# How to detect SARS-CoV-2: A brief review about molecular diagnosis techniques

## SARS-CoV-2 nasıl tespit edilir: Moleküler tanı teknikleri hakkında kısa bir inceleme

Ahmet ÇARHAN[1] (ID), Ender ŞİMŞEK[1] (ID), Özen ÖZENSOY GÜLER[1] (ID)

**ABSTRACT**

A new type of coronavirus (SARS-CoV-2) was detected in patients with acute respiratory disease in 2019. SARS-CoV-2 is a virus that can cause fever, dry cough, shortness of breath, anorexia, fatigue and sore throat, which can be transmitted through respiration and has started to spread around the world and has become a global problem. The disease caused by SARS-COV-2 has been named Coronavirus Disease 2019 (Covid-19) by the World Health Organization (WHO). According to the WHO, Covid-19 is a pandemic and it is stated that humanity has not experienced such an epidemic in the last hundred years. The coronavirus originated from bats and was transmitted to human through unknown animals in Wuhan province of China in December 2019. On January 2021, 96.658.420 confirmed cases and 2.092.062 deaths have occurred in the world. There is a growing need in the detection of SARS-CoV-2. A number of problems and difficulties are observed in the diagnosis and treatment of Covid-19. Chest tomography and molecular tests are used to detect SARS-CoV-2. The molecular techniques used are PCR-based and non-PCR-based methods. Usually detection of SARS-CoV-2 is based on PCR, but isothermal nucleic acid amplification tests have also been promising alternatives. Currently,

**ÖZET**

Yeni tip koronavirüs (SARS-CoV-2) 2019 yılında akut solunum yolu hastalığı olan hastalarda tespit edilmiştir. SARS-CoV-2'nin sebep olduğu en önemli semptomlar; ateş, kuru öksürük, nefes darlığı, iştahsızlık, yorgunluk ve boğaz ağrısıdır. Solunum yoluyla bulaşabilen ve tüm dünyaya yayılmaya başlayan bu virüs küresel bir sorun haline gelmiştir. Bu virüsün sebep olduğu hastalığa Dünya Sağlık Örgütü (DSÖ) tarafından Covid-19 adı verilmiştir. DSÖ'ye göre Covid-19 bir pandemidir ve insanlığın son yüz yıldır böyle bir salgın yaşamadığı belirtilmiştir. Yarasalardan köken alan bu virüs Aralık 2019'da Çin'in Wuhan eyaletinde insana bulaşmıştır. Bu virüsten kaynaklı 2021 Ocak'ta, tüm dünyada 96,658,420 onaylanmış vaka ve 2,092,062 ölüm meydana gelmiştir. Bu durum ise, bu virüsün tespit edilebilmesinin önemini her geçen gün arttırmaktadır. SARS-CoV-2'nin tanı ve tedavisinde birtakım sorunlar ve zorluklar gözlenmektedir. Bu hastalığı tespit edebilmek için klinik olarak göğüs tomografisi, röntgen ve moleküler testler kullanılmaktadır. Kullanılan moleküler teknikler, PCR tabanlı ve PCR tabanlı olmayan yöntemlerdir. Genellikle SARS-CoV-2'nin tespiti PCR yöntemine dayanmaktadır, ancak izotermal nükleik asit amplifikasyon testleri de bu tür enfeksiyonların tanısı

[1]Ankara Yıldırım Beyazıt University, Department of Medical Biology, Ankara

İletişim / Corresponding Author : Özen ÖZENSOY GÜLER
Ankara YBÜ Tıp Fakültesi Temel Tıp Bilimleri Binası Bilkent Yolu 3. Km Bilkent Ankara - Türkiye
E-posta / E-mail : oozensoy@hotmail.com

Geliş Tarihi / Received : 02.03.2021
Kabul Tarihi / Accepted : 07.09.2021

DOI ID : 10.5505/TurkHijyen.2022.87533

Çarhan A, Şimşek E, Özensoy Güler Ö. How to detect SARS-CoV-2: A brief review about molecular diagnosis techniques
Turk Hij Den Biyol Derg, 2022; 79(4): 762 - 775

qRT-PCR is a golden assay and it is widely used although many alternative assays have been developed for recent years. The current testing capacity and availability can not meet the unprecedented global demands for rapid, reliable and widely accessible molecular diagnosis. In addition, there is a need for a comprehensive strategy compare the molecular techniques used in the field. Since in this review we aim to give a summary of molecular diagnosis techniques to detect Covid 19.

**Anahtar Kelimeler:** Covid-19, molecular techniques, pandemia, PCR, SARS-COV-2

için umut verici alternatifler olmuştur. Mevcut tanı seçenekleri arasında, qRT-PCR testinin kullanımı altın standart bir test olarak düşünülmektedir. Ancak bu yöntemin de test kapasitesi ve kullanılabilirliği, hızlı, güvenilir ve yaygın olarak erişilebilir moleküler tanı için küresel talepleri karşılayamamaktadır. Bu derlemede, SARS-CoV-2 virüsünün hızlı tespiti ve kolay teşhisi için moleküler tanı tekniklerinin kullanımına dair bir çalışma sunulmuştur.

**Key Words:** Covid-19, moleküler teknikler, pandemi, PCR, SARS-COV-2

## INTRODUCTION

In this review we aim to evaluate the several molecular techniques to detect SARS-CoV-2 with limitations and predominances such as RT PCR, immunoassay techniques for antibody detections. But as we all know from the current status in the world that the most commonly used laboratory detection method for the clinical diagnosis of SARS-CoV-2 is real-time reverse transcriptase polymerase chain reaction (RT-PCR).

### What is Covid-19?

Coronaviruses are a large family of viruses, and certain types of this family pose a threat to human health. Orthocoronavirinae, subfamily of coronaviruses, consists of 4 genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus (1). α and ß coronaviruses usually infect mammals and human while ϒ and ð coronaviruses infect birds (2).

SARS-CoV-2 belongs to ß coronaviruses (2). The first human coronavirus belonging to the Coronoviridae family, severe acute respiratory syndrome coronavirus, was identified in 2003. So far, the identified human corona viruses are: HCoV-229E, HCoV-HKU1, HCoV-OC43, HCoV, NL63, Severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2). Among these, SARS-CoV, MERS-Covand SARS-CoV-2 caused pandemics (3, 4). SARS-CoV-2 is associated with SARS-CoV and MERS-CoV (5). Although the mortality rate is higher in SARS (about 10%) and MERS (about 40%), the spread of SARS-CoV-2 is much higher compared to predecessors (6). The disease caused by SARS-CoV-2 has been named Coronavirus Disease 2019 (Covid-19) by the WHO (3, 4). According to the World Health Organization (WHO), Covid-19 is a pandemic and it is stated that humanity has not experienced such an epidemic in the last 100 years (7).

