REVIEW

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COVID-19 Pathogenesis and Diagnosis

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ABSTRACT

The end of 2019 marked the beginning of a pandemic that shook of the whole world. This newly emerged viral agent was quickly identified with the help of molecular techniques and classified as a novel coronavirus severe acute respiratory syndrome-coronavirus-2. In the first part of this article, the general virological features and pathogenesis of this viral agent, which caused many deaths at the beginning and changed in the pathogenesis with different variants over time, were discussed. In the second part of the article, information is given about the diagnostic tests applied in pandemic conditions.

Keywords: COVID-19, SARS-CoV-2, pathogenesis, molecular tests, serological tests

Introduction

A new coronavirus emerged in late 2019 in China and spread throughout the world. This virus is identified as the third member of the coronaviruses causing severe acute respiratory syndrome (SARS) and still continues its pandemic. The disease caused by this new coronavirus was named as coronavirus disease-19 (COVID-19) by the World Health Organization (WHO) on February 11, 2020. At similar dates, the International Committee on Taxonomy of Viruses named this new coronavirus as SARS-coronavirus-2 (CoV-2). The first case of COVID-19 in our country was detected on March 11, when the WHO declared the disease as a pandemic (1,2,3,4,5). In this article, the basic virology pathogenesis and new approaches and diagnostic methods of SARS-CoV-2, which causes COVID-19, are reviewed.

Basic Virology

Coronaviruses belong to the order Nidovirales in the family coronaviridae and includes four genera, alpha, beta, gamma, and delta coronavirus. Alpha-coronaviruses include HCoV-229E, NL63, beta-coronaviruses include Middle East respiratory syndrome (MERS)-CoV, SARS-CoV, HCoV-OC43, HCoV-HKU1. SARS-CoV-2 is closely related to the beta-coronaviruses according to phylogenetic analysis. Comparing the DNA viruses RNA viruses have a higher mutation rate and shorter replication times so the SARS-CoV-2 has high mutation rate and variants (5,6).

The virus particle is 60-100 nm in diameter and has a round or oval appearance with an envelope. Similar to other betacoronaviruses, the SARS-CoV-2 genome is a positive-sense single-stranded RNA and lenth is about 29.9 kb. SARS-CoV-2 genome, has 5' and 3' terminal sequences (265 nt at 5' end and 229 nt at 3' end) with open reading frame (ORF) 1ab-spike (S)- envelope (E) - arranged in membrane (M) -nucleocapsid (N) regions. The predicted *S* (3822 bp), *ORF3a* (828 bp),

E (228 bp) M (669 bp) and N genes (1260 bp) of SARS-CoV-2 are, nucleotides long, respectively. Similar to SARS-CoV-1, the SARS-CoV-2 genome carries a predicted *ORF8* gene (366 nt long) located between the *M* and *N ORF* genes (5,6,7,8). ORF1a/b covers about 65% of viral genome encodes two polyproteins pp1a and orpp1ab. pp1a clavage products are nsp1-11, orpp1ab products are nsp1-16. S protein is critical protein for viral attachment to cell receptor, which is angiotensin-converting enzyme-2 (ACE-2). S protein includes two unit, S1 is responsible for receptor binding and S2 is mainly responsible for cell membrane fusion (5,6). Six amino acids are critical for receptor binding that are L455, F486, Q493, S494, N501, and Y505 (6). Many mammalian body tissue cells (lung cells, kidney cells, gastrointestinal tract cells, heart, liver, and blood vessels epithelial cells) express ACE-2 receptors on their surface (9).

The N protein mainly attaches and covers the viral genome. It also involves an RNA replication process, formation of virion structure and immune evasion. The M protein mainly responsible assembly and budding of viral particles interacts with N protein. This protein is conserved part of genomes. The E protein responsible for production, maturation and releasing of viron. This is the smallest part of virion structure (10,11).

