Detection and Diagnosis of SARS-CoV-2/COVID-19

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Latest weeks the humanity is faced with the spread of a new coronavirus, SARS-CoV-2 that causes a respiratory illness with high mortality rates, COVID-19. Since there is no approved treatment or vaccination against that specific coronavirus the reduce in virus spread is essential. That is based in the use of appropriate tools, enabling the accurate and early detection. Molecular biology and immunological techniques are widely used in order to predict the COVID-19 cases in a very short period of time. These are commonly based either in identification of the SARS-CoV-2's genetic material or in detection of antibodies that have been produced by the immune system against the virus. Many of the above mentioned tests have been validated and approved by local authorities. However, there are much more companies that provide detection tests, without basic validation processes, contributing in non-precise data. The present review aim to analyze the most common platforms that are used in COVID-19 detection, analyzing their advantages and weaknesses. Therefore, each physician will be equipped with appropriate information required for each test.

Keywords: SARS-CoV-2 ; COVID-19 ; Coronavirus ; Detection ; Diagnosis

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the family of Coronaviridae and is a betacoronavirus with positive-sense single-stranded RNA virus, and was first identified in China (Wuhan) on December 2019 [1]. The genetic material contains information about four proteins, the spike (S), envelope (E), membrane (M) and nucleocapsid (N). Spike protein is responsible for attachment and further fusion of virus into the host cell [2], while in combination with the envelope and membrane proteins are responsible for viral envelope formation. The nucleocapsid protein holds the viral RNA [3]. The detection of SARS-CoV-2 based in detection of virus's genetic material, through molecular biology techniques, or in detection of antibodies that have been produced by host's immune system. The Polymerase Chain Reaction (PCR) enable the detection and amplification of specific DNA or RNA (through cDNA) molecules from a mixture of molecules in a sample [4]. The technique is based on amplification of regions that are determined by using specific primers (oligonucleotides). Whether the DNA/cDNA sequence is present, even at very low amounts, the primers will bind to that, and then enable the amplification of the region, while the absence of product usually means absence of DNA/cDNA. The evolution of PCR is the Real-Time PCR, where scientists not only can observe the presence or absence of genetic material, but also to quantify it, therefore alterations can be monitored [5]. On the other hand, detection of SARS-CoV-2 can be achieved with serology tests, which enable identification of antibodies in the serum. Antibodies are produced by the immune system as a response to an infection, against foreign proteins, or against to own's proteins. Serological tests are based on antibodies ability to recognize and bind to specific antigens [6]. Alternative technologies include also nucleic acid isothermal amplification and imaging methods (X-ray). Although scientists can use different methods for detection, they should always take into consideration potential limitations of them. This review describes the widely used diagnostic tests for COVID-19 detection and their advantages and weaknesses.

2. CDC Guidelines Test

The Centers for Disease Control and Prevention (CDC) suggest the use of molecular biology techniques for detection of SARS-CoV-2. The test is based on Real-Time Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR), and consumables are available from difference companies (BioSearch Technologies, KIT-nCoV-PP1- 1000, Integrated DNA Technologies (IDT), 10006606,). The basic principle behind that test is the detection of the virus nucleocapsid gene (N), using specific primers and probes. The test includes another set of primers/probes for the detection of the human RNase P gene (RP) as internal control. The sampling is performed with isolation of specimens from upper and lower respiratory systems (nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate. RNA is isolated from the above specimens and used as template in RT-PCR reactions using the following primer/probe sets [7].

Table 1: Primer/probe information of CDC diagnostic test based on Real-Time PCR

Description	Oligonucleotide Sequence (5'-3')
2019-nCoV_N1 Forward Primer	GAC CCC AAA ATC AGC GAA AT
2019-nCoV_N1 Reverse Primer	TCT GGT TAC TGC CAG TTG AAT CTG
2019-nCoV_N1 Probe	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1
2019-nCoV_N2 Forward Primer	TTA CAA ACA TTG GCC GCA AA
2019-nCoV_N2 Reverse Primer	GCG CGA CAT TCC GAA GAA
2019-nCoV_N2 Probe	FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1
2019-nCoV_N3 Forward Primer	GGG AGC CTT GAA TAC ACC AAA A
2019-nCoV_N3 Reverse Primer	TGT AGC ACG ATT GCA GCA TTG
2019-nCoV_N3 Probe	FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1
RNAse P Forward Primer	AGA TTT GGA CCT GCG AGC G
RNAse P Reverse Primer	GAG CGG CTG TCT CCA CAA GT
RNAse P Probe	FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1