SARS-CoV-2 originated from bats and was transmitted to human through unknown animals in Wuhan province of China in December 2019 (1). The chronology related to the spread of Covid-19 is presented in the Table 1.

**Tablo 1.** Covid-19 situation in worldwide

| Date | Case | Reference |
|---|---|---|
| 26 December 2019 | First case in Wuhan Central Hospital | 67 |
| 31 December 2019 | Most of the cases occurred in the Huanan Seafood Market | 1 |
| 1 January 2020 | Seafood market was closed | 1 |
| 7 January 2020 | New type of coronavirus was isolated. | 68 |
| 5 March 2020 | 96,000 cases have been reported in 87 countries. Number of cases has started to increase in countries such as South Korea, Italy and Iran. | 1 |
| 10 March 2020. | In Turkey, the first cases were seen in Covid-19. | 67 |
| 15 March 2020 | SARS-CoV-2 has spread to 34 provinces and cities in China, the disease has been reported in five continents and additionally 144 countries. | 6 |

When the coronaviruses are examined with an electron microscope, there are protrusions on the surface with a crown appearance (Figure 1) (8). SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virüs with diameter 50-200 nm (7).

The life cycle of SARS-CoV-2 begins with the binding of the S protein on the virus to the ACE2 receptor. After binding to the receptor, S protein undergoes conformational change. Viral membrane fusion towards the cell membrane is facilitated and the SARS-CoV-2 RNA is sent into the cell. RNA is translated with viral replicas, pp1a and pp1ab. The product obtained is then cut with viral proteinases. Polymerases produce subgenomic mRNAs, then they are translated into viral proteins. Viral proteins and RNA combine in ER and golgi apparatus. They are released out of the cell by vesicles (Figure 2) (9).

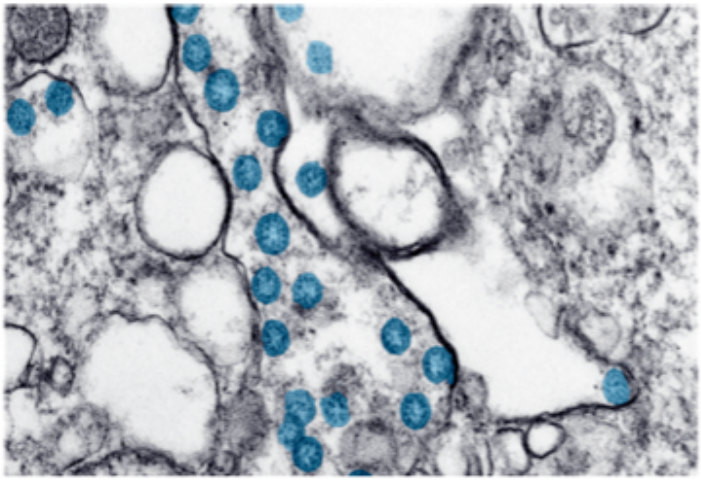

**Figure 1.** SARS-CoV-2 morphology (8).

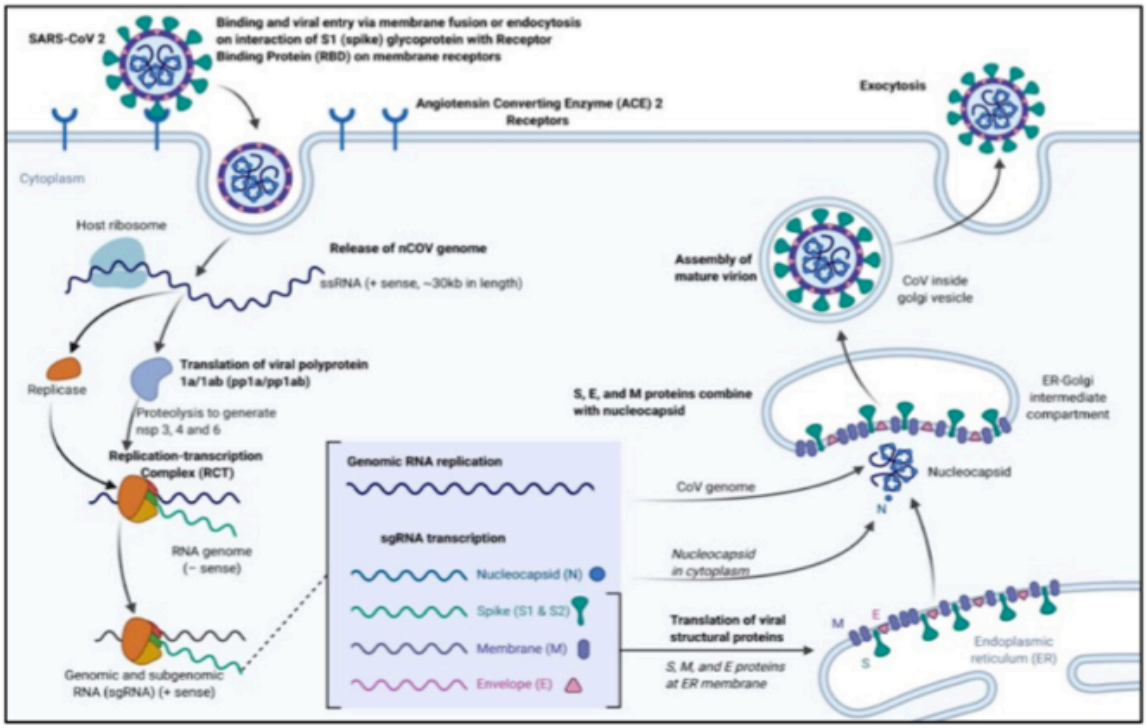


**Figure 2.** The life cycle of SARS-CoV-2 (7).

Structurally, it has four protein branches; nucleocapsid protein (NP), spike protein (SP), envelope protein (EP) and membrane protein (MP) (Figure 3) (7).

