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MOLECULAR AND SEROLOGICAL PROSPECTS FOR SARS-COV2 EPIDEMIOLOGY

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ABSTRACT.

The sudden outbreak of the 2019 novel coronavirus "Severe Acute Respiratory Syndrome coronavirus 2" (SARS-CoV-2) in Wuhan, China, has affected the healthcare system and economy by its rapidly worldwide spreading. The present review intends to describe some of the current molecular and immunological assays used in SARS-CoV-2 detection from an epidemiological perspective, highlighting the importance of rapid test development and improvement. In this purpose the database as Databases (NCBI/PubMed, medRxiv) were used for searching, identifying and synthesizing data regarding SARS-CoV2 diagnosis.This report presents the optimal assays that can discriminate between different stages of the infection pointing out their utility in diagnosis, treatment, and vaccine development. Taken into account SARS-CoV-2 pandemic evolution and the dynamic character of COVID-19 diagnostic assays, the development of a joint effort by the scientific community is necessary for identifying future diagnostic directions that might help the disease management.

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Introduction

The pandemic caused by the new coronavirus that is ongoing has been a public health problem. The new coronavirus, "Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)" has been detected in 218 countries and territories, with a high number of cases, being in dynamics [1-4]. The genome of the novel virus, confirmed to belong to β-coronavirus family, was first sequenced in early January 2020 in a sample collected from a patient in Wuhan, and the disease was later named COVID-19 (Corona Virus Disease-2019). [5, 6] As the infection spread rapidly worldwide, the need for rapid diagnosis became crucial.

Methodology

PubMed database was interrogated for articles selection, and the following topics: SARS-CoV2 testing methods, SARS-CoV2 RT-PCR tests, SARS-CoV2 rapid antigen test, SARS-CoV2 RT-PCR false positive rate, tracking SARS-CoV2 using NGS, SARS-CoV2 antibody tests . Along with PubMed the search for articles was done in medRvix, PMC, Google Scholar, MedlinePlus.

Molecular Biology of SARS-CoV-2

SARS-CoV-2 is an enveloped virus with a non-segmented positive single-stranded RNA genome that encodes 4 structural proteins, 16 non-structural proteins (NSPs), and several (4-8) accessory proteins. Non-structural and accessory proteins are involved in viral genome replication and infection and structural proteins, spike (S), small envelope (E), membrane (M), and nucleocapsid (N) are required for the synthesis of the viral particle. [7] S, a transmembrane glycoprotein which facilitates the virus binding to ACE2 (angiotensin-converting enzyme 2) host cell receptors, entry, and transmission, pierces the viral

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envelope giving the characteristic shape of coronaviruses. S protein is cleaved by the host furin-like protease into subunits S1 (contains the receptor-binding domain), RBD (interacts with its host cell receptor, conferring cell tropism), and S2 (mediates the virus fusion with host cell membranes). [8] The envelope is the smallest viral protein which has an ion channel activity important in virus-host interaction and virulence. [9, 10] Glycoprotein M is the most abundant structural one, with a role in determining the shape of the viral envelope by interacting with all other viral structural proteins. [11] Although necessary for maintaining S in the Golgi complex, the S-M interaction is dispensable for the virion assembly process. In contrast, the interaction of M with N stabilizes the nucleocapsid and is essential for virion assembly while the interaction of M and E is required for viral envelope formation and the production and release of VLPs. [9] The nucleocapsid (N) is located in the endoplasmic reticulum and binds to viral RNA, being involved in the viral replication and host cells' response to viral infections. [12]

Molecular Methods

- **Comparative RT-PCR assays regarding viral gene evaluation (N, Orf1ab, E, RdRp) used in SARS-CoV2 diagnosis**

The primary testing method worldwide used for SARS-CoV2 was RT-PCR, as this type of test is developing very fast. The first test protocol was developed two weeks after the public presentation of the viral genome sequences which formed the basis of the first test kits distributed by WHO in January 2020. [13] While RT-PCR is the most widely used technique for COVID-19 diagnosis, several technologies are used or are being developed and implemented such as reverse transcription loop-mediated isothermal amplification (RT-LAMP), CRISPR-Cas, or digital droplet PCR (ddPCR). [14]

The first molecular assay being authorized by the U.S. Food and Drug Administration (FDA) under certain conditions and receiving an emergency use authorization (EUA) on February 4, 2020, was "Centers for Disease Control and Prevention (CDC) 2019-Novel Coronavirus (2019-nCov) Real-Time Reverse Transcriptase (RT)-PCR Diagnostic Panel (CDC 2019-nCoV Real-Time RT-PCR)''. [15] The kit uses specimens from the lower and upper respiratory tract and primers and probes are designed to target two regions from the SARS-CoV-2 nucleocapsid gene (N1, N2). As an internal control, a set of primers/probes targeting the human Ribonuclease P gene (RNase P) was added. Another strategy proposed by Corman et al., 2020 and recommended by WHO uses different sets of primers/probes that target, in addition to N gene, RNA-dependent RNA polymerase (RdRP), and envelope (E) genes. [13] Globally, there are different strategies for targeting the viral genome, the tests differing depending on the number of genes/regions (one or more), with the most common targets being N, E, ORF1ab/RdRp, and S gene.