The use of Real-Time RT-PCR is very sensitive and able to identify the genetic material even at very low amounts. However the main limitation is the requirement of specimens only from upper and lower respiratory system. It is also suggested to collect specimens (types and time points) from the same patient, since a negative results should checked again, and does not quarantine absence of infection. The test has not been established from blood specimens while there is a limit of detection at 10-5 copies/ul or RNA. On the other hand, the use of three different primer/probe sets increase

the accuracy or results, compared with other tests targeting only one region. The above diagnostic kit has been tested in other viruses and pathogens and ensuring no significant combined homologies with them or any other part of human genome.

3. Real-Time PCR Tests

Up to now there are more than 250 available tests based on PCR reactions, but only 98 are CE-IVD (In vitro diagnostic). Due to the circumstances many of the kits are authorized as Emergency Use Authorization (EUA), or have been authorized by US FDA. On the contrary more than 95 are used for Research Use Only (RUO) and should not be included for diagnosis. The majorities of these tests do not follow the same guidelines of CDC and are based on detection of other virus's proteins. Furthermore, in many kits there is targeted only one region, and are not used replicated of the experiment, increasing the possibility of false negative results [8]. Almost all kits use internal controls, the majority do not require positive control samples. The difference between internal and positive control is that the first one is used to ensure the isolation of genetic material from the specimen, while the second is used as control of the PCR reaction. It is recommended that the minimum guidelines required for publication in experiments with Real-Time PCR, should be followed also in all diagnostic kits [9].

Another important issue for all kits is the lack of clinical evidence, concerning positive and negative predictive values (PPV-NPV). PPV is defined as the probability of a test to be positive, when the sample has the specified disease, while NPV referred to the probability of a negative test, when it is really negative [10]. The calculation of PPV and NPV requires the blind test of the kit in patients and healthy volunteers. There have been recorded cases where kits have withdrawn or revised since the sensitivity of the assay has been found to be lower than expected.

4. Biosafety of Laboratories

The National Authorities have published guidelines not only for the diagnostic tests, but also for the biosafety of laboratories. In general, all PCR laboratories should be divided in pre and post-PCR areas reducing the chance of contamination. On the pre-PCR area is taking place the nucleic acid extraction and preparation of reaction, while the post-PCR is appropriate for amplification and post-amplification processes [11]. The processing of suspect specimens should be performed on a BSL-2 laboratory (Biosafety level-2), where virus isolation in cell culture must be conducted in BSL-3 laboratory [12]. Inappropriate sampling conditions put in jeopardy both healthcare stuff and healthy individual as well.

5. Sampling

Specimen type is one major issue regarding molecular diagnostic tests. Since SARS-CoV-2 infects mainly upper and lower respiratory system it is suggested sampling of upper and lower respiratory tract. Therefore it is recommended using nasopharyngeal or oropharyngeal swabs, nasal aspirate specimen, nasal mid-turbinate swab or anterior nares specimen. Regarding the lower respiratory tract it is recommended bronchoalveolar lavage, tracheal aspirate, pleural fluid, lung biopsy or sputum. Based on recent data the viral load is detected soon after symptoms onset and the load is higher in nose than in throat [13]. In addition the use of lingual swabs, although is easier to use and requires no-expertise personnel, provide lower positive rate than throat swabs. On the same study it was demonstrated that training or experience of personnel might affect the sampling process [14]. In general the higher positive rates have been shown in bronchoalveolar lavage fluid specimens (93%) followed by sputum (72%) and nasal swabs (63%). The positive rates decrease in other types of specimens as follow: fibrobronchoscope brush biopsy (46%, pharyngeal swabs (32%), feces (29%), blood (1%) and no detection in urine [15]. Therefore the use of different specimen types for each individual is highly recommended. It is noteworthy that has been detected a patient with meningitis/encephalitis, where SARS-CoV-2 was not detected in nasopharyngeal swab but was detected in a cerebrospinal fluid (CSF) [16]. Although that is surprisingly, SARS coronavirus RNA has been detected in the CSF of a patient with severe acute respiratory syndrome a few years ago [17].