SP is considered as an important target for neutralizing antibodies and vaccines. SARS-CoV-2 infects human respiratory epithelial cells through the ACE2 receptor (10). SP protein plays a central role in the transmission of SARS-CoV-2. SP is a glycoprotein consisting of two subunits, S1 and S2. The SP is the main target for neutralizing antibodies and vaccines. S1 has the receptor binding domain (RBD) responsible for recognising and binding by the host cell receptor ACE2. The receptor binding domain consists of 424 to 494 amino acids. This domain connects with ACE2. S protein has two cleavage regions, at arginine R667 and R797. Proteases such as cathepsin L, trypsin, and elastase can disrupt this region. This cleavage in S protein is important for binding to ACE2 and membrane fusion. The S2 subunit contains the essential components for membrane fusion (7, 11). Changes in S protein cause host coronavirus diversity (10).

NP is a phosphoprotein and it forms a nucleocapsid which can be detected in serum samples (7). It plays a role in the replication of viral RNA and cellular response as a host for the infection. In some species, the N protein increases the production of virus-like particules (5).

EP is the smallest main protein that plays a role in pathogenesis (7). While EP is expressed abundantly during replication, it is expressed in a small part of the virion envelope. EP protein is localized in the ER, Golgi apparatus and ER-Golgi apparataus intermediate compartments. The EP is associated with the M protein, contributing to the viral order, promoting the release of virions with the hydrophobic transmembrane domain (10).

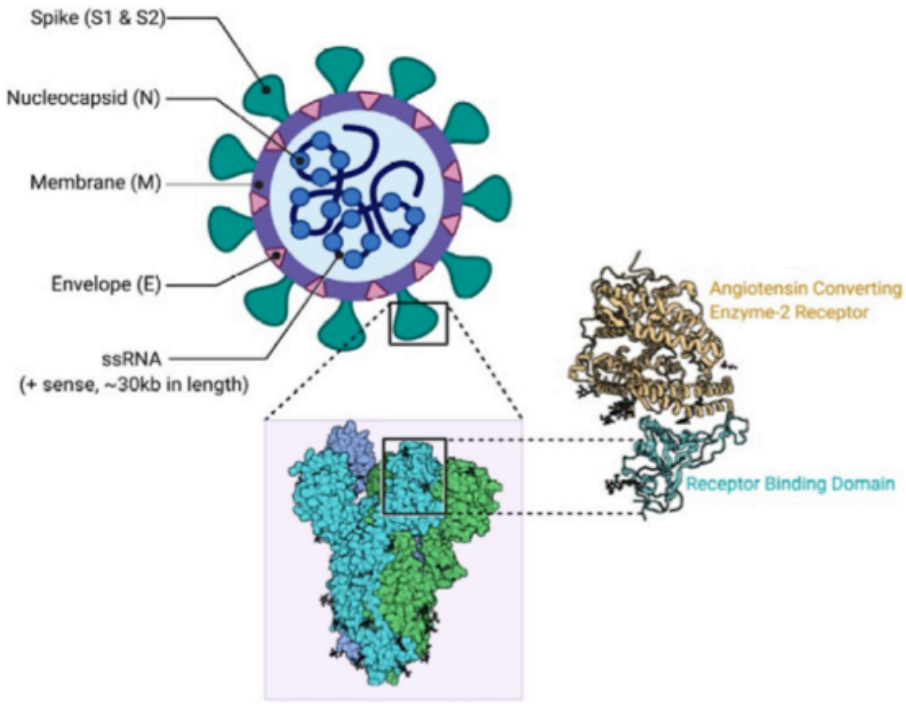


**Figure 3.** The Structure of SARS-CoV-2 virus and ACE2 protein (7).

MP is the most abundant protein in the SARS-CoV-2 structure (7) and have a central role in the assembly of cell membranes to form new virüs particles. SARS-CoV, SARS-CoV-2, MERS-CoV M proteins are located around the Golgi apparatus. Studies have shown that MP plays a role in excluding some host membrane proteins from the viral envelope (10).

SARS-CoV-2 contains ~30,000 nucleotides. The genome encodes 27 proteins containing RNA-dependent-RNA polymerase (RdRP) and E, M, N, S proteins (Figure 4). RdRP plays an important role in viral replication, involves in maintaining genome integrity and is one of the main targets in anti-SARS treatments. The SARS-CoV-2 RdRP gene has been shown to be highly similar to the bat coronavirus, RaTG13 RdRP gene (8, 12). 5'-noncoding region and an ORF1a/b-coding region are the other parts in the genome. Polyprotein encoded from the ORF1a/b region can be cut with proteinases to generate RdRP and helicase, which are involved in virüs replication, transcription and translation (2). SARS-CoV-2 mutates very quickly as there are deficiencies in the error correction mechanisms (13, 14).

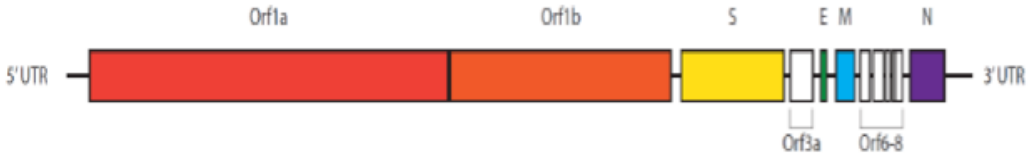


**Figure 4.** SARS-CoV-2 genome (15).

### The Detection of SARS-CoV-2

The important issue in the diagnosis of SARS-CoV-2 is to take samples from the patient properly and quickly. It is recommended to collect and test sputum/bronchoalveolar lavage fluid samples (BAL) for the detection of SARS-CoV-2 (16).

Wang et al. conducted a study using different types of clinical samples for the detection of SARS-CoV-2. In this study, nasopharyngeal swab samples were used to confirm clinical diagnosis. The lower respiratory tract samples have been tested positive for the virus. They showed that SARS-Cov-2 samples were found 32% in oropharyngeal swab samples and 63% in nasal swab samples. In addition, virus was also detected in fecal samples. This suggests that the disease may be systemic (17). Wölfel et al. noted that the virus was easily isolated from throat and lung samples, but was not isolated from fecal samples despite high virus RNA concentration (18). Tang et al. reported that the viral load was high in the upper and lower respiratory tract in the early stages of the disease. They suggested that the use of nasopharyngeal and oropharyngeal swab samples would be more appropriate. Anal and blood samples were positive for the virus in the late stage of the disease. Therefore, they claim that the disease can transmit from the oral route to the fecal route (19).

The symptoms observed in Covid-19 patients are similar too the respiratory diseases. Therefore, they are not specific and cannot be used for diagnosis. According to the general definitions of National and International Health Autorities (NIH), the most common symptoms of Covid-19 are fever, cough, sore throat, general weakness and muscular pain. The incubation period of this disease is on average 2-14 days.

