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Review Article

A Brief Review on the Advancement of the Molecular and Serological Diagnosis of SARS-CoV-2

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Abstract

The novel coronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is causative agent for coronavirus disease 2019 (COVID-19) since the 2019 December. Human coronaviruses are classified in Nidovirales order and Coronavirdiae family. This family includes four genera. The SARS-CoV-2 is a member of Betacoronavirus genera and Sarbecovirus linage (linage B). There is a great number of conducted researches for the therapeutic options, epidemiological aspects, clinical and radiological features and molecular or serological diagnosis for the SARS-CoV-2. There is a verity of the commercially available serological and molecular kits for COVID-19 diagnosis. Also, the WHO recommended molecular approaches for the diagnosis. This recommended list contains different primer and probe sets for SARS-CoV-2 diagnosis and different authors assessed the specificity and sensitivity of this primer and probe sets. In this review, we tried to gather comprehensive information about these diagnosis strategies. Also, there are some studies focused on the antibody response to SARS-CoV-2. By considering the growing amount of the available researches in the field of the serological and molecular diagnosis in SARS-CoV-2 detection, current study was aimed to briefly review the most important advancements in this particular subject area.

Keywords: SARS-CoV-2; COVID-19; PCR, Serology; Antibody; Diagnosis

Introduction

The novel coronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is causative agent for coronavirus disease 2019 (COVID-19) (1). Human coronaviruses are classified in Nidovirales order and Coronavirdiae family. This family includes four genera. The SARS-CoV-2 is a member of Betacoronavirus genera and Sarbecovirus linage (linage B) (1, 2). The SARS-CoV-2 genome contains different open reading frames (ORFs), such as all other Sarbecovirus the SARS-CoV-2 encodes major and accessory ORFs. The major ORFs are ORF1a/b (contains 16 non-structural proteins (NSP), named as NSP-1 to NSP-16), S (Spike), M (Membrane), E (Envelop) and N (Nucleo-capsid).

Also, SARS-CoV-2 encoded accessory proteins include ORF3a, ORF3b, ORF6, ORF7a, ORF7b and ORF8 (1). Patients with COVID-19 are rapidly growing around the word. Based on the World Health Organization (WHO) Situation Report, there are 4789205 confirmed cases and 318789 deaths around the world by the date of 20th May, 2020 (3).

As a result of the rapid distribution of the SARS-CoV-2 around the word, a reliable and fast diagnostic approach seems to be necessary (4, 5). Classic virological approaches such as virus culture in cell lines or electron micro-scopy are time consuming and not applicable

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in fast responses for virus detection. But advances in virus detection have resulted in the different, reliable and faster techniques (4, 6, 7). Real time PCR as one of molecular methods seems to be a method of choice for the SARS-CoV-2 detection. Regardless of advantages of Real time PCR, this method depends on the highly trained staffs and expensive equipment (8, 9). As a result of the early sequencing of the SARS-CoV-2 and full genome release, different molecular assays for diagnosis of the SARS-CoV-2 were developed. In this regard, there is a list for these primer probe-based real time PCR molecular assays, suggested for the diagnosis by the WHO (10). Furthermore, antibody response is a major element for limiting viral infections (11).

Using the antibody assessment could be beneficial to overcome into some of the molecular diagnostic challenges (12). In this matter, by considering the growing amount of the available researches in the field of the serological and molecular diagnosis in SARS-CoV-2 detection, current study was aimed to briefly review the most important advancements in this particular subject area.

Molecular diagnosis of SARS-CoV-2

In the current months, different molecular approaches have been introduced for SARS-CoV-2 detection (10, 13-15).

Currently, the molecular techniques are considered as the method of choice by the WHO for the diagnosis of the SARS-CoV-2 in COVID-19 patients (14). By considering this, there are some studies for assessment of these methods for sensitivity, specificity, efficacy and throughput.

Vogels et al. (16) assessed the efficacy of the PCR primer and probe sets recommended by the WHO. The results were promising for all of the recommended primer and probe sets by the WHO. Also, the E-Sarbeco primer and probe set and HKU-ORF1 have shown the most sensitivity (all of the mentioned primer and probe sets are listed in the Table 1 and the following link: https://www.who.int/ emergencies/diseases/novel-coronavirus-2019/ technicalguidance/laboratory-guidance (14).

