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Review Article

COVID-19; History, Taxonomy, and Diagnostic Molecular and Immunological Techniques

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In recent years, viral epidemics such as the coronavirus disease (COVID-19) have spread, and this outbreak is thought to be the result of animal-to-human transmission. Hence, accurate diagnostic tests to detect COVID-19 and antiviral antibodies in infected individuals are of utmost importance. This report describes the structure, history, taxonomy, and molecular and immunological techniques for diagnosing this disease. Tests for early diagnosis of COVID-19 depend on the reverse transcription-polymerase chain reaction (RT-PCR). However, tests based on isothermal amplification and clustered regularly interspaced short palindromic repeats (CRISPR)-based methods are promising options. Identifying people whose activated antibodies require serological tests, including enzyme-linked immunosorbent assays (ELISA). The search for efficient, cost-effective, and accurate laboratory techniques that can be used on a large scale continues. The RT-PCR technique is a dominant technique for the detection of viral RNA. Other acidic nucleic-based assays such as isothermal amplification, microarray hybridization, amplicon-based metagenomics sequencing, and CRISPER-based techniques have been developed. Along with molecular methods, different efficient serological and immunological methods such as ELISA, rapid antigen test, lateral flow immunoassay, luminescent Immunoassay, and biosensors are also developed.

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Introduction

Coronaviruses (COVs) are the most widely seen viruses in mammals and birds and mainly cause respiratory, gastrointestinal, and sometimes neurological diseases or hepatitis. Infections are usually transmitted through the respiratory tract or the stool and mouth, and infections can be acute or chronic. Coronaviruses are usually specified to infect a particular animal species and are transmitted from one species to another. These viruses are known for having structures on their surface called spikes. Historically, coronaviruses were officially identified as a new viral family in the 1960s following the discovery of several new human respiratory pathogens. Almost forty years after identifying this group of viruses, and in late 2002 and early 2003, a coronavirus caused severe respiratory complications in humans known as severe acute respiratory syndrome (SARS-CoV). The sudden onset of SARS led to new research to understand the main mechanisms of reproduction and pathogenicity of members of this viral family to control them worldwide [1-6].

In December 2019, a newly diagnosed case of pneumonia caused by a β-coronavirus in Wuhan City, Hubei Province, China, was associated with symptoms of acute respiratory syndrome, and within a few months, it became a pandemic. It is the seventh most common coronavirus to cause disease in humans [7- 8]. Chinese researchers quickly quarantined a patient with coronavirus disease (COVID-19) in January 2020 and sequenced the virus genome [9]. Studies have estimated the initial rate of reproduction of the COVID-19 virus at about 2.2 [7, 10].

Taxonomy and genomic structure of COVID 19

Coronaviruses belong to the family Coronaviridae and subfamily Orthocoronavirinae and fall into four general classified: Alphacoronaviruses (α), Betacoronaviruses (β), Gammacoronaviruses (γ), And Deltacoronaviruses (δ). Coronaviruses have been coated with a single-strand RNA-positive genome with a length of about 26,000 to 32,000 bp. One of the most prominent features of this viral family is the size of their genome. Coronaviruses have the largest genome size of all RNA viruses [11-12].

The genomes of these viruses contain a cap structure at the 5 'head and a poly (A) tail at the 3' head, which allows it to act as mRNA to translate the required polyproteins. The viral genome contains a leader sequence and a non-coding region (UTR) that contains several structures required for RNA replication and transcription. SARS CoV-2 is a new species of beta-coronavirus that has the typical genomic structure of coronaviruses. It has a unique accessory protein that interferes with the host's innate immune system [13]. The Cov genome consists of a variable number [6-12], open reading frame (ORF). Besides, two-thirds of the viral RNA is mainly located in the first ORF (ORF1 a / b), which encodes two polypeptides called pp1a and pp1ab, while other ORFs encode structural proteins [7, 14] (Fig. 1).