Viral Life Cycle and Pathogenesis

In SARS-CoV-2 infections, clinical findings including fever, dry cough, shortness of breath, myalgia, fatigue, normal or decreased leukocyte counts and radiological pneumonia are also seen in individuals with infection or clinical disease due to COVID-19 or other factors. While most COVID-19 patients (>80%) are asymptomatic or have a mild course, up to 5% of them may develop acute respiratory distress syndrome (ARDS), shock, and single or multiple organ failure, as in some other severe coronavirus infections (5,12). Our knowledge of the physicochemical properties of SARS-CoV-2 has also increased, since it is an enveloped virus, it is sensitive to external environments and SARS-CoV-2 can be inactivated by UV or heating at 56°C for 30 min, also diethyl ether, 75% ethanol and chlorine. It is inactivated by most disinfectants such as peracetic acid and chloroform. SARS-CoV-2 can remain stable on plastic and steel surfaces for longer periods than copper and cardboard surfaces, and live virus can be detected up to 72 h after virus application on these surfaces (7).

The S protein plays the main role in entry into the host cell. The process of entry into the cell begins with the binding of the S glycoprotein, which consists of trimeric S1 and S2 subunits, to its receptor on the cell. After SARS-CoV-2 binds to ACE-2 on the host cell with the S1 subunit, various proteases of the host cell such as cathepsin L, trypsin, elastase, serine transmembrane proteases (TMPRSSs) cleave the S1 and S2 units from the junction site and the S2 subunit becomes active, initiating membrane fusion. SARS-CoV-2 has a higher affinity for ACE-2 than SARS-COV-1. There is an insertion of 4 amino acids between the S1 and S2 subunits of SARS-CoV-2 that functions as the cleavage site of proteases and furins; these 4 amino acid regions are not found in any other coronavirus and are thought to have important contributions to the transmission ability of SARS-CoV-2 (5,13,14). ACE-2 is expressed in the oral mucosa, gastrointestinal tract, cardiovascular system, and kidneys, and these tissues can be infected by SARS-CoV. Clinical trials have been conducted after some studies have shown that TMPRSS2 has a key role in cutting S and S2 subunits at entry into the host cell and that blocking this enzyme may be a treatment option (14).

After the virus enters the cells, the viral RNA genome is released in the cytoplasm and ORF1ab polyproteins are first synthesized; synthesized polyproteins are formed by autocatalytic mechanisms, especially functional non-structural proteins required for replication. Then, the negative-sense complement of the viral genome is synthesized, and this newly synthesized RNA and positive-sense genomic RNA is replicated and m-RNAs of structural proteins are synthesized. The synthesized envelope glycoproteins pass through the endoplasmic reticulum or Golgi apparatus and are formed by the combination of nucleocapsid, genomic RNA, and nucleocapsid protein. Next, the viral particles bud into the endoplasmic reticulum-Golgi intermediate compartment. Finally, the vesicles containing the virus particles then fuse with the plasma membrane to release the virus (4,5,7).

The antigenic structures of the intracellular virus are presented to the antigen presenting cells, which play a key role in the antiviral immune system, via the major tissue compatibility complex [(MHC); or human leukocyte antigen (HLA) in humans]. These structures are then recognized by virus-specific cytotoxic and helper T-lymphocytes. Therefore, understanding the antigen presentation of SARS-CoV-2 is important for understanding the pathogenesis of COVID-19. Antigen presentation of SARS-CoV is mainly dependent on MHC I molecules, but MHC II also contributes to its presentation. Again, based on previous data, individuals with allerines HLA-B * 4601, HLA-B * 0703, HLA-DR B1*1202 and HLA-Cw*0801 are more susceptible to infections caused by SARS-CoV, HLA-DR0301, HLA. There are publications reporting that -Cw1502 and HLA-A * 0201 alleles are associated with resistance to infection. In silico studies, HLA-B*46:01 binds weakly to SARS-2 peptides; it has been reported that HLA-B*15:03 has the highest capacity to present peptides from SARS-2, so people

with this tissue group may be more resistant. Additionally, mannose-binding lectin gene polymorphisms associated with antigen presentation have been associated with the risk of SARS-CoV infection. These studies and future studies will provide valuable clues for the treatment and understanding of the pathogenesis of COVID-19 (4,5,11,15,16).

