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Impact of COVID-19 on the development of detection methods: a narrative review.

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Abstract
COVID-19 is without a doubt the most extensively researched infectious disease in history, with over 130,000 PubMed and NCBI citations between January 2020 and September 2023. This compares to around 8,000 citations for influenza and 10,000 citations for tuberculosis during the same period. The value of molecular diagnostics in COVID-19 assessment in terms of lowering morbidity and death is widely acknowledged. This review assesses the impact of COVID-19 on the development of detection methods and emphasizes the specificity and sensitivity of PCR testing.

Keywords: Covid-19, PCR, diagnostics, antigen, pooling, CT

1. Introduction

Engineers and medical practitioners have collaborated for decades to build equipment and instruments that help doctors diagnose diseases faster. The transition from time-consuming imaging and cell culture-based diagnostics to rapid high-throughput genomic and protein analysis was made possible because to this concentrated effort. These approaches have been improved in recent years by the incorporation of robotic sample handling and preparation, as well as artificial intelligence-based data analytics (AI). These advancements have cut across all sectors of medicine as platform technologies, increasing patient care [1]. While many of these breakthroughs were prompted by cancer and heart disease, with the emergence of COVID-19, some technologies were immediately repurposed to combat the global epidemic. Furthermore, many university research groups have shifted their focus from basic science to more applied research in response to a clear worldwide demand. However, the medical and financial requirements for these two types of diseases, which range from chronic illnesses to acute infections, are vastly different. As a result, it became clear that some assays were more suited to this transition than others [2-3].

2. Sensitivity and specificity

Sensitivity and specificity are the two most important features to consider when evaluating a diagnostic sensor. The genuine positive rate, or, in the case of a diagnostic, the proportion of sick individuals who test "positive," is referred to as sensitivity. The true negative rate is calculated by multiplying the fraction of healthy people who test "negative" by the specificity. Many factors, including the detecting mechanism, sample type, and sample preparation requirements, can influence these parameters depending on the specific diagnostic test [4]. In China, the sensitivity of the RT-PCR diagnostic test was estimated to be 0.707 (95% CI: 0.668, 0.749), while the specificity was 0.851 (95% CI: 0.774, 0.941) [5-6].

3. Detection methods for Covid-19

It takes years to translate a technology from a research setting to usage in patient care. During the spring and summer of 2020, however, this process was hastened by changes in regulatory processes and increased government financing to confront the immediate societal threat posed by COVID-19. This funding fueled diagnostic development in both university and industrial research labs.

Other coronaviruses, like as SARS and MERS, already had diagnostic and antibody testing capabilities at the time of the COVID-19 epidemic. Developing, validating, and mass-producing assays for the detection of SARS-CoV-2 in symptomatic and asymptomatic persons, on the other hand, provided a considerable challenge that necessitated large-scale international and multistakeholder cooperation.

Most of COVID-19 nucleic acid tests use on reverse transcription (RT) to convert viral ribonucleic acid (RNA) to deoxyribonucleic acid (DNA), and then polymerase chain reaction to amplify the DNA concentration (PCR). The number of stages and specific methodologies utilized to obtain the final DNA read-out distinguish the various types of nucleic acid–based testing. However, all RT-PCR techniques have the drawback of being unable to detect earlier infections. SARS-CoV-2 PCR recognizes and copies target areas in the SARS-CoV-2 genome using a SARS-CoV-2 primer and probe combination. As an internal control or standard, a second primer and probe set that detects human RNase P (RP) can be employed [7]. After that, PCR techniques are used to amplify the SARS-CoV-2 markers and control targets. Earlier experiments iterated similar steps, converting RNA to complementary DNA (cDNA) first, then amplifying and detecting that cDNA using fluorescence or barcoding techniques. It was able to develop a test that could be conducted in a single vial by optimizing testing parameters and sample pretreatment. This rapid RT-qPCR test has several benefits, the most important of which is that it reduces the amount of reagent required, reducing resource constraints on test capacity, facilitating a faster Turnaround Time (TAT), improving the effectiveness of quarantine procedures, and allowing testing at the point of care (POC). RT-PCR, in particular, may diagnose both symptomatic and asymptomatic patients. [8-9].

3.1. LAMP Test

Loop-mediated isothermal amplification (LAMP) technique is a type of RT-qPCR that has gained popularity in the last year. RT-LAMP uses a DNA polymerase that isothermally melts and synthesizes DNA, similar to several RT-qPCR procedures. It also has an RNA transcriptase, which allows reverse transcription and DNA amplification to happen at the same time [10]. DNA amplification will occur if the unique viral gene targeted by the assay is present in the sample. This increase in DNA lowers the pH of the sample, resulting in a color shift that can be detected with a cell phone without requiring laboratory processing. However, the reagents are unreliable and difficult to get at this moment, restricting their usage [11].

3.2. Antigen Test

SARS-CoV-2 viral proteins are detected in respiratory samples using the fast antigen COVID-19 test, which is an immunoassay. The test uses a sandwich assay, which involves mixing the sample with a solution that contains two antibodies. If SARS-CoV-2 viral proteins are present, both antibodies will bind to them. The first antibody will bind the viral protein to a detection sheet, and the second antibody will tag the viral protein for fluorescence detection. The antigen tests take longer to develop and validate than the RT-PCR tests because they require a SARS-CoV-2-specific antibody pair. While antigen testing is useful for identifying likely infectious persons or those in a group with a high number of positive cases, RT-PCR assays remain the gold standard due to their better sensitivity, specificity, and scalability [12]. Antigen testing, on the other hand, has lately grown in popularity due to its low cost, quick turnaround time, and ability to be delivered at the point of care (POC) without the use of complicated equipment [13].

