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SARS-CoV-2 (COVID-19) Diagnostic Tools: New Advancements and New Tools

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Abstract:

Background: SARS-CoV-2, the virus responsible for the COVID-19 pandemic, represents a significant challenge in terms of diagnostics due to its rapid spread and the emergence of various variants. Initially identified in December 2019 in Wuhan, China, SARS-CoV-2 has led to a global health crisis, prompting the need for efficient and accurate diagnostic tools. Early detection is crucial for controlling the spread of the virus, necessitating continuous advancements in diagnostic methodologies.

Aim: This article aims to review the current state of SARS-CoV-2 diagnostic tools, focusing on novel advancements and emerging technologies that promise to improve detection accuracy, speed, and cost-efficiency.

Methods: The article synthesizes various diagnostic approaches, including traditional nucleic acid-based methods like RT-PCR, protein-based tests such as antigen and antibody assays, imaging techniques like CT scans, and innovative CRISPR/Cas systems. Studies comparing the sensitivity, specificity, and application of these methods in different clinical settings are discussed.

Results: While RT-PCR remains the gold standard for SARS-CoV-2 detection, new tools, including CRISPR-based diagnostics and lateral flow immunoassays, have shown potential for rapid and accurate detection. Antigen tests offer fast results but lack sensitivity in later stages of infection.

Imaging techniques like CT scans provide supplementary diagnostic information, although they are not standalone solutions. Recent advancements in diagnostic technology emphasize the need for multimodal approaches to improve detection accuracy.

Conclusion: The ongoing development of diagnostic tools for SARS-CoV-2 is crucial in the fight against COVID-19. Although RT-PCR remains the standard, the emergence of newer methods, such as CRISPR-based diagnostics and antigen tests, offers promising alternatives for faster and more accessible detection. A multimodal diagnostic approach will likely become the standard for comprehensive COVID-19 diagnosis.

Keywords: SARS-CoV-2, COVID-19, diagnostic tools, RT-PCR, CRISPR, antigen tests, antibody tests, CT scans, lateral flow immunoassay.

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Introduction:

The seventh coronavirus known to infect humans is SARS-CoV-2, which is also the cause of the current COVID-19 epidemic. SARS-CoV-2 has spread widely, in contrast to its predecessors [1,2]. Initially discovered in 2002, SARS-CoV affected 8,000 people and had a 10% fatality rate before being contained in 2004. Approximately 30% of deaths were caused by the Middle East Respiratory Syndrome Coronavirus (MERS-CoV), which surfaced ten years later [3]. By early January 2020, a novel coronavirus was found to be the cause of several pneumonia cases in China that had been reported by December 2019 but had no known cause. Later, this virus was identified as SARS-CoV-2 and proved to be the cause of COVID-19 [4,5,6]. The World Health Organization (WHO) declared COVID-19 a pandemic in March 2020 after the virus quickly spread over the world despite massive efforts to contain the outbreak in China [7,8]. Due mainly to changes in the spike protein, SARS-CoV-2 has since developed into a number of variations, including alpha, beta, gamma, delta, omicron, and others.

Genetic Composition of SARS-CoV-2

There are 14 open reading frames (ORFs) in the SARS-CoV-2 genome, which code for 27 viral proteins [9]. RNA-dependent RNA polymerase (RdRp), an enzyme necessary for viral replication, is encoded by the ORF1ab region [10]. The spike (S) glycoprotein, small envelope (E) protein, matrix (M) protein, and nucleocapsid (N) protein are among the major structural proteins that the viral genome encodes [11]. The spike protein's S2 domain enables membrane fusion, while its S1 domain aids in binding to the angiotensin-converting enzyme 2 (ACE2) receptor [12]. The existence of a functional polybasic (furin) cleavage site at the S1–S2 junction and the virus's unique capacity to bind to human ACE2 receptors with high affinity both contribute to its increased capacity to infiltrate host cells [13]. The most changeable part of coronaviruses is their receptor-binding domain (RBD) in the spike protein, which controls their affinity for ACE2 receptors on the surface of the host cell [14]. Protease activity, including furin, is facilitated by SARS-CoV-2's strong binding affinity for ACE2 receptors and polybasic cleavage site, which maximizes viral entry into host cells [15].

