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Editorial

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Analysis of Laboratory Tests for Diagnosis of Covid 19

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Widespread availability of accurate and rapid testing procedures is extremely important in unraveling the complex dynamics involved in Covid 19 infection and immunity. To this achieve, laboratories, universities, and companies around the world have been racing to develop and produce critically needed diagnostic test kits. The pandemic of corona itself is a big challenge for containing the spread of COVID-19 is the ability to identify asymptomatic cases that result in spreading of the virus to close contacts. The actual number of SARS-CoV-2-infected individuals may be much higher than currently accounted for based on positive test results. For getting accurate, convenient, and rapid testing for widespread deployment can aid in eliminating the silent spread of COVID-19 by asymptomatic viral carriers. Because COVID-19 exhibits a great range of clinical manifestations, from mild flu-like symptoms to life-threatening conditions, it is important to have efficient testing during the early stages of infection to identify COVID-19 patients from those with other illnesses. Which avoids unnecessary quarantines of negative individuals and the spread of infection by positive individuals. Early diagnosis permits physicians to provide prompt intervention for patients who are at higher risk for developing more serious complications from COVID-19 illness.

Presently commercially available COVID-19 tests currently fall into two major categories. The first category includes molecular assays for detection of SARS-CoV-2 viral RNA using polymerase chain reaction (PCR)-based techniques or nucleic acid hybridization-related strategies. The second category includes serological and immunological assays that largely rely on detecting antibodies produced by individuals as a result of exposure to the virus or on detection of antigenic proteins in infected individuals. It is important to reemphasize that these two categories of tests serve overlapping purposes in management of the COVID-19 pandemic. Testing for SARS-CoV-2 viral RNA identifies SARS-CoV-2-infected individuals during the acute phase of infection. Serological testing subsequently identifies individuals who have developed antibodies to the virus and could be potential convalescent plasma donors. It also furthers the ability to conduct contact tracing and monitor the immune status of individuals and groups over time. Timely diagnosis, effective treatment, and future prevention are key to management of COVID-19. The current race to develop cost-effective point-of-contact test kits and efficient laboratory techniques for confirmation of SARS-CoV-2 infection has fueled a new frontier of diagnostic innovation.

Molecular Assays for Detection of Viral Nucleic Acids

SARS-CoV-2 is a single-stranded, positive-sense RNA virus, and as its entire genetic sequence was uploaded to the Global Initiative on Sharing All Influenza Data (GISAID) platform on January 10, 2020, companies and research groups in a matter of weeks have

developed a range of diagnostic kits for COVID-19. Generall sample required for such tests are throat swab, nasal swab, sputum and bronchio-aveolar lavage etc. The availability of sequence data has facilitated the design of primers and probes needed for the development of SARSCoV-2-specific testing. Following diagnostic molecular assays are available to detect viral nucleic acid.

1. RT- PCR (Reverse Transcription Polymerase Chain Reaction)
2. Isothermal Nucleic Acid Amplification.
3. Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP)
4. Transcription-Mediated Amplification (TMA).
5. CRISPR-Based Assays. Clustered Regularly Interspaced Short Palindromic Repeats
6. Nucleic Acid Hybridization Using Microarray.
7. Amplicon-Based Metagenomic Sequencing.
8. High-Level Overview of Current Molecular Genetic

Serological and Immunological Assays

While RT-PCR-based viral RNA detection has been widely used in diagnosis of COVID-19, it cannot be used to monitor the progress of the disease stages and cannot be applied to broad identification of past infection and immunity. Serological testing is defined as an analysis of blood serum or plasma and has been operationally expanded to include testing of saliva, sputum, and other biological fluids for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies. This test plays an important role in epidemiology and vaccine development, providing an assessment of both short-term (days to weeks) and long-term (years or permanence) trajectories of antibody response, as well as antibody abundance and diversity. IgM first becomes detectable in serum after a few days and lasts a couple of weeks upon infection and is followed by a switch to IgG. Thus, IgM can be an indicator of early stage infection, and IgG can be an indicator of current or prior infection. IgG may also be used to suggest the presence of post-infection immunity. Following tests are serological or immunological assays.

1. Enzyme-Linked Immunosorbent Assay (ELISA).
2. Lateral Flow Immunoassay.
3. Neutralization Assay
4. Luminescent Immunoassay
5. Biosensor Test.
6. Rapid Antigen Test.
7. High-Level Overview of Current Serological and Immunological Assays for COVID-19 Diagnosis.

RT-PCR

From very beginning Polymerase Chain Reaction testing method which detect viral nucleic acid was available for covid 19 viral infection was available but it was too costly, too much time consuming and laborious. It also requires special room with negative pressure. With simple PCR method only DNA can be detected, but Covid 19 is RNA virus, so it requires reverse transcriptase PCR (RT-PCR). The sensitivity of this test is about 61%, and oral or throat swabs are to be taken and these swabs are to be transported to PCR laboratory within 72 hours in viral transport medium under 40C temperature. As RT PCR is very popular, every one knows too much, it is not needed to discuss about in detail.

RT-PCR test are constantly evolving with the improved detection methods and more automated procedures. Although RT-PCR is the most common widely used method for detection of SARS-CoV2 infections, it has the disadvantage of requiring expensive laboratory instruments, highly skilled laboratory staff, and take too much long time, can take days to generate results. So, many companies and laboratories through out the world are searching to further improving the efficacy and timeliness of the RT-PCR technologies and develop various other techniques.