In this review we aim to evaluate the detection techniques of SARS-CoV-2 infection categorized as clinical findings and subsequent laboratory tests.

### The Clinical Findings of the Covid-19 Patients

Covid-19 is a respiratory disease and clinicians can use chest imaging or X ray techniques to diagnose people who have Covid-19 symptoms, while awaiting RT-PCR results or when RT-PCR results are negative, and the person still has Covid-19 symptoms. Chest tomography (CT) and X-ray techniques are used to diagnose. (3, 8).

So people need to know quickly whether they are infected of SARS-CoV-2 or not because of this suspection appropriate treatment and self-isolating are more crucial to inform close contacts.

### Chest Tomography

Chest Tomography (CT) is an imaging method used to detect a number of chest diseases (20). Abnormal CT scans are used to detect SARS-CoV-2 in patients with negative molecular diagnosis. CT imaging shows infiltrates, ground-glass opacities and subsegmental consolidation (1). CT gives crucial information about the stages and severity of the disease.

Molecular techniques such as quantitative Real Time PCR (qRT-PCR) and Isothermal amplification methods are carried out as an alternative or combinatory approach to CT scan (5).

### Tomography and X ray Techniques

X-rays or scans produce an image of the organs and structures in the chest. X-rays radiography use radiation to produce a 2-D image by a radiographer. Computed tomography (CT) scans use a computer to merge 2-D X-ray images to convert them into a 3-D image. This techniques require highly specialised equipment by a specialist radiographer.

### Subsequent Laboratory Tests

#### a) qRT-PCR

The diagnosis of SARS-CoV-2 is confirmed by detection of viral RNA. Generally, high purity RNA is isolated by commercially available kits. However, kits are expensive and prolong analysis time. Eliminating the RNA isolation step can increase laboratory efficiency and reduce test costand time (21).

Marzinotto et al. have developed a method to bypass RNA isolation step in the detection.

They treated the SARS-CoV-2 RNA sample with ProteinaseK and bypassed the RNA extraction step by performing heating-cooling before amplification. They demonstrated that higher amounts of viral RNA were obtained compared to the commercially kits. In addition, 100 µL of sample is sufficient to isolate RNA with this method, while 200 µL of sample is required in classical RNA isolation methods (21).

Won et al. isolated RNA from the pharyngeal swab samples with TRIzol™ and then detected them by PCR. This method used for reducing the cost and provided a biosafety advantage. TRIzol™ inactivated viral particles in infectious samples and reduced biosafety measures (22).

Smyrlaki et al. eliminated the RNA extraction step by lysing the samples with detergent and heat inactivating. As a result, they stated that SARS-CoV-2 detection can be performed using simpler protocols with out the use of extraction kits (23).

A protocol that does not require RNA extraction and lasts only 5 minutes was developed by Ladha et al. They proposed a method of single-step and column-free RNA isolation. They suggested that this method is applicable to the SARS-CoV-2 RT-qPCR test protocol. In the study, the solutions were used to lyse the virus and QuickExtractTM DNA Extraction Solution (Lucigen, US) demonstrated similar results as the FDA approved RNA extraction kits (24).

In brief, the protocols without RNA extraction can provide cost benefits, reduce the time in lab, increase the number of test.

Polymerase Chain Reaction (PCR) has high sensitivity and specificity in identifying viral infections (3,8). Quantitative Real-time reverse transcriptase PCR (qRT-PCR) is a method used to detect many viruses and gives opportunity for early diagnosis compared to conventional PCR methods (3). qRT-PCR is currently regarded as the gold standart for diagnosing in SARS-CoV-2 (25).

Since pandemia, many qRT-PCR kits have been developed for SARS-CoV-2 detection in nasopharyngeal swab samples, alveolar lavagefluid, sputum and blood samples. This method is used in many fields such as mRNA expression studies, copy number measurements in genomic and viral DNA/RNA and expression studies (4, 26).

The main difference between qRT-PCR and conventional PCR is quantification of nucleic acid measurements (27). Since the conventional PCR can only amplify the DNA up to 2000 nucleotides but at the same time RT-PCR or qPCR can amplify DNA as well as quantify the amount of DNA as well. Product measurement is performed in realtime using fluorescent probes/dyes in qRT-PCR (28). Fluorescent probes/dyes used in qRT-PCR bind to the target region and emit fluorescence during amplification. This fluorescent signal is read by the instrument. There are many different qRT-PCR protocols used for SARS-CoV-2, but not all of these protocols target the same regions of the genome. Many protocols target the conservative domain of N protein to obtain consistent results.

It is stated that qRT-PCR can not detect SARS-CoV-2 in the early stages of infection and may produce false negative results. Therefore, primer and probe design are very important in qRT-PCR. The primers used in the PCR are single-stranded DNA molecules that bind to specific regions in the genome during the amplification process. The sensitivity and specificity of the primers used in the detection of SARS-CoV-2 are very important. The higher the sensitivity of the primers, the less viral RNA is required and the less false negative rate decreases. After the design of the primers and probes, the test conditions are optimized (7, 15).

Van Elden et al. developed a qRT-PCR method based on Taq-Man probe for the N gene and detected coronavirus in only 28% of 261 clinic specimens with patients suffering from a cold/pneumonia (28).

Corman et al. analyzed the viral genome sequence of SARS-CoV-2 using a set of primers and probes. As a result of the study, they identified three conserved areas: RdRP gene located in the open reading frame ORF1ab, E gene and N gene. It is stated that RdRP and

E gene have higher sensitivity than N gene (29).

Chu et al analyzed samples using the primer for the N and ORF1b genes, and they found that the experiment for the N gene was 10 times more sensitive than the ORF-1b gene for detecting positive clinical samples (30).

A highly specific qRT-PCR experiment was developed by Tib-MolBiol. And does not cross-react with other coronaviruses. The Rd gene was used for validation, while the E gene was used for screening purposes (31).

Chan et al. developed three different qRT-PCR tests targeting RdRp / Helicase (Hel), S and N genes. Of these three tests, RdRp /Hel had the lowest detection limit. TheRdRp / Hel test did not cross-react with cell culture and other pathogenic viruses in clinical samples. As a result of their study, they claimed that the RdRp/Hel test may improve the Covid-19 laboratory diagnosis (32).