After antigen presentation, humoral and cellular immunity in the body is mediated by virus-specific B and T-cells. As with other viral infections, antibody production against SARS-CoV virus classically takes the form of immunoglobulin M (IgM) and IgG production. IgM antibodies specific for SARS disappear at the end of the 12th week of the disease, while the IgG antibody can remain detectable in the body for a long time. Although antibodies develop against many proteins of the virus, they are specific antibodies to the protective neutralizing antibodies, especially S and N antigens (6,11). There is more research on the cellular immune response to coronaviruses compared to the humoral immune response, and current data show that the number of CD4 and CD8 T-cells in the peripheral blood of SARS-CoV-2 infected patients is significantly reduced, while the acute phase response in SARS-CoV patients is significantly reduced by CD4+. It has been reported to be associated with severe reduction in T and CD8+ T-cells. Although SARS-CoV-2 infects monocytes, macrophages and T-lymphocytes, the mechanism by which they enter these cells is unknown due to the low expression of ACE-2 in these cells. Even in the absence of antigenic stimulation, CD4+ and CD8+ memory T-cells can remain active for up to four years in individuals with SARS-CoV infection, so these clones are capable of T-cell proliferation, delayed-type hypersensitivity response and interferon (IFN-γ) production (2,7,17). Six years after SARS-CoV infection, 14 of 23 recovered SARS patients had T-cell memory responses specific to the SARS-CoV S peptide library. The specific CD8+ T-cells also exert a similar effect on the clearance of MERS-CoV in mice. When the data are examined, it shows that ARDS is the underlying cause of deaths due to COVID-19. ARDS is the common immunopathological event in SARS-CoV-2, SARS-CoV, and MERS-CoV infections. One of the main mechanisms in the development of ARDS is the uncontrolled release of large amounts of pro-inflammatory cytokines (IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, TGFβ, and the release of chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10, etc.) by immune effector cells (cytokine storm). When patients with SARS-CoV and MERS-CoV infections are classified according to their severity, individuals with severe symptoms have higher serum levels of IL-6, IFN-α and CCL5, CXCL8, CXCL-10 compared to those with mild-to-moderate symptoms. It is thought that cytokine storm can cause a severe immune response in the body by the immune system, causing ARDS and multi-organ

failure and may lead to death in severe cases of SARS-CoV-2 infection, as in SARS-CoV and MERS-CoV infection (4,18,19).

Breathlessness, fatigue, and decreased exercise tolerance are persistent symptoms after acute SARS-CoV-2 infection. These findings called "long COVID" and biological mechanisms underlying "long COVID" syndrome remain unknown. Pulmonary endotheliopathy and microvascular immunothrombosis in may play key role acute COVID-19 and long COVID. Autopsy studies also support these hypothesis (20).

A lesson learned from SARS-CoV-2 is that this agent uses multiple strategies to better survive and inhibit the immune response in host cells. Evolutionarily conserved microbial structures called pathogen-associated molecular patterns (PAMPs) can be recognized by pattern recognition receptors (PRRs). Because of prevention detection of RNA genomes by the host cell.

SARS-CoV-2 can induce the production of double-membrane vesicles that lack PRRs and then replicate in these vesicles. IFN-I (IFN-α and IFN-β) has a protective effect on SARS-CoV and MERS-CoV infection, but inhibition of the IFN-I pathway has been shown in infected mice. The accessory protein 4a of MERS-CoV is thought to block IFN induction at the level of MDA5 activation through direct interaction with double-stranded RNA. Additionally, MERSCoV's ORF4a, ORF4b, ORF5, and membrane proteins inhibit nuclear transport of IFN regulatory factor 3 and activation of the IFN β promoter. Again, gene expression due to the antigen presentation decreases after MERS-CoV infection. These factors may be the basic mechanisms that SARSCoV-2 uses to escape in the immune system (4,6,11,18).