6. Isothermal nucleic amplification

Another method used for detection of SARS-CoV-2's RNA is the isothermal nucleic amplification, which is not limited by the constraint of thermal cycling. It has been used in the past for rapid detection of severe acute respiratory syndrome coronavirus [18], however only a few data are available about specificity and sensitivity against SARS-CoV-2 detection.

7. Serology Tests

Detection of SARS-CoV-2 is not limited in RNA detection and identification but also in serology tests, based on detection of specific antibodies produced by the immune system. Immunoglobulins (Ig) or antibodies are proteins produced by the immune system against infections or foreign substances. Antibodies divided in five classes or isotypes, IgA, IgD, IgE, IgG and IgM. Immunoglobulin M (IgM) is the first antibody produced as response of the immune system. Immunoglobulin G (IgG) released by plasma B cells, later than IgM. Both antibodies circulate in serum and their presence indicates infection by a specific antigen [19]. SARS-CoV-2 upon infection is recognized by the immune system leading to production of IgM and later IgG against specific epitope of the virus. At the moment more than 250 tests are available in the market, and 144 of them obtained CE-IVD license. The majority of tests based on detection of IgM and /or IgG, while other uses IgA or combination of COVID-19 with influenza. As in molecular techniques, the antibodies that are used recognize the proteins of the virus, mainly N protein. Identification of IgM indicates infection in the early phase, while IgG observed approximately 10 days post infection. Simplicity in specimen and quick results are the main advantages of these diagnostic tests based on serological assays, like enzyme-linked immunoassay (ELISA) or chemiluminescence [20]. Based on CDC's guidelines, the use of serology tests is not recommended until their evaluation by different authorities and laboratories, and comparison with already established molecular biology techniques. Immunology-based tests do not require specific equipment or well-experience personnel and the results are obtained even in a few minutes.

Comparing serological assays with PCR, there have been mentioned several cases where patients were positive with real-time RT-PCR by negative with antibodies test, while fewer cases, where the molecular technique was negative and the immunology method was positive, were recorded [21]. Data about specificity and sensitivity of serological assays are placed almost in manufacturers' specifications sheets, however very few have been published. Among them, the sensitivity of immunology assays is 88,66% and the specificity 90,63%, when they compared with PCR in patients [22]. One weakness of serological tests is the time frame that can be used. IgM and IgA detected approximately 5 days post infection while IgG on 14 days after symptom onset. Therefore, these tests should not recommend as screening but as diagnostic tests [23]. On the same study, scientists demonstrated that the combination of IgM with PCR increase the positive detection rate up to 98,6 %.

Taking everything into consideration, it is well-understood the need of simple, quick and accurate diagnostic tests for COVID-19. Real-time RT-PCR provides sensitive and reliable data, however the sampling and preparation processes require well-trained and experienced personnel. The selection of the specimen is of primary importance, since inappropriate specimens lead to negative results. The combination of specimens from different parts of upper or lower respiratory tract increase the specificity of assays. It is also noteworthy that all procedures should be conducted on laboratories under restrict biosafety levels, ensuring protection of personnel and contamination of tested samples. Scientists and healthcare experts providing molecular diagnostic tests need to obey the rules of MIQES, including appropriate controls in each case. On the contrary, diagnosis of COVID-19 based on antibodies detection, is very simple, requiring blood, plasma or serum and does not implicate specialized equipment. Nevertheless, the detection of antibodies supposes activation of the immune system, therefore in most of the times symptoms. Serological tests are more suitable diagnosis of individuals with symptoms, rather than for screening.

Recapitulating this brief review, each test has both advantages and disadvantages that must be taken into from national authorities and healthcare experts. Reliable and accurate tests are preferable to simple and quick tests.

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