Sampling Methods for SARS-CoV-2 Diagnosis

Accurate sample collection from specific tissues or organs is essential for the diagnosis of SARS-CoV-2 in order to guarantee exact detection and prompt treatment action. Along with oropharyngeal swabs, saline washes, and nasopharyngeal washes, which target the upper respiratory tract, nasopharyngeal swabs (NPS) are frequently used for sample collection. Sputum,

tracheal aspirates, and bronchoalveolar lavage fluid (BLF) are examples of lower respiratory tract sample techniques [16]. In a comparative investigation, Tapia et al. used quantitative RT-PCR to assess NPS, nasal mid-turbinate swabs (NMTS), and saliva samples. The study found that the preferable use of NPS for clinical diagnoses was supported by positivity rates of 96.4%, 85.7%, and 78.6% for NPS, saliva, and NMTS, respectively [17]. Although antigen detection tests, which may also be performed using urine samples, are used in serological diagnostic techniques, their limited sensitivity restricts their utility as a diagnostic standard [18,19]. Both oropharyngeal and nasopharyngeal specimens are taken in a single tube to increase diagnostic precision. One swab is utilized for both nostrils for doing anterior nasal sampling [20].

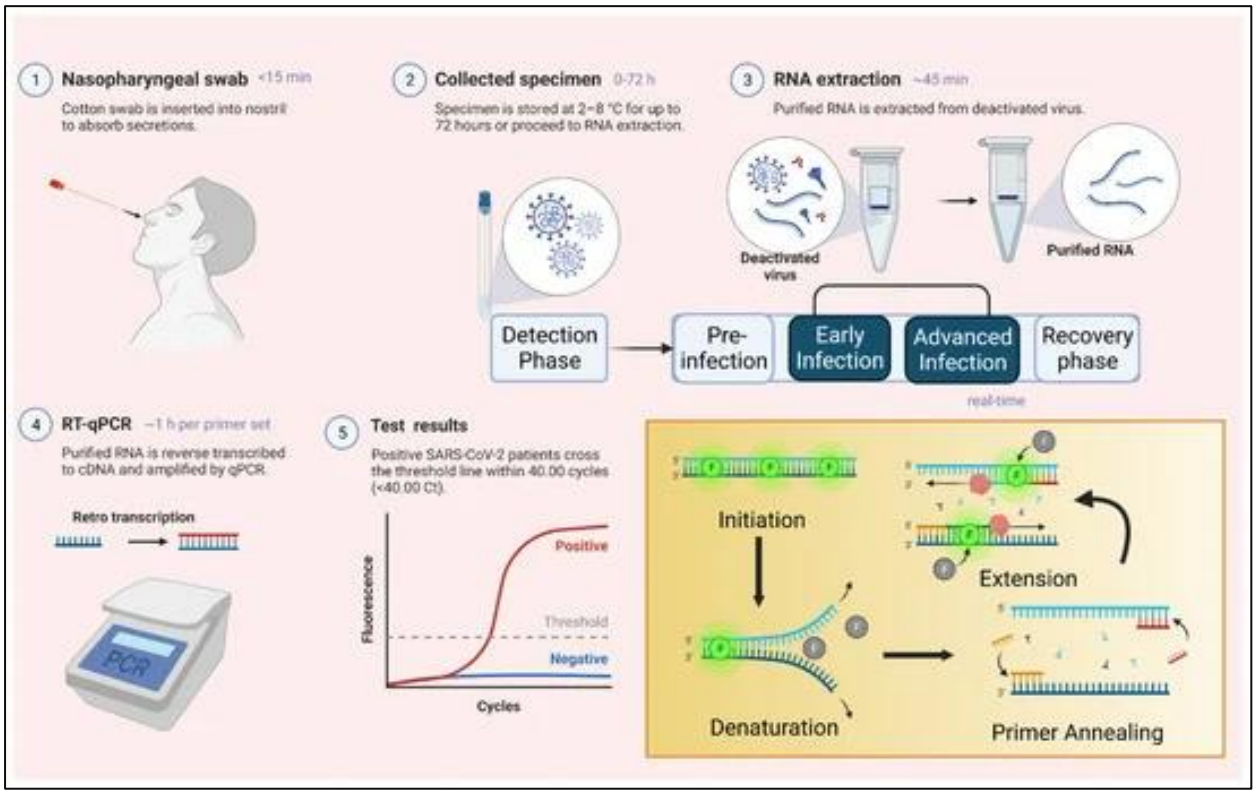

Figure 1: Mechanism of COVID-19 RTPCR.

Diagnostic Techniques for SARS-CoV-2: Clinical Importance and Efficacy

The accurate and timely diagnosis of SARS-CoV-2 infections plays a pivotal role in mitigating the spread of COVID-19. Although nucleic acid-based testing is regarded as the gold standard, challenges related to sensitivity, affordability, efficiency, and capacity have prompted the development of alternative diagnostic strategies [22,23]. This section discusses the primary diagnostic modalities currently employed for SARS-CoV-2 detection.