In Table 1, main proteins and their properties, mutation rates during pandemics and roles presented for SARS-CoV-2 (21,22).

COVID-19 Diagnosis

Clinical signs and respiratory symptoms of patients infected with SARS-CoV-2 are generally the same with the other upper respiratory infections. Therefore, patient anamnesis is the starting point for the diagnosis of COVID-19, and the epidemiological history, clinical signs, computed tomography (CT) scan, blood count, and biochemistry test results needs confirmation for diagnosis. Nucleic acid testing strategies, which includes real-time polymerase chain reaction (RT-PCR) assays and serological tests [mainly, lateral flow tests, point-of-care tests (POCT)] that detects IgM/IgG antibodies or antigen tests are used. CT is highly instructive for rapid pre-diagnosis and prognosis estimation, especially during periods of high

Table 1. Main proteins and functions of SARS-CoV-2 (21,22)

Gene	Amino acid length in SARS-CoV-2	Number of amino acid residues with mutation rate greater than 0.01 during the pandemic (n)	Functional name	Function
Nsp1	180	0	Virulent factor	Inhibits host translation Invasion from immune response efficient viral gene expression
Nsp2	628	5	Endosome-associated protein	It is entirely unknown Mitochondrial biogenesis ???
Nsp3	1922	10	Cutting and untagging protein	Interacts with other viral nsps and RNA Removes tags from old proteins set for destruction
Nsp4	500	1	Double-membrane vesicle maker	Probably nucleate and anchor viral replication complexes on double-membrane vesicles in the cytoplasm.
Nsp5	306	5	Protease (3CLpro)	3CL protease
Nsp6	390	6	Double-membrane vesicle factory	Probably nucleate and anchor viral replication complexes on double-membrane vesicles in the cytoplasm.
Nsp7	83	1	Copy assistant	Dimerizes and interacts with other proteins
Nsp8	198	0	Primase	*De novo* initiation of replication and has been proposed to operate as primase
Nsp9	113	1	RNA-binding protein	Single-stranded RNA-binding protein.
Nsp10	139	0	Methyltransferase stimulator	Stimulates nsp16 to execute S-adenosyl-L-methionine-dependent methyltransferase activity
Nsp11	13	0		Unclear
Nsp12	932	7	RNA-dependent RNA polymerase	Core of RNA dependet RNA polymerase
Nsp13	601	5	Helicase	Unwinds dsRNA or DNA with 5′→3′ polarity
Nsp14	527	2	Proofreading exonuclease	N-terminal exoribonuclease domain, which has a proofreading role Prevents lethal mutagenesis
Nsp15	346	4	Endonuclease	Cleaves 3′ of uridines in a manganese dependent manner. Antiviral defance
Nsp16	298	1	Methyltransferase	Methyltransferase
S	1273	21	Spike protein	Attachment of virus
ORF3a	275	9	Accessory protein	Interactions S, M, and E proteins Inducing apoptosis (*in vitro*)
ORF3b	151	-	Accessory protein	Induces apoptosis, necrosis and hinders the antiviral innate immune response
E	75	0	Envelope protein	Production, maturation and releasing of viron
M	222	0	Matrix protein	M protein mainly responsible assembly and budding of viral particles interacts with N protein
ORF6	61	0	Accessory protein	Suppress IFN induction and IFN signaling pathway
ORF7a	121	1	-	May play a pivotal function in the recruitment of monocytes to the lung During COVID-19
ORF7b	43	1	-	Interfere with some cellular processes Involving leucine zipper formation and epithelial cell-cell adhesion
ORF8	121	2	-	Apoptosis and antagonizing the IFN signaling pathway Supporting replication

Table 1 continued

N	419	16	Nucleocapsid protein	Assembly and budding of viral particles interacts with N protein
ORF9a	97	-	-	Modulate the host immune response by compromising type I IFN synthesis
ORF9b	73	4	-	Impaired interferon signaling, antigen processing and presentation, complement signaling and induced IL-6 signaling
ORF10	38	-	Hypothetical protein	Biological functions have not been described yet, Immun modulation ???