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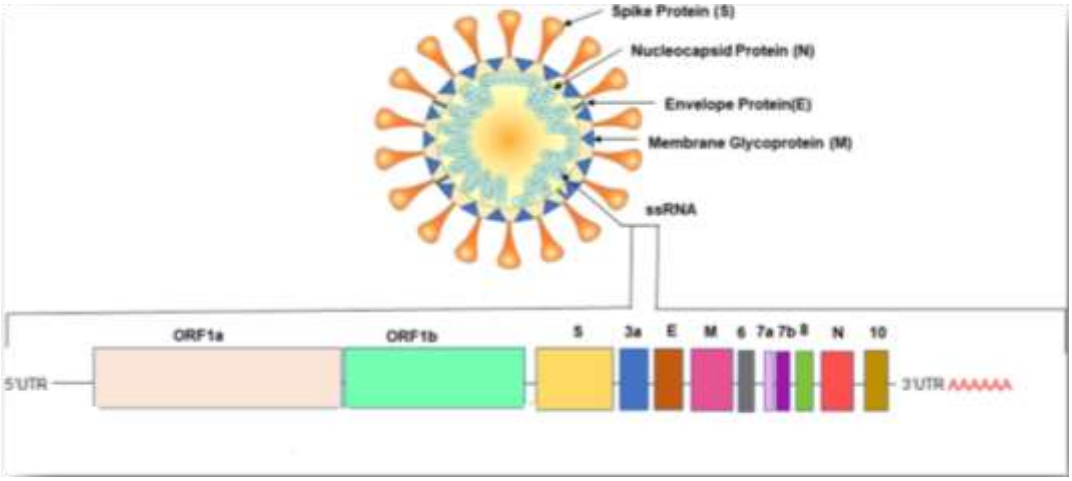

Fig. 1. COVID-19 genome structure

The findings indicate that the COVID-19 genomic sequence is 96.2% similar to that found in bats. Based on the results of virus sequencing and studies, bats are considered the natural hosts of the virus, which is transmitted to humans through an unspecified intermediate interface. COVID-19 uses the enzyme angiotensin-converting enzyme 2 (ACE2)-its receptor- to infect humans [7, 15].

The importance of molecular detection

COVID-19 has a wide range of clinical presentations, ranging from mild symptoms like the flu to life-threatening disorders. Thus, it is critical to do effective diagnostic testing first to rule out other diseases. It prevents the transmission of the disease by testing positive people and avoids unnecessary quarantine for test-negative people. Early detection helps doctors provide treatment interventions to individuals at a higher risk of problems related to COVID-19. More complex tests based on viral genome sequencing are necessary for determining the prevalence and diversity of COVID-19 alterations, as well as identifying emerging strains of the virus in order to produce effective vaccinations. Identifying people affected by COVID-19, with or without symptoms, and those who have gained antiviral immunity is critical until an effective commercial vaccine is accessible to all people. It enables researchers to assess the immune system's strength and power in the general population. COVID-19 diagnostic tests are divided into two categories on the commercial market. The first category includes molecular methods for detecting viral RNA that uses polymerase chain reaction (PCR) techniques or nucleic acid hybridization methodologies. Serological and immunological tests belong to the second group, and they depend heavily on antibodies or antigenic proteins produced by people who have been exposed to the virus (Fig. 2). Of course, these two sets of tests overlap in the diagnosis of COVID-19. Viral RNA testing identifies people who are in the acute stage of infection. In addition, serological tests can identify potential plasma donors. It also makes it possible to follow up with infected people and assess their immune status over time [16-18]. COVID-19 must be diagnosed early, treated effectively, and prevented. The current competition is to create low-cost laboratory kits that are quick to diagnose.

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Fig. 2. Tests used to diagnose SARS – CoV-2/COVID-19 [19]

Molecular detection methods for identification of viral RNA

Due to its whole genetic sequence uploaded in Global Initiative on Sharing All Influenza Data (GISAID), COVID-19 is a positive single-stranded RNA virus, and companies and research groups have expanded a range of diagnostic kits. The availability of virus genetic sequences has assisted the development of particular testing by making it easier to design primers and probes [19]. In this section, we look at COVID-19 molecular diagnostic trends and approaches, including clustered regularly interspaced short palindromic repeats (CRISPR) and traditional technologies.