Also, the results of a study performed by Vogels et al. suggested that the N2 primer probe set (developed by the US Centers for Disease Control and Prevention (CDC)) shows the background color. Meanwhile, in the study conducted by Brown and colleagues (17), the N2 primer probe set of the CDC was the most sensitive primer probe set for the N region of the SARS-CoV-2 genome.

One of the time-consuming processes before the real time PCR is nucleic acid extraction. In the suggested method by Beltrán-Pavez and colleges, PCR detection without RNA extraction was explained (14). Also, using the pre-heated swab samples before the PCR test suggested by Alcoba-Florez et al. (15) and this method introduced as an alternative method for increasing the throughput and decreasing the time in SARS-CoV-2 diagnosis.

Also, Arumugam et al. (18) suggested a rapid PCR protocol, leading to the results in 12 minutes by using specific alterations.

Regardless of the mentioned studies, there are other molecular approaches for SASR-CoV-2 diagnosis (19). For instance, we could mention the ID NOW platform by Abbot. This method is fast and accurate isothermal amplification method for SARS-CoV-2 diagnosis. This specific method can decrease the time of detection to 5 minutes, but the specificity and sensitivity need to be improved (19). Also, a recently new approach such as Digital PCR assay has been suggested to be useful. Based on the study conducted by Romeo and colleagues (20), using a Droplet Digital PCR assay could improve the limit of detection (LOD) in the PCR assay for SARS-CoV-2 diagnosis. Also, it has been suggested that there are valuable information about pre-analytical, sampling and post-analytical issues reviewed by Tang et al (21).

By the current time, there are plenty of the commercially available diagnostic kits for SARS-CoV-2. Recently, by the date of the 8th June, there are 63 US FDA approved molecular based commercial diagnostic kits for SARS-CoV-2 (22). Recent FDA approved Xpress SARS-CoV-2 (Cepheid's GeneXpert® Systems) is a reliable and fast diagnostic tool.

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Table 1. Primers and probe sets suggested by the WHO (14)

Institute	Primer and probe sets		Target gene
China CDC	F	5'-CCCTGTGGGTTTTACACTTAA-3'	ORF1a/b
	R	5'-ACGATTGTGCATCAGCTGA-3'	
	P	FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1	
	F	5'-GGGGAACTTCTCCTGCTAGAAT-3'	N
	R	5'-CAGACATTTTGCTCTCAAGCTG-3'	
	P	FAM-TTGCTGCTGCTTGACAGATT-TAMRA	
Institute Pasteur	F	5'-ATGAGCTTAGTCCTGTTG-3'	RdRp
	R	5'-CTCCCTTTGTTGTGTTGT-3'	
	P	Hex-AGATGTCTTGTGCTGCCGGTA-BHQ-1	
	F	5'- GGTAACTGGTATGATTTCG -3'	RdRp
	R	5'- CTGGTCAAGGTTAATATAGG-3'	
	P	Fam-TCATACAAACCACGCCAGG-BHQ-1	
	F	5'- ACAGGTACGTTAATAGTTAATAGCGT-3'	E
	R	5'- ATATTGCAGCAGTACGCACACA-3'	
	P	Fam-ACACTAGCCATCCTTACTGCGCTTCG-BHQ-1	
US CDC	F	5'-GACCCCAAAATCAGCGAAAT-3'	N-1
	R	5'-TCTGGTTACTGCCAGTTGAATCTG-3'	
	P	FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ-1	
	F	5'- TTACAAACATTGGCCGCAAA-3'	N-2
	R	5'-GCGCGACATTCCGAAGAA-3'	
	P	Fam-ACAATTTGCCCCCAGCGCTTCAG-BHQ-1	
	F	5'-GGGAGCCTTGAATACACCAAAA-3'	N-3
	R	5'-TGTAGCACGATTGCAGCATTG-3'	
	P	Fam-AYCACATTG GCACCCGCAATCCTG-BHQ-1	
	F	5'-AGATTTGGACCTGCGAGC G-3'	Host RNAse P
	R	5'-GAGCGGCTGTCTCCACAAGT-3'	
	P	Fam-TTCTGACCTGAA GGC TCTGCGCG-BHQ-1	
Japan National Institute of Infectious Diseases	F	5'- AAATTTTGGGGACCAGGAAC-3'	N
	R	5'- TGGCAGCTGTGTAGGTCAAC-3'	
	P	FAM-ATGTCGCGCATTGGCATGGA-BHQ	
Charité, Germany	F	5'- GTGARATGGTCATGTGTGGCGG-3'	RdRp
	R	5'- CARATGTTAAASACACTATTAGCATA-3'	
	P	FAMCCAGGTGGWACRTCATCMGGTGATGCBBQ	Pan sarbeco
	P	FAM-CAGGTGGAACCTCATCAGGAGATGCBBQ	SARS-CoV-2
	F	5'-ACAGGTACGTTAATAGTTAATAGCGT-3'	E
	R	5'-ATATTGCAGCAGTACGCACACA-3'	
	P	FAM-ACACTAGCCATCCTTACTGCGCTTCGBBQ	
HKU (Hong Kong University)	F	5'- TGGGGYTTTACRGGTAACCT-3'	ORF1b
	R	5'- AACRCGCTTAACAAAGCACTC-3'	
	P	FAM-TAGTTGTGATGCWATCATGACTAG-TAMRA	
	F	5'- TAATCAGACAAGGAACTGATTA-3'	N
	R	5'- CGAAGGTGTGACTTCCATG-3'	
	P	FAM-GCAAATTGTGCAATTTGCGG-TAMRA	
Thailand National Institute of Health	F	5'- CGTTTGGTGGACCCTCAGAT-3'	N
	R	5'- CCCCACTGCGTTCTCCATT-3'	
	P	FAM-CAACTGGCAGTAACCA-BQH1	