SARS-CoV-2: Severe acute respiratory syndrome-coronavirus-2, IFN: Interferon, COVID-19: Coronavirus disease

prevalence, in suspected SARS-CoV-2 infection. Typical thorax CT images classically show a ground glass image with bilateral pulmonary parenchymal involvement and consolidations. However, it should be kept in mind that these findings cannot be distinguished from other viral pneumonia and similar images can be obtained in some different clinical situations (4,6,18,23).

The virus begins to spread in the upper respiratory tract 1-3 days before the onset of symptoms and is especially high in the first week of the disease. However, starting from the second week, the virus load in the upper respiratory tract decreases and it is found in greater amounts in the lower respiratory tract samples. In the diagnosis of COVID-19, upper respiratory samples are generally recommended, but sputum or lower respiratory tract samples such as bronco-alveolar lavage fluid (BALF), endotracheal aspirate can also be taken in patients who cannot produce sputum. Lower respiratory tract samples should be preferred, especially if they can be taken in the second week of the disease. Upper respiratory tract samples include nasopharyngeal and oropharyngeal swab samples, either alone or preferably in combination, nasopharyngeal washes and nasal aspiration fluids. These can be taken by health personnel or the patients can give an example themselves. Similar results to nasopharyngeal swabs can be obtained with nasal swabs obtained by the patient their self. Apart from this, more reproducible results can be obtained, especially with saliva samples originating from the posterior wall of the pharynx. Caution should be exercised during the collection of lower respiratory tract specimens such as induced sputum, BALF, or endotracheal aspirates, as there is a high risk of aerosol generation. In the first 14 days after disease onset, the most reliable specimen for detecting SARS-CoV-2 has been reported to be a nasopharyngeal swab followed by sputum, followed by a throat swab. Additionally, the virus begins to be excreted with faces, especially from the second week and continues to be excreted with faces for a long time. Especially in the advanced stages of the disease, stool samples can also be used for diagnosis, since viral excretion in upper respiratory

tract samples may be low or irregular. However, since the viral load varies according to the samples and severity/stage of the disease, a negative test result obtained especially with the samples taken from the upper respiratory tract does not exclude the disease, and in case of clinical suspicion, the test should be repeated a second time or with a different sample with higher sensitivity (4,6,18). In the diagnosis of COVID-19, factors such as sample collection technique (experience of the sample taker), timing, amount, where the sample was taken, sample transport and storage, performance of the molecular test used and the severity of the disease can seriously affect the test results. Sample collection at the right time and with the right technique greatly affects the sensitivity of molecular tests. Single nasopharyngeal swab samples are also becoming the more preferred sampling because they are better tolerated by the patient and safer for the sampler. In a real life experience, 3 different samples (taken by the healthcare worker, by the patient and saliva) performance compared and the sensitivity of the three samples in the diagnosis of the COVID-19 was 100%, 98.7%, and 96.1%, respectively. The authors conclude these results are accepted to be accurate (24).

The widely used technology for SARS-CoV-2 is test RT-PCR technology. RT-PCR is currently used in many microbiology laboratories in the form of syndromic panels for the diagnosis of different infectious agents, pathogen identification and quantitation in single form, and disease diagnosis and follow-up. Following the emergence and rapid spread of SARS-CoV-2 in China, many organizations published diagnostic protocols, and then different commercial companies released RT-PCR test kits for diagnosis, some of them such as Food and Drug Administration (FDA), WHO. It has been pre-approved or approved by different institutions/organisations (18,25).