Reverse transcription-polymerase chain reaction (RT-PCR)

The standard gold technique for identifying the Covid 19 virus is RT-PCR, which can amplify a small amount of viral genetic material in a sample. RT-PCR testing is performed on samples taken from the upper respiratory tract using a swab. In addition, serum, feces, and ocular secretions have been used in a small number of investigations (9-13]. Recently, the Rutgers Clinical Genomics Laboratory has developed an RT-PCR-based method called the Taq Path COVID19-Combo kit, which uses saliva samples collected by the patient himself, which is faster than other methods of collecting the patient sample, causes less pain, also has a lower risk for the treatment staff and provide a larger sample size [16].

As shown in Fig. 3, RT-PCR begins with the reverse transcriptase enzyme converting genomic RNA to DNA in vitro. This reaction requires short DNA primers to identify complementary sequences in the viral genome's RNA and the reverse transcriptase enzyme to produce a small copy of the viral RNA's DNA (cDNA).

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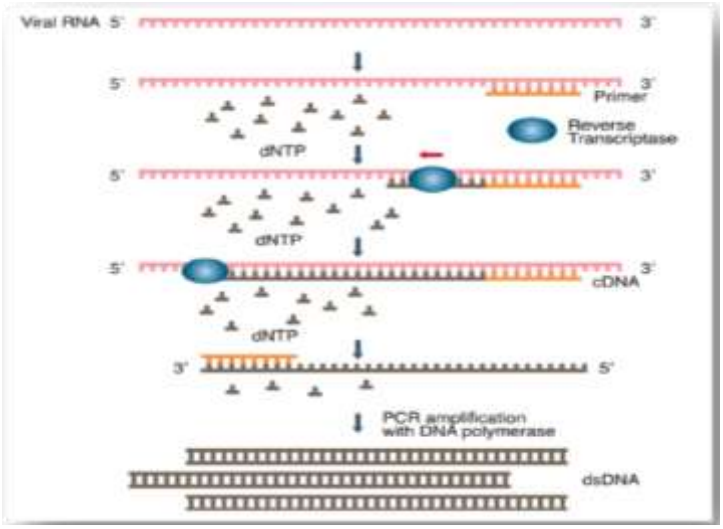

Fig. 3. Reverse polymerase chain reaction generates a cDNA copy of a specific segment of viral RNA, which is then transformed to double-stranded DNA and exponentially amplified [16].

Similar to the TaqMan method, fluorescent dye or probe labeled with a fluorescent molecule as well as a quencher are observed in this real-time amplification reaction, and when the PCR reaction proceeds, an automated system repeats the amplification process for about 40 cycles until the viral cDNA is visible with a fluorescent or electrical signal [16, 20]. One-step or two-step RT-PCR reactions are available. The one-step reaction is carried out in a microtube containing all the necessary primers. Multiple microtubes are used in the two-step RT-PCR procedure to perform separate reverse transcription and replication operations. The step shows higher flexibility and sensitivity, requires less material to initiate the reaction, and can store c-DNA for other purposes [19]. Typically, the one-step method is preferred for detecting COVID-19 because it can be set up quickly and does not require much sample management, and can be done quickly, so pipetting risks and contaminations in reverse transcription and replication stages are reduced.

To date, most molecular diagnostic tests have used RT-PCR technology that covers different regions of the virus genome, including ORF1b or ORF8, nucleocapsid (N), spike (S), DNA-dependent RNA polymerase (RdRp), or the enveloping gene (E). Have been targeted [21]. Lower respiratory tract samples and oral and nasopharyngeal can be tested in patients with pneumonia. The results of various samples are different from patient to patient and in different courses of the patients' illnesses. Patients with pneumonia appear to have a positive lower respiratory tract samples test but a negative nasal or oropharyngeal samples test [22]. While RT-PCR is the most popular approach for detecting COVID-19 infections, it has some downsides, such as the necessity for precise laboratory equipment and highly competent laboratory staff and the time it takes to receive a result which can take several days. As a result, several companies and laboratories worldwide are attempting to improve RT-PCR technologies and develop new ones [16].

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Isothermal nucleic acid amplification

Each cycle of RT-PCR requires several temperature alterations, necessitating specialized temperature equipment. Isothermal nucleic acid amplification is a method that does not require a temperature cycle and allows for amplification at a constant temperature. As a result, various methods based on this principle have been developed [23-25].