Nucleic Acid-Based Tests:

The gold standard for identifying SARS-CoV-2 is the reverse transcription-polymerase chain reaction (RT-PCR). Through a multi-step procedure that includes sample collection (usually using nasopharyngeal swabs), RNA extraction, reverse transcription to complementary DNA (cDNA), amplification, and detection, this approach detects and amplifies the genetic material of the virus [22,24]. In order to distinguish SARS-CoV-2 from its predecessors, the World Health Organization's procedure, which was created in early 2020, places a strong emphasis on primers that target the E and RNA-dependent RNA polymerase (RdRp) genes for specificity [25]. While

the U.S. Food and Drug Administration (FDA) has released guidelines targeting the N gene, other primers targeting the ORF1 and N genes have also been created in places like China, Hong Kong, and Japan [10,27-29]. Even while RT-PCR is dependable, it has drawbacks. As demonstrated by systematic reviews that documented up to 54% false-negative rates in initial testing and case studies of false positives despite normal CT findings and negative antibody tests, sensitivity problems might result in false-negative or false-positive outcomes [30–33]. Both the presence of detectable viral RNA and the quality of sample collection are critical factors in RT-PCR accuracy. To confirm infection and improve diagnostic reliability, complementary diagnostic techniques like as CT scans and serological testing are frequently advised [34]. According to a comparative research, the sensitivity of RT-PCR was 64%, which is lower than the sensitivity of viral culture (84%) [36]. Newer technologies, including the Cobas 6800, have shown higher sensitivity, identifying cases that RT-PCR missed because of undiscovered RdRp and N genes [37].

Computed Tomography (CT) Scanning:

By obtaining cross-sectional lung pictures, non-invasive chest CT scans are a valuable diagnostic tool that radiologists evaluate for anomalies. Bilateral and peripheral ground-glass opacities are common lung abnormalities seen in COVID-19 patients in the early stages (0–4 days), which progress to irregular patterns and lung consolidation as the disease worsens [39–41]. Although CT scans have a high sensitivity (86–98%), their specificity is limited because their results may overlap with those of other viral pneumonia cases [42]. Compared to RT-PCR, CT has a lower probability of false-negative results (9%) but is more expensive per diagnostic usefulness, according to comparative studies [43]. Despite its advantages, CT is typically used in conjunction with RT-PCR to offer a thorough diagnosis [45].

Protein-Based Tests:

Protein-based diagnostics use antigen-antibody interactions to find viral proteins, glycans, or antibodies. In contrast to RT-PCR, these assays are quick, easy to use, and need little experience, which makes them a desirable substitute in environments with limited resources [46–48]. The main targets of antigen-based assays are SARS-CoV-2-specific spike, glycan, and nucleocapsid proteins. The first week of infection is when these tests show the highest sensitivity (80%), which drops to 76% in the second week and 19% in the third. In contrast, the sensitivity of antibody-based testing is low (26.8%) in the early stages of infection but increases to 76% by the 14th day and reaches 95–100% by the fourth week [50–53]. While antibody testing indicate immune responses after recovery, antigen tests identify the virus during its most virulent phase [51,52]. Overall, even though every diagnostic technique has special benefits, using them all together improves diagnostic precision and makes it possible to treat COVID-19 clinically.

Antibodies are included in diagnostic procedures to provide quick, simple, and highly sensitive results. The first line of defense against many viral infections is immunoglobulin M (IgM) [62]. Nevertheless, IgG and IgM-based assays have intrinsic limitations even though they are widely used and accepted. In particular, their efficacy is usually only noticed 5–10 days following the onset of symptoms [63]. A number of additional diagnostic methods are also used, such as antigen testing, CRISPR/Cas systems, and reverse transcription-polymerase chain reaction (RT-PCR). Because of its specificity, quick reaction time, and capacity to identify IgG and IgM antibodies in as little as 15 minutes, the lateral flow immunoassay (LFIA) approach is unique among them [58,64]. Acceptable sensitivity and specificity rates of 88.66% and 90.63%, respectively, are demonstrated using the LFIA approach [64]. Notwithstanding its benefits, there

are drawbacks to the approach. The intricacy of multi-step processes, ineffective protein immobilization, and irregular protein–probe conjugation are some of the major causes of false-negative outcomes [65].

According to a study that used the RIAT technique to evaluate the false-positive outcomes of an IgG-mediated diagnostic kit, 57% of patients had false positive results. Human common cold coronavirus pneumonia was the final diagnosis made for these patients [66]. This result emphasizes the unpredictability of diagnostic results. Furthermore, determining the stages of an infection depends heavily on the detection of antibodies, particularly IgG, IgM, and IgA. After being exposed to SARS-CoV-2, IgM levels usually increase during the first week, but IgG levels rise during the second week and continue to climb for a long time. On the other hand, IgA levels become detectable 4–10 days after infection, while IgM levels tend to decrease over time [47]. These changes in antibody levels may be used as markers to track the course of the illness. To improve diagnostic sensitivity and specificity, diagnostic kits that can detect numerous immunoglobulins at once have been created. For example, Li et al.'s fast immunoassay showed better diagnosis accuracy when it detected both IgG and IgM at the same time [58]. Comparable increases in sensitivity and specificity have also been observed in tests that combine the detection of IgA, IgG, and IgM [69].