The first step in RT-PCR is the synthesis of complementary DNA (cDNA) from the RNA genome. The cDNA is then amplified and detected using unique primer probe systems. The development process for these tests generally includes two main steps: i. selection of appropriate gene regions, conserved region in known sequences, and primer/probe design ii. optimization

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and validation of the developed test. In a study by Corman et al. (25), viral genomes associated with SARS include the *RdRP* gene (RNA-linked RNA polymerase gene), ORF1ab region, *E* gene (envelope protein gene), and *N* gene (nucleocapsid protein gene) conserved regions. These regions are still frequently used target regions for RT-PCR. Studies on these gene regions have reported that both RdRP and E genes have high analytical sensitivity (3.6 and 3.9 copies per reaction), and the *N* gene has weaker analytical sensitivity (8.3 copies per reaction). Reaction buffers, realtime PCR instruments extraction devices used for nucleic acid extraction may also effect performance of test (18,25,26).

The pooling can be a good strategy spatially limited testing resources and reagents. Testing by pooling samples requires very well established RNA extraction step for increasing sensitivity. Also careful monitoring of RT-PCR test sensitivity can avoid false negative results due to low-viral load. Therefore, laboratories should arrange their own validation of pooling

Studies based on the prevalence of COVID-19 (27).

In the initial period in the United States, the CDC recommended two nucleocapsid protein targets (N1 and N2), while WHO recommended initial screening with the *E* gene, followed by a confirmatory test using the *RdRp* gene. Because viral genes are present in equal copy numbers, the assay needs to be well optimized, as assay performance is usually determined by the reagent design and not the target itself. At least two molecular targets should be included in the analysis to avoid false positive or negative results from potential genetic alterations of SARS-CoV-2, as well as potential cross-reactivity with other endemic coronaviruses. However, WHO states that single-target test formats with high specificity can also be used alone, especially during the epidemic period. Molecular methods are being developed and evaluated worldwide, unlike RT-PCR. These include loop-mediated isothermal amplification (LAMP), multiplex isothermal amplification methods, followed by microarray detection and CRISPR/Cas systems. The Cas13-based specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) platform is used in patient samples at concentrations as low as 1 copy per microliter. It has been used to detect Zika and dengue viruses. Currently, Tang et al. (28) they used a CRISPR/Cas13-based SHERLOCK technology to detect SARS-CoV-2, and the test has received FDA pre-approval (5,6,23,29).

Droplet digital PCR (ddPCR) is an sensitive alternative of realtime PCR technique. It enables us to detect a few copies and can do absolute quantification. ddPCR allows quantitative data that more insightful than regular RT-PCR. Because of specific instrument needs and cost, the ddPCR assays are still very rarely studied in COVID-19 (30,31). Recently, FDA approved SARS-CoV-2

ddPCR kit developed assay detected as low as 0.260 to 0.351 copies/µL.

LAMP is a new isothermal nucleic acid amplification method. This method can give rapid results and no need complicated equipments. So these properties allows to decreasing cost of SARS-CoV-2 RNA detection. It can detecte a few copies (3.4 copies) of RNA. Penn RAMP technology is a modification of LAMP technology and mainly an updated two-step LAMP protocol. For amplification of target this system combines recombinase polymerase amplification with LAMP so sensitivity increased approximately 10-100 times (32). A meta-analysis result showed that the overall pooled sensitivity of RT-PCR was 0.96 [95% confidence interval (CI), 0.93-0.98] and RT-LAMP was 0.92 (95% CI, 0.85-0.96). RT-PCR had 0.06 (95% CI, 0.04-0.08) and RT-LAMP had 0.12 (95% CI, 0.06-0.16) false-negative rates. Mixed sampling and multiple target selection for diagnostic methods had better value than single site sampling and single target (33).