Loop-mediated isothermal amplification (RT-LAMP)

The RT-LAMP test was created as a quick and economical alternative to the COVID-19 test. To improve sensitivity, as illustrated in Figure 4, RT-LAMP uses four primers that are specific for the gene or target region and combines LAMP with a reverse transcription step to detect RNA. Photometry, which measures the amount of turbid produced by magnesium, can be used to see the result of this amplification. This reaction can be observed in real-time by utilizing intercalating dyes to measure opacity or fluorescence light. The simplicity and sensitivity of real-time RT-LAMP diagnostic testing, which involves heat and visual inspection, make it a good choice for virus identification [26].

Few molecular tests are available to identify COVID-19 using the RT-LAMP technique. This test is rapid (13 minutes or less) and uses upper respiratory tract swabs to detect viral RNA, but it is also limited to a single sample run [16]. Zhang et al. propose the RT-LAMP test, which uses reverse transcriptase to convert viral RNA to cDNA, subsequently amplified by DNA-dependent DNA polymerase and detected with a DNA-binding dye. The enzyme is a silicon-based RNA-driven DNA polymerase that binds to an aptamer that prevents RTx activity below 40˚C. LAMP is a speedy and straightforward method of replacing viral RNA in cell lysates with approximately 480 copies of RNA and is a rapid and simple method of replacement by RT-PCR [16, 27-28].

Transcription-Mediated Amplification (TMA)

TMA is a single-tube isothermal amplifier utilized in the post-retroviral replication phase to amplify specific regions of DNA or RNA more efficiently than RT-PCR. Retroviral reverse transcriptase and RNA polymerase T7 are employed in this procedure to detect nucleic acids from a variety of pathogens. Both RT-PCR and TMA processes can be performed using Hologic's Panther fusion platform [16]. TMA is a unique technology that may simultaneously detect other common respiratory viruses whose symptoms overlap with COVID-19 using the same patient sample due to its high efficiency (maximum 1000 tests in 24 hours). The first step involves trapping viral RNA on magnetic microparticles and hybridizing it to a particular probe and oligonucleotides carrying the T7 protomer primer. The trapped target RNA is subsequently transcribed into cDNA after being hybridized into the T7 promoter. As a result, the reverse transcriptase's RNaseH activity removes the target RNA strand from the duplex RNA-cDNA, leaving just a single cDNA strand with the T7 promoter.

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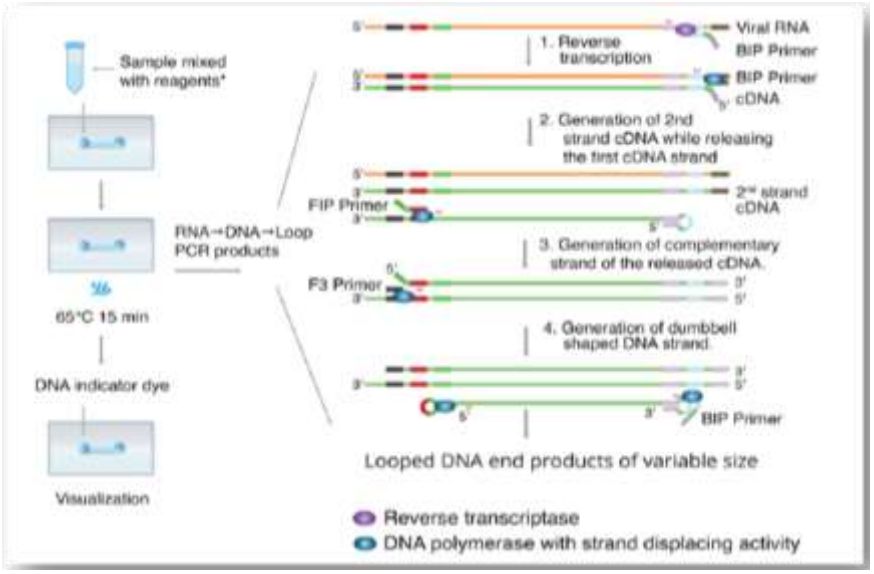

Fig. 4. Loop-mediated isothermal amplification [16]

To make double-stranded DNA, another primer is used. T7 RNA polymerase then converts the DNA amplicons to RNA amplicons. These new RNA amplicons are then sent through the TMA process, producing billions of RNA amplicons in less than an hour. The detection process is performed using single-stranded nucleic acid hybridization to the RNA amplicons. This process depends on a fluorophore and quencher. The fluorophore can generate a signal when excited when the nucleic acid is hybridized to single-stranded RNA [29-30].