Cepheid has developed an Xpert®Xpress SARS-CoV-2 test on the qRT-PCR. The test targets the N and E regions of the viral genome and results with in 30 minutes. The test can be performed on the nasopharyngeal, nasal, oropharyngeal swab samples and nasal aspirates (33). The performance of Xpert®Xpress SARS-CoV-2 test was evaluated with clinical nasopharyngeal swab samples. The positive agreement percentage (PPA) of the test was 97.8%, and the negative agreement percentage (NPA) was 95.6% (34).

Lieberman et al tested 169 nasopharyngeal swab samples which qRT-PCR which was performed on ABI 7500 real-time PCR devices using N1, N2 and RP primers. They compared their developed method with other four tests on the market (Cepheid, DiaSorin, HologicPanther and RocheCobas). Among these tests, CepheidXpertXpress and their method found to be the most sensitive (35).

The Rutgers Clinic Genomic Laboratory has developed more practical qRT-PCR test (TaqPath Covid-19 Combo Kit) using saliva samples (36).

In additon, groups from some countries used the same regions in the diagnosis. China has developed primers and probes targeting both the ORF1ab and N gene. The University of Hong Kong proposed RT-PCR for the N gene and the ORF1b test as a control of this test. The Thai Ministry of Public Health has suggested targeting the N gene. The National Institute of Infectious Diseases of Japan proposed a method that targets the ORFla and S gene by nested RT-PCR method, and the S gene by RT-PCR. The U.S. CDC has developed primers to target the N gene (25). The studies performed in those institutions have not avaliable yet.

However, qRT-PCR method is applicable with high cost materials longer reaction times, with high-tech laboratory equipments. Therefore, shorter analysis times, easily applicable and inexpensive costs will be an important step in combating SARS-CoV-2. Therefore, isoothermal nucleic acid amplification technique is also a promising approach for SARS-CoV-2 detection. The processing time of this method is shorter and it doesn't require a thermocycler (3, 7). Non-PCR-based approaches are also another alternative method to detect coronavirus RNA (3).

In our country, local real-time PCR kits have been developed based on the protocols published by international reference centers and it has been widely used throughout the country (37). Another PCR kit at national level is produced by Spinarayscientific (38).

The Sentebiolab Senteligo™ SARS-CoV-2 (COVID-19) Multiplex qPCR Detection Kit is an in vitro national diagnostic test, based on real-time polymerase chain reaction technology. It tests for the presence or absence of ribonucleic acid (RNA) of SARS-CoV-2 coronavirus, specifically, in lower and upper respiratory tract samples from patients suspected of SARS-CoV-2 viral infection (39).

Another application entitled with Respiratory Pathogens Panel, stands out among the Real Time PCR diagnostic kits produced by Anatolia Geneworks under the brand "Bosphore". This panel is strategically important for detecting infections whose symptoms are similar to each other and for

appropriate treatment. It is recommended by health authorities that the possibilities of other respiratory pathogens be evaluated for the patient to be tested with suspicion of novel coronavirus. In the content of Bosphore Respiratory Pathogens Panel Kits that detects 33 viruses and bacteria from a single sample of respiratory samples, Coronavirus 229E, Coronavirus OC43, Coronavirus NL63 and Coronavirus HKU1 are already included. (40).

### b) Antibody Tests

SARS-CoV-2 antibody tests can detect whether an individual has developed antibodies against SARS-CoV-2 or not. The tests help identify people infected with SARS-CoV-2 (38).

The antibody tests for SARS-CoV-2 detect the presence of IgA, IgM, or IgG antibodies produced by B cells (41). Tests used to detect SARS-CoV-2 antibodies are ELISA, neutralization tests and chemiluminescent immunological tests. However, there is still no standard method for detecting SARS-CoV-2 antibodies (41).

The IgM and IgG response to SARS-CoV-2 can occur 3-6 days after the onset of symptoms. Up to three weeks almost all patients are seroconverted and antibodies persist for at least two months, IgG is more persistent, but the exact duration of immunity for both immunoglobulins is not yet known (42, 43).

Antibody tests offer significant advantages compared to other methods due to the rapid test results and the ability to be performed outside the laboratory (44).

It was first suggested by Zhu et al. That SARS-CoV-2 can be detected by antibody tests (45). Bastos et al. Performed a meta-analysis of approximately 40 trials to compare antibody tests with each other. In this study, enzyme linked immunosorbent assay (ELISA), lateral flow immunoassay (LFIA) and chemiluminescent immunoassay (CLIA) methods were compared. It has been reported that the method with the highest sensitivity is CLIA and the method with the lowest sensitivity is LFIA (46).

Antibody tests show which individuals are immunized and what It should be used in parallel with analyzes for nucleic acid detection to determine the uptake. Frequent, large-scale serological testing helps determine which part of the population may be immune to SARS-CoV-2 and which individuals can rejoin the workforce (47).

### c) Antigen Tests

According to the time consuming and rate availiability of SARS-CoV-2 detection, PCR tests require hours or days to get results in a special laboratory. But antigen tests can be revealed in about 15 minutes. Antibody testing determines whether you had SARS-CoV-2 in the past and now have antibodies against the virus (48)https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html.

Apart from the most widely used real time RT-PCR analysis for the diagnosis of SARS-CoV-2, Isothermal amplification, CRISPR and next generation sequencing (NGS) analyzes are also used (49).

Isothermal amplification is an alternative method that allows constant temperature amplification and eliminates the need for thermal cycler (36). It gives opportunity to investigate 2-3 regions using 4-6 primers will help us to increase the positivity rate in lab diagnosis. Therefore, new techniques were required other than RT-PCR (26). RT-PCR and/or qRT-PCR methods are used to detect RNA viruses (50). LAMP (Loop-Mediated Isothermal Amplification) method is an isothermal nucleic acid amplification with high efficiency (24). LAMP accelerates nucleic acid amplification with both DNA polymerase and 4 to 6 specific primers. In this technique, reverse transcription and amplification are used simultaneously (15, 31, 51,52,53). It is allowed to read the amplification reaction with pH-dependent color change (32). For this reason, LAMP method allows easy identification directly with color change (50). Amplification becomes visible with the use of magnesium pyrophosphate or fluorescent dyes.

The method has high sensitivity and specificity and is widely used for amplification of DNA and RNA. Moreover, it is fast and cost effective. Therefore, performing the LAMP test may work to reduce the detection cost of the coronavirus (24). Even though the background signal is low, optimizing primers and reaction conditions take time (8). In the RT-LAMP method, four region-specific primer sets are required to increase sensitivity. RT-LAMP is a promising candidate for Covid-19 detection, as it required sheating and visual inspection (36).

