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COVID-19 Testing – Between Diagnostic Accuracy, False Positives, And Human Gatherings

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Abstract:

COVID-19 is a highly communicable disease. The outspread of the novel coronavirus and the increasing incidence required emergency approval of several diagnostic testing methods. This study covers the types of available testing options and their accuracy. There are mainly two types of tests for detecting COVID-19 infection; diagnostic tests (molecular and antigen tests) that involve active detection of virus in the body, showing active infection. It is of two types antigen and molecular tests. The second type of test is antibody tests. Similarly, a rapid test is also available based on the diagnostic testing techniques that show results in a lesser time, allowing faster detection and easy quarantine of the individual. However, all of them show variable accuracy and precision. That is why slowly lesser accurate techniques were detected and replaced by advanced and accurate ones. Currently, rRT-PCR is the best available test for detecting active infection, and the ELISA test is for the presence of antibodies.

Introduction:

The novel COVID-19 is highly communicable and can be spread through respiratory droplets, airborne aerosols, or immediate contact. (1)To prevent the spread of SARS-CoV-2, health and administrative officials worldwide have prioritized the rapid and adequate distribution of COVID-19 testing resources. Individuals who have COVID-19 symptoms or who have been exposed to people who have suspected or proven COVID-19 sickness should have a COVID-19 diagnostic test. The COVID-19 testing is also recommended for travel, social or professional gatherings, and employers can enforce it at work.(2)For tracking SARS-CoV-2 spread, understanding the epidemiology, disease management, lowering the transmission, and quarantine purposes, fast and reliable testing for COVID-19 is crucial. Early test results aid in making educated suggestions to patients, protecting front-line personnel, and preventing the spread of COVID-19 infection.(3)

The emergence of asymptomatic carriers has altered the symptom-based-diagnostic scenario, emphasizing the need for correct identification of the diseased population to prevent the rapid spread of the virus. Positive cases must be diagnosed quickly to provide timely care to those afflicted and prevent infection from spreading further across the population. For effective molecular identification, samples must be collected at the right time and from the correct anatomical place. (4) However, false-negative diagnosis, especially at this point of the pandemic, might have serious effects by allowing the infected people to propagate the virus, sabotaging efforts to restrict the virus. Additional screening approaches that can detect the infection despite the small viral titers are extremely helpful in ensuring that all COVID-19 patients are diagnosed as soon as possible.(3)

Methodology:

The aim of this study is to present a comprehensive evaluation of commercially available in-vitro diagnostic (IVD) tests for detecting the novel SARS-CoV-2, emphasizing tests approved by the Food and Drug Administration (FDA). Because according to FDA, there are mainly two types of tests for detecting the novel coronavirus:

1. Diagnostic tests
2. Antibody tests

Diagnostic tests:

If one has an active COVID-19 infection, diagnostic tests can reveal if you need to quarantine or segregate yourself from other people. There are further two types of diagnostic tests:

a) Molecular tests
b) Antigen tests

Antibody tests:

Antibody testing involves assessing the antibodies produced by our immune system in response to the COVID-19 virus. However, these antibody testingsare not beneficial in the diagnosis of an active COVID-19 infection.

This study aims to determine if commercially accessible, quick, point-of-care molecular and antigen tests are valid enough to detect COVID-19 infection accurately and if any accuracy differs between those who have and do not have symptoms.

Results:

Several in vitro diagnostic tests for SARS-CoV-2 were given the Emergency Use Authorization (EUA) by the FDA on the emergence of this novel coronavirus. They are chiefly divided into two types. Active COVID-19 infection can be diagnosed using a variety of SARS-CoV-2 in vitro diagnostic techniques (i.e., if there is an actively dividing virus). By using saliva or throat or nasal swab, the samples for this diagnostic test can be gathered. Both sputum and broncho-alveolar fluid come from our lower respiratory tract and are sometimes utilized as confirmatory testing. Assays that evaluate the appearance of antibodies to SARS-CoV-2 in serum from a finger prick or veins are called serology or antibody tests. If antibodies are present in the blood samples, it indicates that the individual has already been exposed to the virus or has a compromised immune system. (2) On the other hand, rapid tests are also introduced based on the same diagnostic testing technique. People who are suspectedof COVID-19 infection need to know their status as infected as fast as possible so that they can isolate themselves, seek treatment, and notify those in their immediate social circle. RT-PCR, a laboratory test that requires specialized equipment and at least 24 hours to give a result, is usually used to confirm the infection with COVID-19. However, patients with and without symptoms could potentially benefit from rapid point-of-care tests that could be used outside of hospital settings.(5)

Discussion:

The Food and Drug Administration (FDA) cautions medical professionals and lab workers about the possibility of receiving a falsely positive result from any laboratory test. Even highly accurate tests, employed to screen the communities with a low prevalence of infection, laboratories governing tests should expect some false-positive results.(6) This study was designed for the comprehension of the accuracy of COVID-19 testing methods and false positives.