CRISPER-based diagnostics

CRISPER's palindromic replication clusters are a type of nucleic acid sequence found in bacteria and other prokaryotic organisms. CRISPER-associated enzymes, including Cas9, Cas12, and Cas13, are bacterial enzymes that can recognize and cleave these sequences. Cas12 and Cas13 enzymes can be designed to target and digest viral RNA sequences specifically.

CRISPER technology's inventors have established two companies, Mammoth Biosciences and Sherlock Biosciences, both of which are investigating the possibilities of using CRISPER with gene editing to detect COVID-19. Sherlock Biosciences created the SHERLOCK technique, which employs Cas13, a protein that can cut reporter RNA sequences in reaction to a specific SARS-CO2-conducting RNA. The Mammoth Biosciences diagnostic test depends on reporter RNA cleavage by Cas12a, which explicitly identifies sequences for the N and E genes. Then, the isothermal amplification of the target region and observation of the target region occurs by one fluorophore [16, 31]. These CRISPER-based methods do not require complex tools, as shown in Figure 5, and the presence of the virus can be detected without decrees in sensitivity and specificity using paper tapes. These tests are time-consuming and cost-effective and can be done in less than an hour [32-34].

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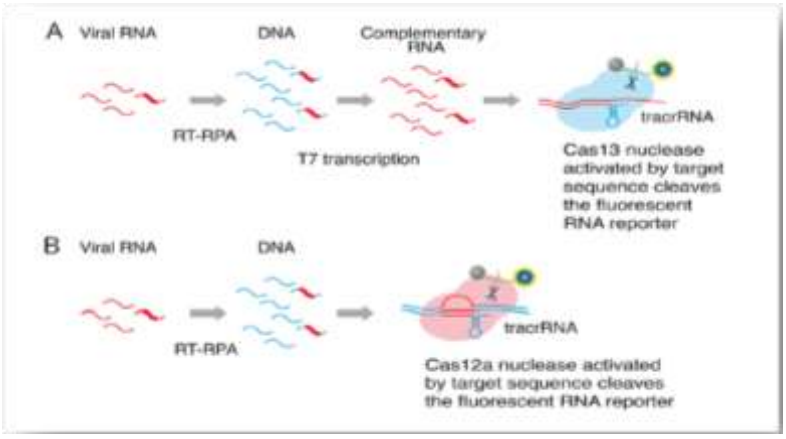

Fig. 5. Two CRISPER alternative techniques for viral RNA detection: A, SHERLOCK test and B, DETECTOR test [16]

Rolling circle amplification (RCA)

Another isothermal method is the RCA method, which has gained a huge amount of attention in the category of tests based on nucleic acid detection, as it is capable of generating 109 amplification signals per cycle in less than 90 minutes. RCA has the advantage of being able to be done in isothermal situations with minimum materials and avoid false-positive results that are common with PCR-based procedures. A SARS-COV RCA for respiratory specimens is available in solid and liquid phases [35-37].

Nucleic acid hybridization using microarray

The microarray approach of nucleic acid hybridization was used to detect SARS-CoV nucleic acids quickly and efficiently. These approaches rely on cDNA synthesis from viral RNA, reverse transcription, and cDNA labeling with specific probes, as shown in Figure 6. The labeled cDNAs are then put into wells with oligonucleotides that have been stabilized on their surfaces. Following the washing of the unbound nucleotides, a signal depends on the concentration of viral acid nucleic acids [16, 38]. Microarray tests are successful at detecting SARS-CoV-related mutations, and they were used to correctly identify 24 single nucleotide polymorphisms linked to SARS-CoV spike gene alterations [39]. Identifying emerging and new SARS-CoV species is becoming increasingly important as the COVID 19 pandemic expands, providing microarray-based diagnostic methods as rapid detection of new species created due to genetic diversity. Although one of the disadvantages of the microarray technique is its high cost, a non-fluorescent oligonucleotide presentation technique has been developed with the ability to identify multiple species of coronaviruses with sensitivity equal to RT-PCR [40]. In addition, nucleic acids of middle east respiratory syndrome viruses such as influenza have been detected using a portable diagnostic platform based on microchips [40-41].