Thai et al. developed a reverse Transcription LAMP (RT-LAMP) test that can be observed by real-time measurement on a photometer to detect SARS-CoV-2. RT-LAMP is an amplification method that reproduces nucleic acid with high efficiency and speed with its special design six primer set that recognizes eight different sequences of the target region. RT-LAMP experiment showed 100 times more sensitivity than RT-PCR. The sensitivity and specificity of the RT-LAMP test were found to be 100% and 87%, respectively. The test procedure was simple and amplification was carried out at 63°C. The result was obtained in less than 1 hour (53).

Yu et al. developed the Isothermal LAMP based method for Covid-19 (iLACO) detecting the RNA orcDNA of the SARS-CoV-2 virus by isothermal amplification of LAMP and the duration is 20-30 minutes at 65°C. The sensitivity of the method was found to be comparable to the Taqman based qRT-PCR method. The method is capable of detecting synthesized RNA equivalent to 10 copies of SARS-CoV-2. Reaction time varies between 15-40 minutes depending on virus load (49).

Zhang et al. detected SARS-CoV-2 purified on cell lysis or RNA using loop-mediated isothermal amplification (LAMP) technique. Moreover, this technique performed equally well to the RT-qPCR test using RNA samples purified from respiratory swabs collected from Covid-19 patients in Wuhan, China. It has been determined that the primers used in this study have higher detection capacity and can detect several hundred copies (54).

El-Tholoth et al. tested loop-mediated isothermal amplification (Covid-19 LAMP) and two-stage isothermal amplification (Covid-19 Penn-RAMP). Both methods can detect colorimetric or fluorescence. In that study, it was observed that Covid-19 LAMP and Covid-19 RT-PCR showed equal performance. When testing purified targets SARS-CoV-2 RAMP has 10 fold better sensitivity than the other techniques. Similarly, 100 times better sensitivity than the other techniques when testing rapidly prepared sample mimics (4).

The ID Now- Covid-19 test developed by Abbott can detect SARS-CoV-2 in 5 minutes. It performs qualitative detection of RNA from the virus using isothermal nucleic acid amplification technology. According to the PCR experiments, the Abbott ID NOW test had detection limits 10 to 100 times higher (55). Recently, the test which is performedwith an easy-to-use device has been approved by FDA EUA (7).

Nucleic acid sequence-based amplification (NASBA) is an in vitro amplification process carried out under isothermal conditions. It is a two-step amplification process. The first step is denaturation and the second step is amplification due to a polymerase which is performed isothermally. This technique has been developed as a multiplex process called multiple real-time nucleic acid sequence-based amplification (RT-NASBA). RT-NASBA has proven to be 10-100 times more sensitive than Multiplex RT-PCR (56). There are many kinds of literature to examine the different diagnostic approaches used by academic labs and clinicians to diagnose COVID-19 disease since the identification of SARS-CoV-2 till now (57).

Chantratita et al. developed a test called NASBA. An RT-LAMP test of SARS-CoV showed 100 times more sensitivity than RT-PCR. NASBA-Beacon on the NucliSens® EasyQ Analyzer was found 10 times more sensitive than RT-PCR TaqMan (ABI7000) and RT-PCR based hybridization probe (LightCycler) (58).

In addition to these, digital pcr (dPCR) based tests have been developed for the detection of SARS

- CoV - 2. In this technique, samples are portioned under microliters and reproducible measurements are made by measuring low DNA concentrations. dPCR is used in detecting low copy number viruses in SARS-CoV-2 detection, measuring viral load, monitoring virus concentration, detection of viral mutations and evaluation of anti-SARS-CoV-2 drugs (58,59,60).

The clustered regularly interspaced short palindromic repeats (CRISPR)-Cas (CRISPR-associated proteins) technology could be an effective approach to target viral RNA. This system has applications in humans against viral infections and is effective to eliminate these infections. The CRISPR-Cas system is under investigation so that SARS-CoV-2 can be diagnosed quickly. It is thought that CRISPR-Cas-based methods can diagnose SARS-CoV-2 infection within a few hours. Besides its diagnostic capability, the CRISPR-Cas system can also be useful in antiviral therapy (61).

With next-generation sequencing, the sequence of close to a million base pairs can be determined in a single experiment. This method is also used in the detection of viral strains. It provides very fast detection of viruses. This method is also an efficient technology that can be used in the detection of SARS-CoV-2. The US Food and Drug Administration (FDA) granted emergency use of Illumina for next-generation sequencing (NGS) use in the detection of SARS-CoV-2 in June. Next generation sequencing methods are candidates for potential use for high-volume screening processes (62).

Various biomarkers, including lymphocyte count, neutrophil-lymphocyte ratio, CRP, troponin T, D-dimer, LDH, procalcitonin, IL-6, and ferritin, are important in predicting disease progression and mortality, although they are not defining markers in the diagnosis of SARS-CoV-2. These laboratory tests play a vital role in identifying patients at risk of complications and guiding treatment interventions (63,64).

Bilkent University's National Nanotechnology Research Center (UNAM) has developed a nanotechnology-based system. This system is an optically based diagnostic and diagnostic system that changes the color of the glow in the presence of viruses, thus detecting viruses with high selectivity. In this system, pathogens are detected with 99% confidence with in 10 seconds by dynamically receiving a fluorescent signal through a pathogen detection chip developed specifically for a biosensor device. Unlike the commonly used PCR tests, the system is not based on sample replication, but on detecting the presence or absence of the virüs with advanced optical methods (65). There is recently another study is revelaed by Deniz et al about Covid-19 patients enzyme levels who they suggest some other approaches for this infection (66).

## CONCLUSION

Covid-19 has a crucial rate on mortality which becomes a pandemic that affects the whole world. The most important step in responding to this pandemic is early diagnosis. Laboratory techniques are important to prevent the spread and infection caused by the Covid-19 pandemic. To prevent the infection caused by SARS-CoV-2 from spreading to humans, it is important to use effective methods in the diagnosis.

So far, many studies have been conducted to develop better diagnostic tests for SARS-CoV-2. There is currently no standard treatment option for this disease, so it still poses a serious threat to the world. Development of new diagnostic tests continues globally. With the studies conducted, the genome structure of the virus will be better elucidated and much more effective methods will be used for diagnosis and treatment. Consequently, there will be more discussions and it will be presented more different test methods occuring to detect SARS-CoV-2 in the near future.