Antigen and molecular tests to detect the active COVID-19 infection:

A host is necessary for the replication of viruses. The virus enslaves the cells of the host in order to multiply its virions. While the coronavirus continues to replicate and reproduce, its genetic material, ribonucleic acid (RNA), is still present in the body. A COVID-19 infection can be diagnosed using diagnostic tests that seek evidence of the replication process—that is, proof that new viruses are being produced. Detection of antigens, tiny proteins that form the virus, in a patient's sample is the primary goal of antigen diagnostic testing.However, molecular assays amplify viral RNA so that a specialized test can be used to identify the presence of a virus. Nucleic acid amplification tests are another name for this type of test (NAAT). A sample of nasal or oral fluid (saliva) from an infected person is taken to check for the presence of the virus. A molecular diagnostic technique can detect millions of copies of SARS-CoV-2

even if the virus is at a very low level in the sample. Diagnostic tests in this area include polymerase chain reaction (PCR) testing, loop-mediated isothermal amplification (LAMP), and CRISPR-based assays. One of the advantages of molecular diagnostics is that results can be obtained more quickly than with classic PCR procedures. LAMP is one of the several quick molecular assays that can give results in minutes rather than hours. ' Since the genetic material in the patient sample is amplified during a rapid molecular test like LAMP, it is both specific and sensitive. Aside from antigen testing, several rapid molecular diagnostic techniques can deliver results in just a few minutes.(7)Following are different types of molecular and antigen diagnostic tests for COVID-19 that are commercially available:

Test	Duration	Explanation
RAT	15-30 minutes	Works by detecting the presence of a specific viral protein and displays results that the eye can easily read.
LAMP	15-60 minutes	It detects the active COVID-19 infection by targeting the gene sequence of SARS-CoV-2. It gives a qualitative result of either presence of infection or no infection.
RPA	15-60 minutes	It also detects the active COVID-19 infection by targeting the gene sequence of SARS-CoV-2. However, it is dependent on the recombinase enzyme. It also gives a qualitative result of either presence of infection or no infection.
CRISPR based diagnostics	15-60 minutes	This test is usually coupled with LAMP. However, it also detects active infection by detecting the genetic sequence of the COVID-19 virus. Its results are easily visible.
rRT-qPCR	2-4 hours	This tests the active infection of COVID-19 with a very low limit of detection. It can give both qualitative and quantitative results.

Antibody tests for the detection of COVID-19:

The goal of antibody assays is to detect antibodies or immunoglobins (Ig) produced by our immune system in response to the coronavirus infection. If a person's SARS-CoV-2 test results come back positive, they may have been exposed. IgM antibodies may show active or recent infection. When IgG antibodies are identified later in infection, they frequently imply a previous infection, but this does not rule out newly infected people who may still be contagious.For an antibody test to be a reliable and effective screening tool, it must have an extremely high positive predictive value (PPV). Prevalence of disease and accuracy of the test (sensitivity and specificity) are critical factors in PPV.(8)the four main types of antibody tests for detecting the COVID-19 virus are rapid diagnostic tests, neutralization assays for antibodies, enzyme-linked immunosorbent assays (ELISA), and chemiluminescent immunoassays. SARS-CoV-2 antibodies cannot currently be detected using a routine antibody test during or following exposure or infection. At first, the antibody tests for SARS-CoV-2 are observed to have a low precision but grow in the second and third weeks after exposure. As a result, current data on antibody testing has several flaws and is prone to bias. Several antibody tests present a high false-negative detection rate. Also, there are high chances of biasness in the selection of participants, application of index tests and the standard utilized as a reference, timing for antibody testing that could inaccurately report the validity of COVID-19 antibody tests.(9)

False-positive results of different types of COVID-19 detecting tests:

To reduce the number of incorrectly tested cases, every available assay's accuracy, precision, and diagnostic capacity must be interpreted considering the background prevalence of coronavirus infection in the areas where they are employed.(10)False positives can occur in a variety of ways in practice. Samples can be mixed up, and several software difficulties can lead to incorrect interpretations of test results, and data entry and communication errors might occur. Due to the fragmentation of the target genetic material by PCR tests, minute amounts of contamination can generate false, barely distinguishable results from true positive results. Contamination at such low levels might be difficult to manage.(11)

A study reported that independent quality studies of PCR tests for other viruses comparable to the coronavirus indicated that half of them had false-positive rates ranging from 0.8 to 4.0 %, with an average of 2.3 %. Later on, data from a few independent quality assessments of diagnosticcoronavirus tests showed false-positive rates varying from under 0.4 percent to 0.7 percent. However, real-world COVID-19 PCR test findings were double-checked with other assays. The false-positive rate in the majority of these was between 0.2 and 0.9 percent. These rates may appear low, but even a minor false-

positive rate can significantly reduce the dependability of positive test results when infection rates are low.(11) Moreover, another study reports that the SC2-RAT had a sensitivity and specificity of 87.9% compared to the 98.5% of RT-PCR.(12)According to another study, IgG-IgM-based ELISA assays had the highest diagnostic test accuracy in overall antibody testing. Furthermore, a combined IgG/IgM test, regardless of method, appears to be a superior choice in terms of sensitivity than assessing either antibody type separately. This study reports that the specificities of all tests were high, ranging from 0.969 to 0.999. IgG-based CLIA had the best sensitivity among the tested methods, indicating that it correctly detected most infections indicated by rRT-PCR. In comparison to LFIA, ELISA and CLIA tests fared one step higher in terms of sensitivity. Except for the ELISA, IgG-based tests fared better than IgM-based testing.(3)

Conclusion:

The timely and accurate detection of the coronavirus infection is essential for preventing the disease's severity and spread. Although many tests are available as per the approval of the FDA, they have variable detection accuracy. Among them, rRT-PCR is accurate for detecting active infection, and the ELISA tests are better for antibody-based tests.

References:

1. Asai T. COVID-19: accurate interpretation of diagnostic tests-a statistical point of view. J Anesth. 2021;35(3):328-32.

2. Benda A, Zerajic L, Ankita A, Cleary E, Park Y, Pandey S. COVID-19 Testing and Diagnostics: A Review of Commercialized Technologies for Cost, Convenience and Quality of Tests. Sensors (Basel). 2021;21(19).

3. Vengesai A, Midzi H, Kasambala M, Mutandadzi H, Mduluza-Jokonya TL, Rusakaniko S, et al. A systematic and meta-analysis review on the diagnostic accuracy of antibodies in the serological diagnosis of COVID-19. Syst Rev. 2021;10(1):155.

4. Islam KU, Iqbal J. An Update on Molecular Diagnostics for COVID-19. Frontiers in cellular and infection microbiology. 2020;10:560616.

5. Dinnes J, Deeks JJ, Berhane S, Taylor M, Adriano A, Davenport C, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 2021;3:CD013705.

6. FDA. Potential for False Positive Results with Antigen Tests for Rapid Detection of SARS-CoV-2 - Letter to Clinical Laboratory Staff and Health Care Providers. Food and Drug Administration. 2020.

7. toolkit C-t. Antigen and Molecular Tests [Available from: https://www.centerforhealthsecurity.org/covid-19TestingToolkit/testing-basics/types-of-COVID-19-tests/antigen-and-molecular-tests.html.

8. Mathur G, Mathur S. Antibody Testing for COVID-19. Am J Clin Pathol. 2020;154(1):1-3.

9. Kopel J, Goyal H, Perisetti A. Antibody tests for COVID-19. Proc (Bayl Univ Med Cent). 2020;34(1):63-72.

10. Mouliou DS, Gourgoulianis KI. False-positive and false-negative COVID-19 cases: respiratory prevention and management strategies, vaccination, and further perspectives. Expert Rev Respir Med. 2021;15(8):993-1002.

11. Cohen AN. False Positives in PCR Tests for COVID-19 2020 [Available from: https://www.icd10monitor.com/false-positives-in-pcr-tests-for-covid-19.

12. Jung C, Levy C, Varon E, Biscardi S, Batard C, Wollner A, et al. Diagnostic Accuracy of SARS-CoV-2 Antigen Detection Test in Children: A Real-Life Study. Front Pediatr. 2021;9:647274.