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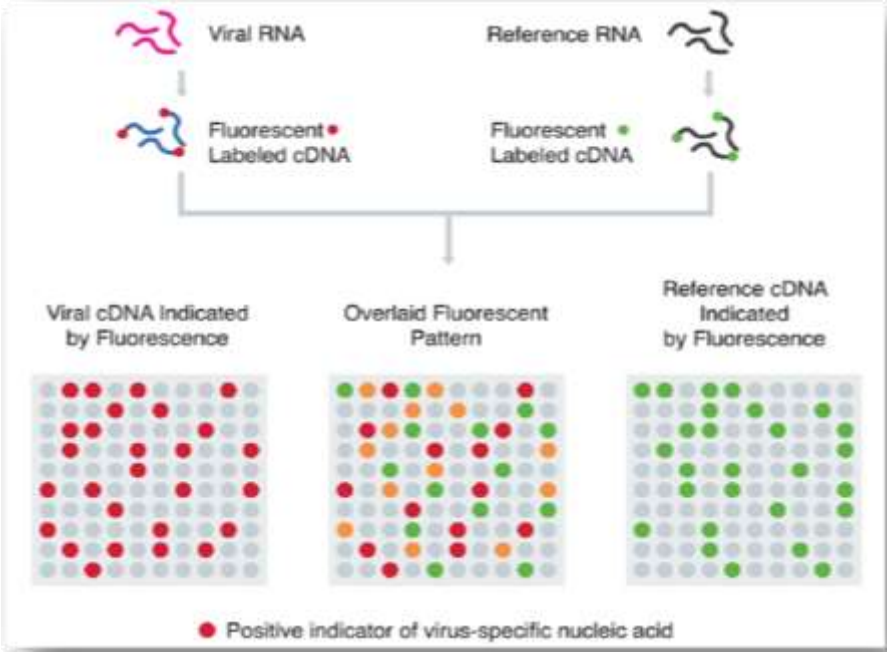

Fig. 6. Nucleic acid hybridization using microarrays. Viral and reference cDNAs are labeled with different fluorescents and placed inside microarray wells covered with specific DNA probes [16].

Metagenomic sequencing based on the implication

This method uses a combination of metagenomic sequencing and amplicon-based sequencing. Metagenomic sequencing is mainly used to determine an infected person's microbiota. This method can quickly identify both the COVID-19 virus and other pathogens that lead to secondary infections which exacerbate the symptoms of COVID 19. Sequencing based on amplicon makes it possible to trace the patient's symptoms and molecular epidemiology and study the evolution of the virus. Metagenomic approaches, including Sequence-independent single primer amplification allow investigations into virus sequence divergence. This technique helps to study the rate of mutation and its possible recombination with other human coronaviruses, which is essential in preparing a vaccine and its antiviral efficacy. Sequencing techniques based on the amplitude and meta-genomics of MinION by Moore et al. were used for rapid sequencing (8 hours) of the COVID-19 genome and other microbiomes in respiratory tract swabs from patients with the disease [44]. This team selected 16 primers that bind to the conserved regions of the virus genome for sequential amplification of 1000 bp fragments with 200 bp homologous regions. After that, the primers were used to generate 30 amplitudes of cDNA, which MinIOn subsequently sequenced. Illumina has developed a sequencing technology based on next-generation sequencing that can detect the presence of several species of coronavirus and several pathogenic organisms in complicated samples [45].