The CRISPR/Cas System

A cutting-edge gene-editing technique that is widely used in many scientific fields is the CRISPR/Cas system (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated gene). When used in conjunction with amplification procedures, its ability to target DNA or RNA makes it very helpful for the simultaneous detection and measurement of viral loads in isolated samples [59]. CRISPR-based diagnostic methods are very effective, quick, portable, and economical when it comes to SARS-CoV-2 detection [70]. CRISPR-based kits specifically designed for COVID-19 detection have been released by innovative firms like Mammoth Biosciences and Sherlock Biosciences. Sherlock Biosciences' SHERLOCK (Specific High Sensitivity Enzymatic Reporter Unlocking) targets the S gene and Orf1ab sequences of SARS-CoV-2, while Mammoth Biosciences' DETECTR (DNA Endonuclease-Targeted CRISPR Trans-reporter) targets the N and E genes using a CRISPR-Cas12-based lateral flow system [60]. Multiple target gene sequences can be detected simultaneously thanks to the improved SHERLOCKv2 [71].

Cas endonucleases are used in the diagnostic process of the CRISPR/Cas system to perform collateral cleavage activity. By identifying a sequence complementary to the spacer region close to a protospacer adjacent motif (PAM), CRISPR RNA (crRNA) directs effector complexes to the target gene [72]. Hou et al. created a CRISPR-based diagnostic kit that showed quick results in 40 minutes, excellent specificity, and single-copy sensitivity [73]. In just 50 minutes, a different approach that Huang et al. tested used the CRISPR-Cas12a/gRNA complex with a fluorescent probe to achieve sensitivity down to two copies per sample—results that were comparable to those of conventional RT-PCR [74]. Single-nucleotide specificity, thermocycling independence, and smooth integration with lateral flow strips are some of the benefits that CRISPR has over RT-PCR [61]. CRISPR technology has drawbacks despite its advantages, most notably off-target consequences. These can result in inaccurate predictions when nucleic acids bind with non-target sequences. To lessen these impacts, screening techniques like DISCOVER, Digenome-Seq, and SELEX can be used [75]. Studies show that Cas9's affinity for non-target DNA strands is greater than the rehybridization forces of DNA, which results in off-target interactions. To combat this, researchers have discovered mutants that decrease off-target binding, like S845K

and L847R [76]. To preserve protein efficiency while reducing off-target effects, structural changes have been investigated, such as adding mutations like Mut268 in Staphylococcus aureus Cas9 (SaCas9) [77].

Limitations of Current Testing Tools

The correct collection of respiratory tract samples during the preanalytical step is essential for an accurate molecular diagnosis of COVID-19. This entails collecting specimens at the ideal moment and at the appropriate anatomical location. When patients have a known history of possible exposure, radiographic evidence compatible with COVID-19 pneumonia, or clinical signs of viral pneumonia, routine testing becomes very important. Interpreting the consequences of a mistaken diagnosis can be difficult, though, especially if the findings have an impact on choices about social seclusion or patient quarantine. When those participating are also healthcare professionals, this problem is even worse [78,79].

Detecting and Monitoring Late-Stage Severe COVID-19 Pneumonia

Lower respiratory tract specimens, such as those derived from sputum or bronchoalveolar lavage (BAL), are recommended for maximizing the detection of viral antigens. Notably, BAL fluid samples have been found to yield the highest concentrations of SARS-CoV-2 RNA, although no robust comparative data are available to evaluate their effectiveness against nasopharyngeal swabs [80,81].

Assay Selection Challenges

Rapid point-of-care immunoassays, which are mostly based on lateral flow, have become a popular method for detecting SARS-CoV-2. Additionally, high-throughput immunological analyzers for screening at the population level are being developed [82]. Lateral flow assays, which are based on past experiences with influenza viruses, have benefits including quick detection times and inexpensive cost. However, because of their limited sensitivity, they are less effective in the early stages of infection. The development of monoclonal antibodies that target SARS-CoV-2 antigens is now underway; however, given the virus's fast rate of mutation, low antigen levels, or irregular sample processing, questions remain about the system's capacity to identify cases. The usefulness of these markers for prompt disease management is diminished because, although IgM responses happen generally, specific IgG responses may take weeks to appear.

