.

Antigen-based tests are diagnostic tests designed to detect fragments of viral proteins. They utilize similar technology to some serology tests, such as the LFA and the ELISA. The advantage of antigen tests is that they can be performed near the patient without the need for a high-complexity laboratory, and a large number of tests can be manufactured and widely distributed due to their simpler design.[18] However, they do suffer from a lack of sensitivity and specificity compared to NAATs. For the first time, the CRISPR-based technology has been authorized under the FDA EUA for direct patient use. The assay uses the SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) method to program a CRISPR molecule to specifically detect the presence of a specific SARS-CoV-2 genetic signature.[19] The advantage of this technology is that it is faster than RT-PCR and can potentially be scaled up to test a large volume of samples. Finally, there are increasing in the development of simple, daily COVID-19 tests. One such test is the paper-strip test, in which a sample of spit in a saline solution would be tested with a strip of paper embedded with protein.[20] Such tests have shown promise and can potentially circumvent some of the issues surrounding the current testing strategies such as cost and testing availability.

CLOSING REMARKS

As it stands, personal hygiene and social distancing procedures are the most effective preventative measures against SARS-CoV-2. When it comes to testing, NAAT and serology testing are the mainstays in clinics and hospitals. In the competitive market of COVID-19 testing, more and more assays are becoming available and being authorized by the regulatory agencies. All the current and emerging assays will keep being used under specific medical and epidemiologic circumstances until the global population reaches herd immunity either by the virus or by the vaccine. The swift response of the medical diagnostic industry to the pandemic highlights the importance of basic biomedical research which is constantly providing scientific and technological knowledge for the health care industry to develop advanced tools and agents to fight diseases and safeguard our population.

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