## ACKNOWLEDGEMENT

The authors would like to thank to Dr. Emine Terzi and Dr. Beyza Ecem Öz Bedir for their invaluable contribution during the paper process.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## KAYNAKLAR

1. Singhal T. A review of coronavirus disease-2019 (COVID-19). Indian J Pediatr, 2020 Apr;87(4):281-6.
2. Yang P, Wang X. COVID-19: a new challenge for human beings. Cell Mol Immunol., 2020 May;17(5):555-7.
3. Shen M, Zhou Y, Ye J, Al-Maskri AA, Kang Y, Zeng S, Cai S. Recent advances and perspectives of nucleic acid detection for coronavirus. J Pharm Anal, 2020 Apr 1;10(2):97-101.
4. El-Tholoth M, Bau HH, Song J. A single and two-stage, closed-tube, molecular test for the 2019 novel coronavirus (COVID-19) at home, clinic, and points of entry. Anal Chem, 2021 Sep 28;93(38):13063-71.
5. Kannan SP, Ali PS, Sheeza A, Hemalatha K. COVID-19 (Novel Coronavirus 2019)-recent trends. Eur Rev Med Pharmacol Sci, 2020 Feb 1;24(4):2006-11.
6. Shi Y, Wang G, Cai XP, Deng JW, Zheng L, Zhu HH, et.al. An overview of COVID-19. J Zhejiang Univ Sci B.2020 May 1:1.
7. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, Evaluation, and Treatment of Coronavirus (COVID-19). 2021 Apr 20. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. PMID: 32150360.
8. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VY, et.al. Diagnosing COVID-19: the disease and tools for detection. ACS nano, 2020 Mar 30;14(4):3822-35.
9. Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. J Adv Res, 2020 Jul 1;24:91-8.
10. Wu F, Zhao S, Yu B, Chen YM, Wang W et al. A new coronavirus associated with human respiratory disease in China. Nature 2020; 579, 265-269. doi: 10.1038/s41586-020-2008-3-7.
11. Shi Y, Wang G, Cai XP, Deng JW, Zheng L, Zhu HH, et.al. An Overview of COVİD-19. J Zhejiang Univ Sci B, 2020 May;21(5):343-60.
12. Xu X, Liu Y, Weiss S, Arnold E, Sarafianos SG, Ding J. Molecular model of SARS coronavirus polymerase: implications for biochemical functions and drug design. Nucleic Acids Res, 2003 Dec 15;31(24):7117-30.
13. Mori M, Capasso C, Carta F, Donald WA, Supuran CT. A deadly spillover: SARS-CoV-2 outbreak. Expert Opin Ther Pat, 2020 Jul 2;30(7):481-5.
14. Oberemok VV, Laikova KV, Yurchenko KA, Fomochkina II, Kubyshkin AV. SARS-CoV-2 will continue to circulate in the human population: an opinion from the point of view of the virus-host relationship. Inflamm. Res, 2020 Jul;69(7):635-40.
15. https://www.centerforhealthsecurity.org/resources/COVID-19/COVID-19-fact-sheets/200410-RT-PCR.pdf. (27.01.2021).
16. Loeffelholz MJ, Tang YW. Laboratory diagnosis of emerging human coronavirus infections-the state of the art. Emerg Microbes Infect, 2020 Jan 1;9(1):747-56
17. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in different types of clinical specimens. Jama, 2020 May 12;323(18):1843-4
18. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et.al. Virological assessment of hospitalized patients with COVID-2019. Nature, 2020 May;581(7809):465-9.

19. Tang YW, Schmitz JE, Persing DH, Stratton CW. Laboratory diagnosis of COVID-19: current issues and challenges. J Clin Microbiol, 2020 May 26;58(6): e00512-20.

20. Singh S, Kalra MK, Khawaja RD, Padole A, Pourjabbar S, Lira D, et.al. Radiation dose optimization and thoracic computed tomography. Radiol Clin, 2014 Jan 1;52(1):1-5.

21. Marzinotto S, Mio C, Verardo R, Pipan C, Schneider C, Curcio F. A streamlined approach to rapidly detect SARS-CoV-2 infection, avoiding RNA extraction. Disease Markers, 2020, Article ID 8869424, 5 pages, 2020.

22. Won J, Lee S, Park M, Kim TY, Park MG, Choi BY, et.al. Development of a Laboratory-safe and Low-cost Detection Protocol for SARS-CoV-2 of the Coronavirus Disease 2019 (COVID-19). Exp Neurobiol, 2020 Apr 30;29(2):107.

23. Smyrlaki I, Ekman M, Lentini A, de Sousa NR, Papanicolaou N, Vondracek, et.al. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. Nat Commun, 2020 Sep 23;11(1):1-2.

24. Ladha A, Joung J, Abudayyeh OO, Gootenberg JS, Zhang F. A 5-min RNA preparation method for COVID-19 detection with RT-qPCR. MedRxiv, 2020 Jan 1. v.20200405 (2020). https://static1.squarespace.com/static/5b7c640be2ccd1703a3da4d3/t/5e8bcd0923fb224b2f3b1356/1586220297644/Ladha+et+al+-+RNA+QE+Extraction.pdf.

25. Yan Y, Chang L, Wang L. Laboratory testing of SARS-CoV, MERS-CoV, and SARS-CoV-2 (2019-nCoV): Current status, challenges, and countermeasures. Rev. Med. Virol. 2020 May;30(3): e2106.

26. Arya M, Shergill IS, Williamson M, Gommersall L, Arya N, Patel HR. Basic principles of real-time quantitative PCR. Expert Rev Mol Diagn, 2005 Mar 1;5(2):209-19.

27. Loftis AD, Reeves, WK. (2012). Principles of real-time PCR. In Chengming W, Bernhard K, Mark DF eds. Veterinary PCR Diagnostics. Sharjah. Bentham Science, 2012: 3-17.

28. Van Elden LJ, Anton M AM, Van Alphen F, Hendriksen KA, Hoepelman AI, Van Kraaij MG, et.al. Frequent detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. J Infect Dis, 2004 Feb 15;189(4):652-7.

29. Corman V, Bleicker T, Brünink S, Drosten C, Zambon M. Diagnostic detection of 2019-nCoV by real-time RT-PCR. World Health Organization, Jan. 2020 Jan 17;17.

30. Chu DK, Pan Y, Cheng SM, Hui KP, Krishnan P, Liu Y, et.al. Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. Clin Chem, 2020 Apr 1;66(4):549-55.

31. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DG. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance, 2020 Jan 23;25(3):2000045.

32. Chan JF, Yip CC, To KK, Tang TH, Wong SC, Leung KH, et.al. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens. J. Clin. Microbiol, 2020 Apr 23;58(5).