Limitations of Random Amplification Techniques

When SARS-CoV-2 first appeared, deep sequencing technologies—such as next-generation sequencing (NGS) and metagenomic techniques—were essential to documenting the virus. Although these methods have the potential to detect viral changes in the future, their complexity and time commitment make them unsuitable for routine COVID-19 diagnosis at this moment [84]. It is crucial that integrated, random-access, point-of-care molecular diagnostic instruments be developed quickly. These instruments are necessary for accurate, real-time SARS-CoV-2 infection detection, prompt treatment, and infection control. When less contagious mutagenic forms of pneumonia overlap and medical resources are limited, this need is more critical [85]. These diagnostics, which are known for their speed, ease of use, and safety, can be readily implemented in nearby clinics and hospitals that already have the tools needed to provide patient treatment [86,87,88].

Viral Variants and Diagnostic Implications

Sequencing data is essential for tracking the advancement of innovative testing techniques and evaluating the ongoing validity of diagnostic procedures. By changing the genetic or protein targets employed in the assays, virus mutations can impair the precision of these diagnostic instruments [89,90]. Researchers can find clinically significant mutations through routine sequencing data processing, which can subsequently be examined to ascertain how they affect diagnostic precision [91,92]. For example, by identifying the mutations causing test failure, sequencing can assist in identifying mutations when diagnostic tests produce unexpected results, such as repeated false negatives [93,94]. Indirect detection techniques that identify anti-SARS-CoV-2-specific antibodies, which indicate a current or previous infection, and direct detection techniques, such as viral RNA or antigens, are the two categories of diagnostic approaches for SARS-CoV-2 [95]. Polymerase chain reaction (PCR)-based diagnostics and other nucleic acid amplification tests (NAATs) continue to be among the most popular and accurate techniques. To accomplish exact distinction, these assays use primers, which are short DNA sequences made to bind to particular viral RNA targets [96]. False negative results, however, may arise from mutations in the primer-binding areas. In spite of this, the majority of NAATs are made to target many genes, guaranteeing that they will continue to operate even if one of them is mutated [106]. If mutations change the virus's protein or structural properties, they may potentially affect antigen and antibody assays. Numerous mutations, especially in the spike (S) gene, are present in well-studied variants such B.1.1.7, B.1.351, and P.1. The accuracy of diagnosis can be impacted by mutations in the S-gene, such as N501Y and P681H, which can alter protein structures or replication initiation locations. For instance, the B.1.1.7 variant's 69/70 deletion mutation prevents primers that target the S-gene from binding, which causes S-gene target failure or dropout in certain PCR tests, such the Thermo Fisher Taq Path assay. In spite of this, the tests' redundancy of several genetic targets guarantees the preservation of overall diagnostic accuracy [108–110, 114].

The gold standard for identifying the mutations causing novel variants is still quantitative reverse transcription PCR (RT-PCR), however it necessitates regular probe configuration changes and validation to account for new mutations. Examples like the FDA's findings on Cepheid test errors caused by a single mutation emphasize how crucial it is to continuously improve diagnostic instruments [111,112]. According to preliminary research, antigen tests may occasionally have higher positive agreement rates than RT-PCR, although initially being less sensitive (90.0% vs. 73.7%, respectively). When examined during early infection, however, the sensitivity of these techniques decreases; antigen tests have a 50% sensitivity, but RT-PCR and viral culture have 64% and 84% sensitivity, respectively [116,117]. The accuracy of diagnosis has not yet been shown to be significantly impacted by mutations in other genes that encode non-antigenic proteins, however the S-gene poses difficulties. However, monitoring the effects of mutations over the entire genome is still essential to preserving the accuracy of the diagnosis. It could be wise to steer clear of focusing on the S-gene in future diagnostic designs due to its high incidence of mutation [4,89,118]. With advantages like less dependence on specialist equipment and transportation media, saliva-based testing presents a viable substitute for RNA extraction-based techniques. Although this method saves time, it is very dependent on a number of variables, including the efficacy of RT-quantitative PCR, extraction procedures, and sample collection techniques [119,120]. Overcoming the difficulties presented by viral variations will need the combination of many approaches as diagnostic strategies continue to develop.

Prediction Models for Diagnosis and Prognosis of COVID-19:

In order to reduce the strain on healthcare systems and provide patients with the best care possible, efficient diagnostic and prognostic methods for COVID-19 are essential. Medical professionals can prioritize patients according to urgency and the availability of limited medical resources with the use of prediction models that combine multiple factors to identify those who are at high risk of developing a serious infection [121]. From simple rule-based scoring systems to complex machine learning algorithms, a variety of models have been created to help public health initiatives and meet the pressing need for sharing important research findings on COVID-19. Interestingly, a large number of these models have been released on open-access platforms without going through a thorough peer review process [122]. Prediction models specifically designed for the COVID-19 pandemic were thoroughly evaluated. These models seek to assess symptomatic patients, predict clinical outcomes, and identify those who are at increased risk of hospitalization. These models are commonly criticized for having a high risk of bias, even though they have shown good to great prediction skills. Subpar reporting techniques, insufficient methodological rigor in participant selection, statistical processes, and predictor descriptions are the causes of this bias [123,124]. Although prediction models showed almost 100% accuracy in identifying COVID-19, poor reporting and a manipulated combination of patients with and without the virus compromise their validity and may introduce biases [125]. These models are intended to aid in medical decision-making by focusing on particular populations that benefit from the forecasts. In order to build and validate these models, representative datasets—ideally made up of consecutive patients—are essential. Determining the target population is also crucial for assessing the accuracy and generalizability of a model.