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Immunological and serological detection methods for identification of viral antibody/ antigen

Despite the success of the RT-PCR method in identifying the virus genome, the progression of the disease, immunity, and past infection identification is not possible to track with this method. Assays that detect IgM, IgG, or total antibodies are the other broad group of tests [46]. The detection of IgM or IgG antibodies specific for several viral antigens, including but not limited to the spike glycoprotein (S1 and S2 subunits, receptor-binding domain) and nucleocapsid protein, is used to determine COVID-19 exposure. Following infection, IgM becomes detectable in serum after a few days and lasts for a few weeks before switching to IgG. IgM and IgG might thus be used as early and late-stage markers, respectively. In addition, IgG may indicate the presence of post-infection immunity [19]. The following are the most common serological/immunological tests:

Enzyme-linked immunosorbent assay (ELISA)

ELISA is a detecting and quantifying substances technique in a plate-based microwell. Indirect ELISA applies for detecting anti-COVID-19 antibodies, and sandwich ELISA detect COVID-19 antigens (It is also called Rapid Antigen Test) (Fig. 7). This qualitative or quantitative test typically takes 1–5 hours.

Although ELISA has advantages in speed, the capacity to test multiple samples, and automation adaptability, it also has several disadvantages, the most notable of which is sensitivity variation [47-51].

Rapid antigen test

Rapid antigen testing is combined with molecular genetic techniques to detect viral antigens [52-53]. Specific monoclonal antibodies are used in these tests to provide a method for capturing viral antigens. These assays are not limited to a specific format; for the detection of COVID-19 nucleocapsid protein, a colorimetric enzyme immunoassay [54], improved chemiluminescent immunoassay [55], and, more recently, a fluorescence lateral flow assay are used [56].

Lateral flow immunoassay

It is a typical qualitative portable test that may be performed at the point of care and results in 10–30 minutes. Samples move across the nitrocellulose membrane via capillary flow in this rapid diagnostic test. If anti-COVID-19 antibodies are present, they bind to the labeled antigen, continue to move, and are captured by immobilized anti-human antibodies. A colorful test band indicates the existence of the captured antibody-antigen complex (Fig. 8). This qualitative test is low-cost and does not necessitate the use of trained staff [57-58].

Neutralization assay

The ability of an antibody to suppress virus infection in cultured cells and the cytopathic effects of viral replication is used in this experiment. Patient samples of diluted blood, serum, or plasma were added to cell cultures for an apparition. Neutralization assays measure an antibody's capacity to prevent virus infection and the cytopathic effects of viral replication in cultured cells. In the presence of neutralizing antibodies, viral replication is inhibited in infected cell cultures, and antibody levels can be determined.

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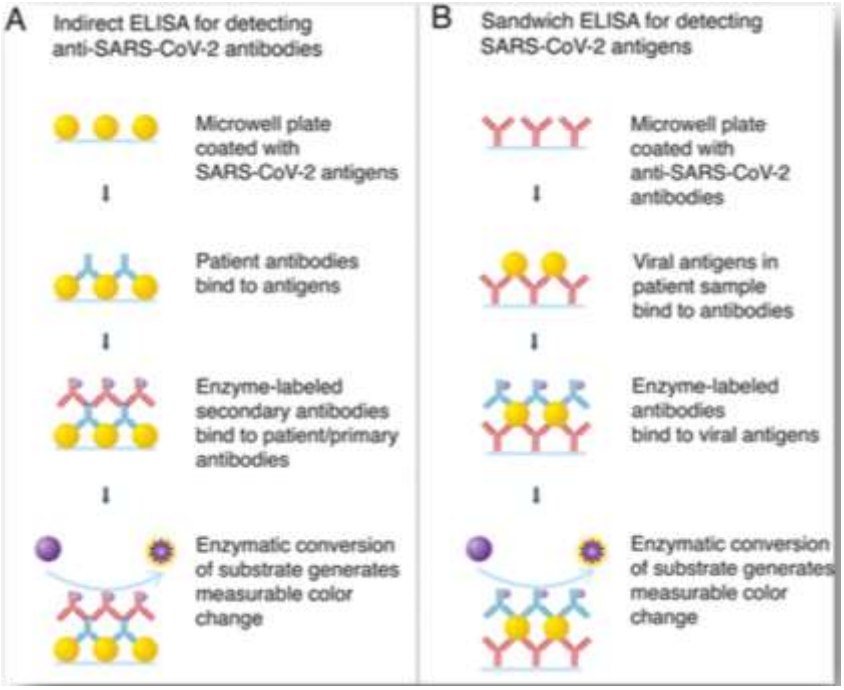