F: forward primer, R: reverse primer, P: probe, N: Nucleocapsid gene, E: envelope gene, RdRp: polymerase gene

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The Xpress SARS-CoV-2 is designed based on the real time PCR method and use two distinct primer and probe sets for N and E genes of SARS-CoV-2 (23). Also, Fast track diagnostics by Siemens health can provides the FDA approval for SARS-CoV-2 real time PCR kit. The Fast track diagnostics SARS-CoV-2 real time PCR kit use the primer and probes for ORF 1a/b and N genes of SARS-CoV-2 (24).

The N gene of the SARS-CoV-2 seems to be the most interested gene for commercially available molecular kits. The N gene was also used by Abbott Real Time SARS-CoV-2 assay (ABBOTT), GeneFinder COVID-19 plus RealAmp Kit (OSANG Healthcare) and the BIO-RAD SARS-CoV-2 Droplet Digital PCR (ddPCR) Kit (25-27). Furthermore, the ORF1a/b and E gene were used in QIAstat-Dx Respiratory SARS-CoV-2 (QIAGEN) (28).

Meanwhile, the only quantitative FDA approved SARS-CoV-2, QuantiVirus SARS-CoV-2 Test kit, manufactured by the DiaCarta used all three mentioned genes (29).

As mentioned there are verities of different commercially available diagnostic kits by using RT-PCR method. For instance the RealStar® SASR-CoV-2 RT-PCR kit 1.0 (Altona Diagnostics GmbH, Hamburg, Germany) is confirmed diagnostic kit by USA and European countries for in vitro diagnostic. The RealStar® SASR-CoV-2 RT-PCR kit 1.0 used three different primer and probe sets for three different channels on Real time PCR termocycler for assessment of the beta coronaviruses E gene, internal control and SARS-CoV-2 S gene. This kit is compatible with most of the available real time PCR termocyclers (30). Meanwhile, the KHB (Shanghai Kehua Bio-Engineering) RT-PCR for Diagnostic SARS-CoV-2 Nucleic Acid use three different viral genes and one internal control in four different channels. This kit use ORF1a/b, E and N genes from SARS-CoV-2 for diagnosis. The DiaPlex Novel Coronavirus (2019-nCoV) Detection Kit (SolGent, Korea) which it were also active in the field of the MERS-CoV diagnostic kits used conserved reigns of N and ORF 1a genes for SARS-CoV-2 diagnostic kit (31). Also, an automated Cobas® SARS-CoV-2 6800/8800 (Roche Diagnostics) is provide great sensitivity and reduce diagnostic process errors to minimum (22).