In order to promote international and multidisciplinary cooperation in data collecting and model building, the WHO has created a novel data gateway to encourage the exchange of anonymized clinical data [126]. This initiative recognizes the importance of collaboration. Many models have been used to predict COVID-19 diagnoses and outcomes, such as Carr's model, QCovid models, the Robust model, Random Forest, Gradient and RUSBoosting, the PRIEST score, and the ISARIC4C Deterioration model. Hospitalization, treatment, shielding, and intervention decisions can be prioritized with the use of the Robust model [129]. Hospitalization outcomes are predicted by the 4C mortality score, whereas mortality risks are estimated by the QCovid model [130,131]. For suspected COVID-19 cases, the PRIEST score helps determine if referrals to the emergency room are necessary [132]. Notwithstanding their potential, COVID-19 diagnostic and prognostic models are frequently criticized for biases brought on by overfitting, arbitrary rejection of non-conforming patient data, and non-representative patient selection. As a result, performance forecasts could be deceptive and unduly optimistic [133]. Transparency in data sharing and the creation of tools to improve, assess, and update COVID-19-related prediction models are necessary to address these problems in subsequent studies [121,134].

Deep Learning Techniques for Diagnosing Novel Coronavirus

As one of the most dangerous illnesses of the decade, the COVID-19 pandemic has presented an unparalleled threat to civilization [102]. Utilizing smart healthcare systems, recent technological developments have made it possible to create novel instruments for illness detection, prevention, and management [137–139]. Imaging techniques like CT scans and X-rays are essential for diagnosing COVID-19. CT scans are recommended because of their three-dimensional imaging capabilities and adaptability, which make them essential for quick diagnosis and pandemic control, even though X-rays are readily accessible and reasonably priced [140]. Medical image analysis has been transformed by artificial intelligence (AI), especially deep

learning (DL) and machine learning (ML), which has greatly aided in the fight against COVID-19. These technologies reduce the need for human labor while producing high-quality diagnostic outputs [141,142]. Pre-trained or custom-built models are being used to construct DL-based diagnosis tools for COVID-19 using CT and X-ray datasets. These methods, which use both public and private datasets for model training and validation, have helped to increase diagnosis, prognosis, prediction, and epidemic forecasting [143–145]. Through iterative learning procedures that include data collection, analysis, feature selection, classification, and performance evaluation, DL algorithms are highly effective in spotting intricate data patterns [146–148]. These technologies provide the sensitivity, specificity, and accuracy needed to assess methods and enhance diagnostic results [149,150]. Cost-effective, quick, and secure diagnoses have been made possible by the incorporation of DL into radiology equipment, which has improved radiologists' decision-making and decreased errors in crucial situations [151,152]. This demonstrates how DL algorithms have the ability to revolutionize healthcare delivery and enhance therapeutic results.