Fig. 7. Antibodies (A) or antigens (B) are detected using ELISA tests [16]

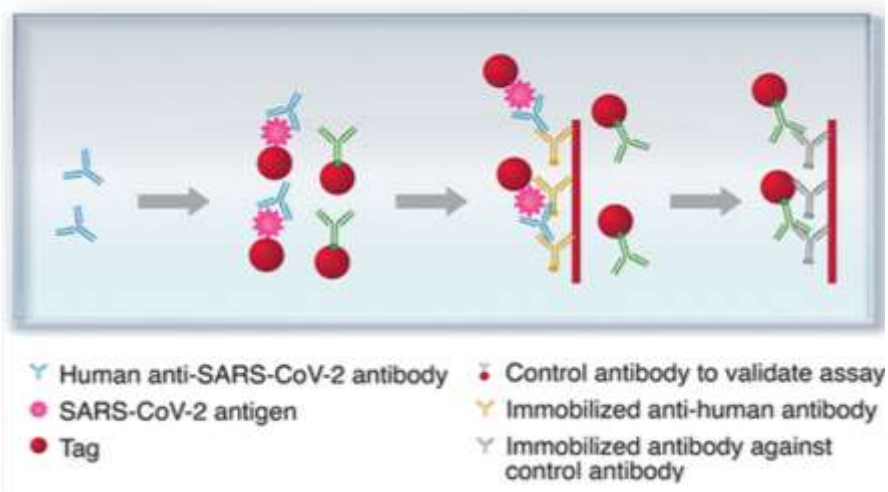

Fig. 8. Anti-COVID-19 antibodies are detected using a lateral flow immunoassay [16]

Neutralization assays normally take 3–5 days to show the results, even though recent developments have reduced this to hours [59-60]. This test requires cell culture facilities and Biosafety Level 3 laboratories for SARS coronavirus. Despite these limitations, determining neutralizing antibodies is critical for the short-term therapeutic application of convalescent plasma and long-term vaccine development.

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Luminescent Immunoassay

Luminescent immunoassays are approaches for reducing the detection limits of antibody-based reagents. So, chemiluminescence and fluorescence are commonly used in these approaches. Cai et al. developed a peptide-based magnetic chemi-luminescence enzyme immunoassay to diagnose COVID-19, and Diazyme Laboratories, Inc. (San Diego, California) announced the availability of two new fully automated COVID-19 serological tests using the Diazyme DZ-lite 3000 Plus chemiluminescence analyzer [61-62].

Biosensor test

Biosensor tests rely on optical, electrical, enzymatic, and other approaches to convert the unique interaction of biomolecules into a quantitative output. Surface plasmon resonance is a technique that detects interference with incident light at a solid boundary due to local disturbances such as an antibody or antigen adsorption. SARS was diagnosed using an surface plasmon resonance-based biosensor using coronaviral surface antigen [SCVme] attached to a gold substrate [63]. This platform provides a result from the capture and signals amplification of the virus in 3-5 min [16].

Discussion

Both types of SARS–CoV-2 testing can be useful in this outbreak. Due to the urgent need to detect COVID-19, manufacturers, commercial laboratories, and molecular laboratories can apply to the food and drug administration (FDA) for approval of their diagnostic assays. FDA-approved tests must be validated and manufactured under optimal conditions for approved positive and negative control samples. Despite the rapid development of COVID-19 diagnosis kits in recent months, the search for more efficient, cost-effective, and accurate laboratory approaches continues. Determining the extent of virus/disease in local groups is likely linked to the acceptability of control strategies that demand individual action, such as social distance.

Conclusions

In conclusion, massive progress in establishing diagnostic tests has been achieved; however, many unresolved questions and challenges remain. Efforts have been made worldwide to facilitate and develop new tests, and various institutions support these efforts to produce and validate new diagnostic methods. It seems that with the development of knowledge in this field, new detection technologies will emerge in the future.

Conflicts of Interest

There was no conflict of interest in this study.

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