Da An Gene Co., Ltd kit (Sun Yat-sen University, China) is suitable for the qualitative detection of SARS-CoV-2 ORFlab and N genes in the different samples of suspected pneumonia patients infected by SARS-CoV-2 such as throat or nasal swabs and sputum specimens. The detection method is based on one-step RT-PCR technique. In practice, ORF1ab and N genes of the SARS-CoV-2 are the target regions for amplification. This kit is consisted of specific primers and fluorescent probes for the detection of SARS-CoV-2 RNA in the specimens.

Also, it includes an endogenous internal standard detection system used for monitoring over the processes of specimen collection and PCR amplification. Thereby, false negative results will reduce (32).

Sansure Biotech (SANSURE BIOTECH INC, China) has developed a simple and fast real-time reverse transcription polymerase chain reaction (rRT-PCR) kit, which is based on its advanced RNA fast-releasing technology. This kit includes the specific primer and probe sets designed to qualitative detect of SARS-CoV-2 ORFlab and N genes in respiratory specimens of suspected patients for COVID-19. The sample mixture can be directly added to the 2019-nCoV-PCR mastermix (2019-nCoV-PCR Mix plus 2019-nCoV-PCR-Enzyme Mix) by one simple step before real time RT-PCR amplification. To avoid false-negative results, there is an internal control targeting the RNase P gene monitor the sample handling, sample collection, and PCR process. The limit of detection (LoD) of the kit is 200 copies/mL (33). Regardless of mentioned kit there is verity of commercially available kits for SARS-CoV-2 diagnosis.

Furthermore, there are general topics about mentioned molecular diagnostic kits. Repeated freezing or thawing of Master Mix should be avoided. The reagents could be frozen in aliquots. Storage between +2°C to +8°C should be only in limited time. Master mixes and probes should be protected from light. The entire mentioned assays only valid by using

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internal Control to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit. In these diagnostic kits Real-time RT-PCR technology by reverse-transcriptase (RT) reaction for converting the RNA into complementary DNA (cDNA) is prior to the polymerase chain reaction (PCR) for the amplification of specific target sequences and probes are used for the detection of the amplified temple DNA.

The probes are attached to fluorescent reporter (emits fluorescent) and quencher (quenching the fluorescent before hydrolysis) dyes. These probes are labeled by verity of fluorophore days includes FAM, Cy5, JOE, HEX, ROX or Yakima yellow. Most of the mentioned diagnostic kits are applicable on Mx 3005P™ QPCR System (Stratagene), VERSANT® kPCR Molecular System AD (Siemens Healthcare), ABI Prism® 7500 SDS (Applied Biosystems) (includes step one and step one plus platforms), Rotor-Gene® 6000 (Corbett Research) (The Rotor-Gene® 6000 is available in different platforms with 2 to 6 plex), CFX96™ Deep Well Dx System (Bio-Rad) (or CFX96™ Real-Time PCR Detection System) and LightCycler® 480 Instrument II (Roche). Also, all of the mentioned diagnostic kits contains the manufactures protocols for the sample preparation, cycling programs, controls or fluorescent detection and limitations of the kits. The data analysis in diagnostic kits is important part, and should be performed based on the manufactures protocols.

Antibody responses and serological assessment of SARS-CoV-2

It has been indicated that the seroconversion in of IgG and IgM in COVID-19 patients could be simultaneous. The antibody rising titer enters to the plateau phase after 6 days from the seroconversion. Also, the IgG positive results could be seen in 100% of the COVID-19 patients on day 19 since the onset of the symptoms (23). The serological assays are useful and these diagnostic techniques can be used for covering the molecular drawbacks. Herein, we mention some of the important researches in the serological assessment of the SARS-CoV-2, and try to highlight the drawbacks and benefits of these methods.