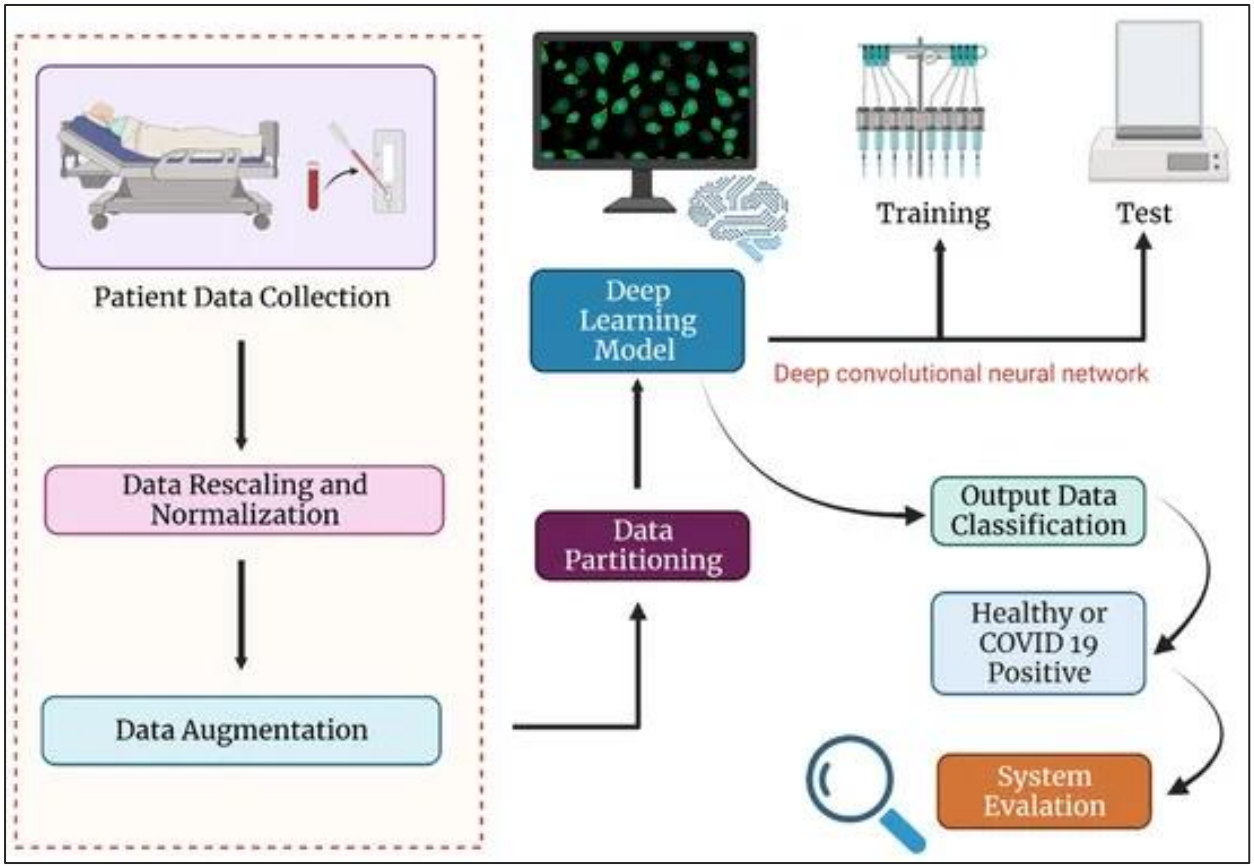

Figure 2: Deep Learning for COVID-19 Diagnosis.

Futuristic Diagnostic Tools and Emerging Research Directions

Rapid, precise, and cost-effective diagnostic methods are required due to the introduction of new COVID-19 variations, repeated waves of infections, and the virus's quick spread. Although RT-PCR is still the gold standard because of its excellent accuracy, it is difficult to meet the needs of large-scale testing because of its labor-intensive and time-consuming nature [22]. Rapid antigen tests and other alternative methods provide in-field diagnosis, but they frequently produce false negative results, highlighting the necessity of confirmatory tests like RT-PCR and other evaluations such chest CT scans for determining the severity of the disease [153,154]. In

the biotechnology and pharmaceutical industries, AI and ML are being used more and more to quickly and accurately identify COVID-19-positive people in congested environments. Rapid bedside diagnostics have been further enhanced by point-of-care testing devices, which allow essential patients to have their disease development tracked [47,155–157]. The promise of cutting-edge diagnostic techniques in the fight against COVID-19 is highlighted by emerging technologies. Extracellular vesicles (EVs), such as exosomes, which facilitate the intercellular trafficking of proteins, enzymes, and viral components, are among the promising developments. Exosomes, which are formed from endosomes, have been linked to the transmission of SARS-CoV-2 by promoting the virus's entry into lung cells through molecules such TMPRSS2 and tetraspanin CD9 [158–160]. These vesicles offer a new diagnostic target because they can be found in a variety of bodily fluids. For example, patients with mild or severe COVID-19 have higher levels of platelet extracellular vesicles (pEVs), which may indicate coagulation problems and organ injury [162]. SARS-CoV-2 components were found in exosomes by Elletra et al., highlighting their function in the spread of infection [163]. Exosome-based diagnostics that make use of CT, PET, MRI, or fluorescent or bioluminescent labeling could reliably identify SARS-CoV-2 and gauge the severity of the illness. However, more work needs to be done on conventional biomarkers and exosome-based diagnostic techniques [164]. Enhancing these technologies will expand their use to additional infectious diseases in addition to improving COVID-19 diagnosis.