3.3. Antibody testing

The COVID-19 antibody test is an immunoassay that identifies IgG and IgM antibodies produced by the immune system in response to SARS-CoV-2, as opposed to antigen assays. Antibody-based diagnostics, unlike

previously discussed procedures, are performed utilizing patient blood samples, often at the point of care, and findings can be available within 30 minutes. The material flows across a substrate tagged with multiple capture IgG and IgM antibodies specific to SARS-CoV-2, as well as a control, in antibody-based assays [14]. The antibodies are labeled with fluorescent tags that allow the SARS-CoV-2 antibodies to be detected in a fluorescence-based test. This method allows the result to be read using the same instruments as a typical enzyme-linked immunosorbent test (ELISA) [15].

3.4. „Pooling“

The demand for PCR-based testing often surpassed capabilities because to the pandemic's magnitude and influence on industry and supply chains. As a result, unique testing methods have been implemented. Pooled testing is one of the most often utilized procedures since it allows for the monitoring of entire populations for epidemics while lowering the cost and number of tests necessary. Pooled testing is not dependent on the diagnostic method employed; nonetheless, due to the availability and advantages of RT-qPCR assays, pooled RT-qPCR was the most common. Pooled RT-qPCR testing is similar to regular RT-qPCR testing [16]. except that samples from numerous patients are mixed and evaluated using RT-qPCR at the same time. Individual samples must be analyzed separately if SARS-CoV-2 is detected in the pooled sample to determine the source of the positive finding. As a result, it is more appropriate when the probability of a positive result is low, such as when illness prevalence in the population is low, to minimize repetitive testing [17]. Beyond the current COVID-19 pandemic, the concept of pooled testing as a surveillance and monitoring technique has a wide range of applications. In the future, this strategy could be employed to contain extremely contagious infections. Because of its great sensitivity, RT-qPCR can detect both symptomatic and asymptomatic cases, and because it can be tailored to detect the RNA or DNA of any pathogen, this testing approach may find various applications in the future [18].

3.5. Chest computed tomography (CT)

As previously stated, RT-qPCR of viral nucleic acid is considered the gold standard, but its false-negative rate and limitations must be taken into account due to the serious ramifications of a missed diagnosis in such circumstances. Chest computed tomography (CT) examination can be an indispensable assistant diagnostic procedure for individuals with a high clinical suspicion of infection, even if RT-qPCR screening is negative, because it is timely, simple to perform, readily available, more sensitive, and can quickly detect lung injuries and make imaging diagnoses at an early stage. The typical CT imaging shows ground glass opacities, crazy-paving pattern, and subsegmental consolidation, especially in the lower lobes, and the CT shows 5 stages, including ultra-early stage, early stage, rapid progression stage, consolidation stage, and dissipation stage, depending on the time of onset and the body's response to the virus [19]. According to certain studies, CT had a lower percentage of missing COVID-19 diagnoses (3.9 percent, 2/51) and had a sensitivity higher than RT-PCR (98 percent vs. 71 percent , respectively). These findings support the use of this test to identify infected patients at the proper moment, allowing them to be isolated and treated. Even with all of these benefits, CT scans have significant flaws, such as indistinguishability from other viral pneumonia with 60–70% specificity and aberrant CT imaging slowness [20].

4. Variables and factors which can affect sensitivity and specificity

The accuracy of molecular assays is affected by the location and quality of sampling, the severity of disease, virus clearance rate, and frequency. Molecular test design variables such as genes targeted and reliance on amplification from many targets must also be taken into account. For these reasons, no test will ever be 100 percent accurate, and the lack of a gold standard against which to assess future performance only compounds the problem [21]. False negatives and positives can be produced using the elements provided. False negatives can lead to infected people circulating in the population, unintentionally perpetuating the pandemic; false positives can be caused by insufficient sample loads, sampling people too early or too late in the genetic disease cycle, or viral genome degradation; and false positives can be caused by insufficient sample loads, sampling

people too early or too late in the genetic disease cycle [22]. Reagent contamination, such as primer contamination or contamination during sample collection and processing, can result in false positives. As a result, it's possible that the number of SARS Cov cases has been underestimated. This portion of the study will look at some of the other relevant parameters related to the sensitivity of these molecular tests that have been highlighted [23].

5. Conclusion

The importance of providing alternate, faster regulatory channels to inspire and catalyze the development of assays has been established by the COVID-19 testing response. The capacity of laboratories to quickly validate pre-existing techniques for SARS-CoV-2 detection, as well as less well-established approaches such as RT-LAMP, resulted in the development of instruments that would have taken years to build otherwise. Advances in signal processing and statistical approaches, in addition to sensor technology, have allowed the area of photonic diagnostics to progress. The influence was particularly noticeable when pooled testing was implemented and automated data analysis technologies were used. This pandemic showed that we need more interaction between doctors and bioengineers especially in less developed countries. Technologists must rethink where sample collection will take place in the future, as well as the user's level of knowledge. Miniaturization and automation of equipment, as well as cost savings, will be critical concerns in the eventual translation from the lab to patient care. Nonetheless, the COVID-19 pandemic has demonstrated the importance of leveraging and adapting existing diagnostic platforms to quickly increase accessibility and availability, as well as continuing to develop emerging technologies that provide new advantages in sensor sensitivity and specificity, as diagnostic technologies advance.

Declaration of competing interest

"The author declares that he has no known financial or non-financial competing interests in any material discussed in this paper."

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