According to WHO, the immediate priority for COVID-19 diagnostic research is the development of nucleic acid and protein testing and the provision of POCT. The longer-term priority is to integrate these tests into multiple panels, as leading multi-parametric respiratory pathogens panel manufacturers have added SARS-2 to their panels. Serological tests using proteins in addition to nucleic acid tests are needed to improve surveillance efforts. These tests have the advantages of post-recovery detection, unlike nucleic acid tests. This enables clinicians to monitor both sick and recovered patients, providing a better estimate of total SARS-CoV-2 infections. POC tests are cost-effective devices used to diagnose patients outside central facilities. These can be run in areas such as community centres to reduce the burden on clinical (6,18).

Pandemic conditions also had a catastrophic effect laboratories and the same time on the world economy and human civilization. So there is an urgent need to develop robust fast technologies and nanotechnology based approaches as nanobiosensors used for detection of SARS-CoV-2 with high sensitivity, specificity, and fast analysis (34).

Serological tests are being developed for the rapid detection of SARS-CoV-2 antigens or antibodies, and some of them have been approved by many institutions. Different commercial companies are developing serological tests for detecting SARS-CoV-2 infection in lateral flow tests, automated chemiluminescence immunoassay systems, ELISA, and other formats. Although antibodies begin to be detected from the seventh day of the disease during COVID-19, IgM responses generally increase to more reliably detectable levels from the second week and IgG responses from the third week. Antibody responses can also be affected by factors such as disease severity and host immune

status. Although IgM responses remain positive for a shorter time, IgG positivity may persist for a long time. The level of protection and duration of antibody responses that develop after SARS-2 virus infections are not yet known precisely. It is estimated that its protection will not be very long (more than 6 months). It is attractive that rapid antigen tests would theoretically allow for low cost and rapid detection of SARS-CoV-2, but as with influenza viruses, their sensitivity would be lower than molecular tests. As a matter of fact, the sensitivity of an antigen test approved by the FDA is 80%. Especially with the development of monoclonal antibodies, more and more sensitive antigen tests are being developed (6,35).

Considering the variability of viral loads and sample diversity in COVID-19 patients, the sensitivity problem of antigen tests will be better understood. Antibody tests, which examine the host's response to the agent, may be more useful in demonstrating the past infection. They can also be used as additional testing to confirm COVID-19 infection; however, in a low prevalence situation, although the specificity of these tests is high, false positivity rates will be very high (for example, if the seroprevalence in the population is 1%), the probability of an antibody positivity obtained with a test with a specificity of 99% will be around 50%. Therefore, the following: For the moment, it is recommended that they be used with caution, considering the limitations mentioned in auxiliary testing and seroepidemiological studies. The use of antibody tests for purposes such as investigating the presence of protective antibodies in people should be avoided.

Previously, this type of serological testing has was central to the epidemiology of SARS and other coronavirus outbreaks. Rapid lateral flow tests that can detect both IgM and IgG antibodies will undoubtedly $1 are central to COVID-19.

However, IgM responses have specificity problems and specific IgG response development takes time. It seems unlikely that biology tests will play a role in active case management, apart from tasks such as diagnosing/confirming late cases of COVID-19 or determining a person's immunity (18,28,35,36).

Next-generation DNA sequencing analysis, although its use is limited in the diagnosis of SARS-CoV-2 due to its high cost, is especially used for understanding and monitoring viral evolution and for genoepidemiological studies. US-FDA has approved the Illumina COVIDSeq that is an amplicon-based NGS platform. This was the qualitative detection method that uses different sets primers and probes leveraged from ARTIC multiplex PCR protocol. Similarly, Thermo Fischer Scientific has launched Ion AmpliSeq SARS-CoV-2, but this is a research panel for NGS. For new generation and long read protocol introduced by Oxford nanotechnology. This protocol has substantial benefits for analytical innovations due to long reads (37).

Ethics

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