33. https://www.cepheid.com/coronavirus. (27.01.2021).

34. https://www.fda.gov/media/136314/download. (27.01.2021).

35. Lieberman JA, Pepper G, Naccache SN, Huang ML, Jerome KR, Greninger AL. Comparison of commercially available and laboratory-developed assays for in vitro detection of SARS-CoV-2 in clinical laboratories. J. Clin. Microbiol, 2020 Jul 23;58(8).

36. Carter LJ, Garner LV, Smoot JW, Li Y, Zhou Q, Saveson CJ, et.al. Assay techniques and test development for COVID-19 diagnosis. 2020.

37. Korukluoğlu G, Altaş AB, Coşgun Y. Ülkemizde kullanılan tanı kitleri ve özellikleri. Çiçek C, editör. Mikrobiyoloji ve COVID-19. 1. Baskı. Ankara: Türkiye Klinikleri; 2020. p.63- 73

38. Hall VJ, Foulkes S, Charlett A, Atti A, Monk EJM, Simmons R, et al. SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN). Lancet, 2021 Apr 17;397(10283):1459-69.

39. (https://sentebiolab.com.tr/corona-virus-tani-kiti-tespit-kiti-test-kiti/)

40. http://www.anatoliageneworks.com/en/index.asp

41. Kopel J, Goyal H, Perisetti, A. (2021, January). Antibody tests for COVID-19. Proc (Bayl Univ Med Cent), 2021;(34(1):63-72.

42. Long, QX., Liu, BZ., Deng, HJ. et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 26, 845–848 (2020).

43. Baraniuk C., Covid-19 antibody tests: a briefing BMJ (2020) Jun 24;369:m2284. doi: 10.1136/bmj.m2284.

44. Jin Y, Wang M, Zuo Z, Fan C, Ye F, Cai Z, et.al. Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019. Int J Infect Dis, 2020 May 1;94:49-52.

45. Zhao R, Li M, Song H, Chen J, Ren W, Feng Y, et.al. Early detection of SARS-CoV-2 antibodies in COVID-19 patients as a serologic marker of infection. Clin Infect Dis, 2020 May 1.

46. Maple PA, Sikora K. How useful is COVID-19 antibody testing-a current assessment for oncologists. Clin. Oncol, 2021 Jan 1;33(1): e73-81.

47. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et.al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med, 2020 Jan 24.

48. Bastos ML, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, et.al. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ, 2020 Jul 1;370.

49. Semen Z, Ekici S, Malas B. Covid-19 İçin Moleküler Tanı Yöntemlerine Genel Bakış. Vet Farm Toks Dern Bult, 2021;11(2):72-9.

50. Yu L, Wu S, Hao X, Dong X, Mao L, Pelechano V, et.al. Rapid detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform Clin Chem, 2020 Jul 1;66(7):975-7.

51. Gonzalez-Reiche AS, Hernandez MM, Sullivan MJ, Ciferri B, Alshammary H, Obla A, et.al. Introductions and early spread of SARS-CoV-2 in the New York City area. Science, 2020 Jul 17;369(6501):297-301.

52. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et.al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. Clin Infect Dis, 2020 Oct 15;71(16):2027-34.

53. https://www.fda.gov/media/134922/download. (27.02.2021).

54. Thai HT, Le MQ, Vuong CD, Parida M, Minekawa H, Notomi T, Hasebe F, Morita K. Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus. J Clin Microbiol, 2004 May 1;42(5):1956-61.

55. Zhang Y, Odiwuor N, Xiong J, Sun L, Nyaruaba RO, Wei H, Tanner NA. Rapid molecular detection of SARS-CoV-2 (COVID-19) virus RNA using colorimetric LAMP. MedRxiv, 2020 Jan 1.

56. Cradic K, Lockhart M, Ozbolt P, Fatica L, Landon L, Lieber M, Yang D, Swickard J, Wongchaowart N, Fuhrman S, Antonara S. Clinical evaluation and utilization of multiple molecular in vitro diagnostic assays for the detection of SARS-CoV-2. Am J Clin Pathol, 2020 Jul 7;154(2):201-7.

57. Islam KU, Iqbal, J. An Update on Molecular Diagnostics for COVID-19. Front Cell Infect Microbiol, 2020;10.

58. Chantratita W, Pongtanapisit W, Piroj W, Srichunrasmi C, Seesuai S. Development and comparison of the real-time amplification based methods-NASBA-Beacon, RT-PCR Taqman and RT-PCR hybridization probe assays-for the qualitative detection of SARS coronavirus. Southeast Asian J Trop Med Public Health, 2004 Sep 1;35:623-9.

59. Çarhan A, Ercan E, Yalçınkaya T. Dijital PZR ve kullanım alanları. Turk Hij Den Biyol Derg, 2016 Jun 1;73(2).

60. Tan C, Fan D, Wang N, Wang F, Wang B, Zhu L, Guo Y. Applications of digital PCR in COVID-19 pandemic. View, 20200082.

61. Kumar P, Malik YS, Ganesh B, Rahangdale S, Saurabh S, Natesan S, Srivastava A, Sharun K, Yatoo MI, Tiwari R, Singh RK. CRISPR-Cas system: An approach with potentials for COVID-19 diagnosis and therapeutics. Front Cell Infect Microbiol, 2020;10.

62. First NGS-based COVİD-19 Diagnostic. Nat Biotechnol, July 2020;38: 769-778.

63. Kumar R, Nagpal S, Kaushik S, Mendiratta S. COVID-19 diagnostic approaches: different roads to the same destination. Virusdisease, 2020 Jun;31(2):97-105.

64. Herold T, Jurinovic V, Arnreich C, Lipworth BJ, Hellmuth JC, von Bergwelt-Baildon M, Klein M, Weinberger T. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J Allergy Clin Immunol, 2020 Jul 1;146(1):128-36.

65. https://w3.bilkent.edu.tr/www/diagnovir/. (27.01.2021).

66. Deniz S, Uysal TK, Capasso C, Supuran CT, Ozensoy Guler O. Is carbonic anhydrase inhibition useful as a complementary therapy of Covid-19 infection? J Enzyme Inhib Med Chem. 2021 Dec;36(1):1230-1235. doi: 10.1080/14756366.2021.1924165. PMID: 34074197.

67. Hasöksüz M, Kiliç S, ve Saraç F. Coronaviruses and SARS-CoV-2. Turk J Med Sci, 2020(50): 549-56.

68. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet, 2020.