Isothermal Nucleic Acid Amplication

On 27 March 2020, the FDA issued an Emergency Use Authorization for a test by Abbott Laboratories, called ID NOW COVID-19, that uses isothermal nucleic acid amplification technology instead of PCR. The assay amplifies a unique region of the virus's RdRp gene; the resulting copies are then detected with "fluorescently-labeled molecular beacons". The test kit uses the company's "toaster-size" ID NOW device which costs $12,000-$15,000. The device can be used in laboratories or in patient care settings, and provides results in 13 minutes or less. But one can perform one test at a time on instrument, so useless in heavy workload. There are currently about 18,000 ID NOW devices in the U.S. and Abbott expects to ramp up manufacturing to deliver 50,000 ID NOW COVID-19 test kits per day.

In a study conducted by the Cleveland Clinic, the ID NOW COVID-19 test detected the virus only in 85.2% of the samples that contained it. According to the director of the study a test should be at least 95% reliable. Abbott said that the issue could have been caused by storing the samples in a special solution instead of inserting them directly into the testing machine.

Serology Tests

Rapid diagnostic test (RDT): This is typically a qualitative (positive or negative) lateral flow assay that is small, portable, and can be used at point of care (POC). These tests may use blood samples from a finger prick, saliva samples, or nasal swab fluids. RDTs are often similar to pregnancy tests, in that the test shows the user colored lines to indicate positive or negative results. In the context of COVID-19, these tests most frequently test for patient antibodies (IgG and IgM), or viral antigen. In some cases, it can be beneficial to measure baseline (before infection) of IgG and IgM titers.

Enzyme-linked immunosorbent assay (ELISA): This test can be qualitative or quantitative and is generally a lab-based test. These tests usually use whole blood, plasma, or serum samples from patients. The test relies on a plate that is coated with a viral protein of interest, such as Spike protein. Patient samples are then incubated with the protein, and if the patient has antibodies to the viral protein they bind together. The bound antibody-protein complex can then be detected with another wash of antibodies that produce a color or fluorescent-based readout. In the context of COVID-19, these tests most frequently test for patient antibodies (IgG and IgM).

Neutralization assay: This test relies on patient antibodies to prevent viral infection of cells in a lab setting. Neutralization assays can tell researchers if a patient has antibodies that are active and effective against the virus, even if they have already cleared the infection. These tests require whole blood, serum, or plasma samples from the patient. Neutralization assays depend on cell culture, a lab-based method of culturing cells that allow SARS-CoV-2 growth (like VeroE6 cells). When virus and cells are grown with decreasing concentrations of patient antibodies, researchers can visualize and quantify how many antibodies in the patient serum are able to block virus replication. This blocking action can happen through the antibody binding to an important cell entry protein on the virus, for example.

Chemiluminescent immunoassay: This test is typically quantitative, lab-based, and uses whole blood, plasma, or serum samples from patients. A variation of this test can use magnetic, protein-coated microparticles, known as a chemiluminescent microparticle immunoassay. The test relies on mixing patient samples with a known viral protein, buffer reagents, and specific enzyme-labeled antibodies that allow a light-based, luminescent read-out. Any antibodies in the patient sample that react to the viral protein will form a complex. Then, (secondary) enzyme-labeled antibodies are added that bind to these complexes. This binding induces a chemical reaction that produces light. The amount of light (radiance) emitted from each sample is then be used to calculate the number of antibodies present in a patient sample. This test can look for multiple types of antibodies, including IgG, IgM, and IgA.

All reports provides a broad survey of molecular genetic assays, and serological and immunological tests for identification of COVID-19 infection. While RT-PCR has been the dominant technique for detection of viral RNA, other nucleic acid assays including isothermal amplification assays, hybridization microarray assays, amplicon-based metagenomics sequencing, and the cutting-edge CRISPR-related technologies are also under development or have resulted in approved tests. The efficiency of such testing has also been significantly improved. Ultra rapid test kits and point of-care tests are a major focus of development in order to speed up the response time for treatment and eliminate the need for elaborate laboratory equipment and waiting time involved with testing in approved laboratories.

The urgent need for accurate and rapid diagnosis of SARSCoV-2 infection remains critical as global healthcare systems continue to operate during the course of the COVID-19 pandemic. In particular, serological and immunological testing of infected asymptomatic and symptomatic individuals, and their close contacts, is expected to be in high demand. In addition to its role complementary to molecular genetic testing to confirm suspected cases, this type of testing would provide valuable information about the course and degree of immune response as well as the durability of immunity in both infected individuals and participants in vaccine clinical trials. The results from these tests may assist epidemiological assessment and can be used to manage the return to normal activities. However, many questions regarding serological tests remain to be addressed, including their degree of sensitivity and specificity. Finally, it remains to be confirmed that the presence of antibodies against SARS-CoV-2 indeed correlates with immunity to the virus.

In summary, significant progress has been made in the development of diagnostic tests despite all the remaining questions and challenges. Ongoing global efforts are working to communicate and facilitate new diagnostic assay development and worldwide test kit delivery. To promote more accurate and faster diagnostic solutions, a number of organizations are supporting these efforts by inviting assay developers to submit their test products for independent evaluation or by providing huge investments for greater

collaboration. As similar initiatives and knowledge sharing become available, including collaborative technological advancements, it is likely that the COVID-19 diagnostic market will continue to thrive well into the future.