Conclusion:

SARS-CoV-2 diagnostic tools have evolved significantly since the early stages of the COVID-19 pandemic. Initially, RT-PCR testing was considered the gold standard due to its high sensitivity and ability to detect viral RNA. However, challenges with test accessibility, time consumption, and sensitivity, especially in asymptomatic or late-stage patients, led to the exploration of alternative diagnostic methods. Among these, antigen tests gained prominence due to their speed and ease of use, although their sensitivity remained an issue, particularly in the later stages of infection. Antibody-based assays provided insights into immune responses and post-infection status, but their utility for early detection was limited. Advancements in CRISPR/Cas technology have introduced innovative, rapid, and potentially cost-effective diagnostic options. CRISPR-based methods, such as the SHERLOCK and DETECTR systems, target specific viral genes and offer high specificity with single-copy sensitivity, making them highly promising for quick diagnostics in resource-limited settings. These systems also demonstrate the potential for simultaneous detection of multiple target genes, further improving diagnostic accuracy. In addition to molecular and protein-based tests, imaging techniques like CT scans are used as complementary diagnostic tools. While CT scans have shown high sensitivity for detecting lung abnormalities typical of COVID-19 infections, their specificity remains lower than that of RT-PCR. Consequently, CT is often used in conjunction with other diagnostic methods to enhance overall diagnostic accuracy. Furthermore, innovations in testing formats, such as lateral flow immunoassays, enable quicker results, making them ideal for point-of-care testing. The integration of these various diagnostic tools is likely to become essential for managing the ongoing COVID-19 pandemic, especially in environments with limited access to healthcare resources. The future of SARS-CoV-2 diagnostics lies in multimodal approaches, where rapid antigen and CRISPR-based tests complement traditional RT-PCR, offering a balance between speed, accuracy, and accessibility. As SARS-CoV-2 continues to evolve, ongoing research into new diagnostic methods will be critical in maintaining effective disease surveillance and control.

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أدوات تشخيص فيروس سارس-كوف-2 (كوفيد-19): التطورات الجديدة والأدوات الحديثة

الملخص:

الخلفية :فيروس سارس-كوف-2، المسؤول عن جائحة كوفيد-19، يمثل تحديًا كبيرًا من حيث التشخيص بسبب انتشاره السريع وظهور العديد من المتغيرات. تم التعرف عليه لأول مرة في ديسمبر 2019 في ووهان، الصين، وقد أدى إلى أزمة صحية عالمية، مما يستدعي الحاجة إلى أدوات تشخيصية فعالة ودقيقة. يعد الكشف المبكر أمرًا بالغ الأهمية للحد من انتشار الفيروس، مما يستدعي تقدمًا مستمرًا في المنهجيات التشخيصية.

الهدف :تهدف هذه المقالة إلى استعراض الحالة الحالية لأدوات تشخيص فيروس سارس-كوف-2، مع التركيز على التطورات الجديدة والتقنيات الناشئة التي تعد بتحسين دقة الكشف وسرعته وكفاءته من حيث التكلفة.

الطرق :تقوم المقالة بتلخيص مختلف الأساليب التشخيصية، بما في ذلك الطرق التقليدية المعتمدة على الأحماض النووية مثلRT-PCR ، واختبارات البروتين مثل اختبارات المستضد والأجسام المضادة، وتقنيات التصوير مثل الأشعة المقطعية(CT) ، وأنظمة CRISPR/Cas المبتكرة. يتم مناقشة الدراسات التي تقارن الحساسية والخصوصية وتطبيق هذه الأساليب في بيئات سريرية مختلفة.

النتائج :بينما يظل RT-PCR هو المعيار الذهبي للكشف عن سارس-كوف-2، أظهرت أدوات جديدة، بما في ذلك التشخيصات المعتمدة على CRISPR واختبارات التدفق الجانبي المناعية، إمكانات للكشف السريع والدقيق. توفر اختبارات المستضد نتائج سريعة، ولكنها تفتقر إلى الحساسية في المراحل المتأخرة من الإصابة. تقدم تقنيات التصوير مثل الأشعة المقطعية معلومات تشخيصية تكميلية، على الرغم من أنها ليست حلولًا مستقلة. تركز التطورات الحديثة في تكنولوجيا التشخيص على الحاجة إلى نهج متعددة الوسائط لتحسين دقة الكشف.

الخاتمة :يعد التطور المستمر للأدوات التشخيصية لفيروس سارس-كوف-2 أمرًا بالغ الأهمية في مكافحة كوفيد-19. على الرغم من أن RT-PCR لا يزال هو المعيار، فإن ظهور أساليب أحدث مثل التشخيصات المعتمدة على CRISPR واختبارات المستضد يقدم بدائل واعدة للكشف الأسرع والأكثر وصولًا. من المرجح أن يصبح النهج التشخيصي متعدد الوسائط هو المعيار لتشخيص كوفيد-19 الشامل.

الكلمات المفتاحية :سارس-كوف-2، كوفيد-19، أدوات التشخيص، RT-PCR، CRISPR، اختبارات المستضد، اختبارات الأجسام المضادة، الأشعة المقطعية، اختبار التدفق الجانبي المناعي.