Lin and colleagues (4) investigated the serological approaches for SARS-CoV-2 detection. They showed that the IgG assessment is more reliable for the COVID-19 patients in comparison with IgM. The IgG assessment showed 82% and 97% sensitivity and specificity for SARS-CoV-2 detection, respectively. Also, Lin et al. suggested that the chemiluminescence-immunoassay method is a better technique for antibody assessment in COVID-19 patients in comparison with ELISA (4). Also, in a study conducted by Liu et al. (8), the IgG-IgM combined antibody test panel assessed for diagnosis of COVID-19. This combined assay showed 85% sensitivity and 91% specificity and the positive predictive value and negative predictive value of the mentioned test were 95.1% and 82.7%, respectively (8). There are several studies regarding the point of care (POC) testing and rapid tests for the assessment of SARS-CoV-2 infection (12, 24, 34). But the data does not seem to be solid and the final conclusion in this matter seems to be controversial .

In a study conducted by Wang et al. (35), the IgM level in COVID-19 patients has been suggested as a prognostic factor in the severity of the disease. However, using the level of the antibodies as prognostic factor in the severity of COVID-19 disease was assessed by Dahlke and colleagues (36). Dahlke et al. (36) suggested that the IgA levels could be a great indicator of the severity of the disease in COVID-19 patients. Also, in a study conducted by Lassaunière and colleges, the specificity and sensitivity of the available serological kits in both ELISA and rapid tests platforms for SARS-CoV-2 were evaluated. In the ELISA platform assessment, the sensitivity ranged from 65-90% and the specificity ranged from 93-100% for different kits and different antibody types (12).

Another serological method such as indirect immunofluorescent assay has been developed by Edouard et al. (37). In this method, the specificity shows 100% for IgA, and 98% and 96% for IgM and IgG, respectively. Edouard et al. (37), has suggested this method as a way for

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the monitoring of exposure to the virus. Also, recently it has been suggested that there might be a cross recitation between SARS-CoV and SARS-CoV-2 antibody response (38).

Furthermore, assessments of different anti genic SARS-CoV-2 proteins show that the antigenicity of the S protein seems to be more specific than N protein of the virus (39).

In the comparison of S antigen there were no differences between the complete S antigen sensitivity in compare with only the R Receptor Binding Domain (RBD) domain (40). These findings might be promising for a selection of the best antigenic site for antibody-antigen based diagnostic approaches. By the date of the 8th June, there are 17 and 1 US FDA approved serologic and antigen based commercial diagnostic kits for SARS-CoV-2, respectively (22).

Most of these serologic approaches are focused in IgG and IgM assessment by using the antibody against SARS-CoV-2 S protein in rapid tests or chemiluminescent immunoassay assay plat-forms (27, 32, 41). Meanwhile, some manufac-tures focused on the IgG assessment in ELISA platforms. For instance, the SARS-CoV-2 IgG assay by ABBOTT, LIAISON SARS-CoV-2 S1/S2 IgG by Diasorin and COVID-19 ELISA IgG Antibody Test by Mount Sinai Laboratory (28, 29, 41).

Conclusion and further perspective

In conclusion, it could be reminded that, all primer and probe sets suggested by WHO could provide a reliable diagnostic approach for the SARS-CoV-2 detection. Also, there are available commercially diagnostic kits in the following link by WHO “https://www.finddx.org/covid-19”. This web site introduces serological available kits with the range from In vitro diagnostics (IVD) by the US or China FDA research use only (41). By considering the great effort of the researchers and commercial companies the COVID-19 diagnostic kits needs to be improved. Also, due to the importance of the serological diagnostic kits for screening programs, serological diagnostic kits needs more improvements. Current study was aimed to briefly review the most important advancements in this particular subject area. A major limitation of the current study was a limitation in the included studies due to the limited number for this particular research area .

By the current researches, it seems that the valuable and great efforts are performing in laboratory diagnosis for SARS-CoV-2 and COVID-19. But, further investigations for a reliable serological method for the diagnosis seem to be desperately necessary.

Also, advancements for a fast and commercially available molecular based diagnostic method should be considered.

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Conflict of interest

There is no any conflict of interest

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