Regarding the interpretation of the results performed on nasal or oral swabs, a positive RT-PCR test generally confirms the diagnosis, but a negative result does not exclude infection as false-negative tests might occur in early and late infection, in asymptomatic or mild infection due to a lower viral load or due to technical errors. The study conducted in Wuhan, China, suggests a false negative rate of 11-25% for sputum samples and 27-46% for nasal samples. [16]

Another aspect of RT-PCR diagnosis is the false-positive test with an unknown rate, but preliminary data showed a low rate between 0.8% and 4.0%. The main source for false positive tests resides in technical problems (contamination during sampling or, by PCR amplicons or reagents contaminations, and sample cross-contamination. [17] Any result of the diagnostic test should be interpreted in the context of symptoms, previous medical history of COVID-19, or presence of antibodies, potential exposure to the virus. [18] An important aspect of Covid-19 management is the prolonged viral RNA shedding, which is known to last for weeks after recovery.[17] To date, there is no information on whether the detection of low levels of viral RNA by RT-CR is equal to infectivity, without confirmation of infectious virus particles in culture-based methods.[19]

- **Tracking SARS-CoV2 using NGS and bioinformatic methods**

Currently, the international scientific community is working to provide the latest information on SARS-CoV-2, including viral genome sequencing to assess variations in the genomic sequences of the virus, the results submitted in international databases (such as NCBI and GISAID https: //nextstrain).org/) being available for phylogenetic analysis and mutation rate. Almost 224,224 viral genomic sequences of SARS-CoV-19 were shared with unprecedented speed via GISAID.

The SARS-CoV-2 viral genome has been sequenced in various regions of the world, and complete genome sequences from 3469 isolates are availabl. [20]

Phan T. performed genetic analyses of 86 complete or near-complete genomes of SARS-CoV-2 and revealed many mutations and deletions in coding and non-coding regions.[21] The whole-genome presented 93 mutations for all genes except envelope one. These data provided evidence of the genetic diversity and rapid evolution of this novel coronavirus. Mutations in the spike surface glycoprotein may induce conformational changes, which probably led to changes in antigenicity. There is no information about the role of mutations upon amino acids involved in conformational changes of the SARS-CoV-2 spike surface glycoprotein structure which may affect SARS-CoV2 antigenicity and should be further explored.

At this moment seven clades were identified (G, GR, GH, O, S, L, V) being scattered around the world in different percentages. Phylogenetic analysis of 160 SARS-Cov-2 sequences (complete genome) indicated - three central variants that are distinguished by specific mutations (A, B, and C). A is the ancestral variant and together with variant C is found in significant proportions outside East Asia, in Europeans and Americans. In contrast, variant B is common in East Asia and

does not appear to have spread outside of East Asia without undergoing new mutations, suggesting the existence of immune or environmental resistance effects outside of Asia. Variant B detached from A after accumulating 2 mutations: a mutation synonymous with the T8782C and a non-synonymous mutation C28144T. Variant C was detached from variant B after the accumulation of the non-synonymous mutation G26144T. [22]

In a recent report, van Dorp et al. estimated the mutation rate over alignment as 9.8×10^4 substitutions per site per year. The authors identified 12,706 mutations, heavily enriched in C→U transitions, of which 398 are recurrent mutations.[23] The authors do not consider that the identified candidate recurrent mutations can increase SARS-CoV-2 transmissibility at this stage, but it is expected that the virus will diverge into phenotypically different lineages in order to establish as an endemic human pathogen.

While Plante et al. in their research have shown that the spike D614G substitution enhances SARS-CoV-2 replication in the upper respiratory tract through increased virion infectivity, van Dorp et al. suggested that the role of D614G mutation should be considered carefully. They consider that the D614G mutation is in linkage disequilibrium (LD) along with three other derived mutations (nucleotide positions 241, 3037, and 14,408) that presented highly similar expansions. [22, 24]

Molecular epidemiology studies analyze the circulating viral strains by sequencing the entire SARS CoV-2 genome. Data obtained establish transmission networks and evolution of viral infection. Identification of some SARS-CoV-2 viral circulating variants allows the evaluation/optimization of detection tests. The presence of selective mutations in SARS-CoV-2 suggests the importance of avoiding certain regions of the viral genome when designing primers and probes for diagnostic tests.

Serology

- **The Antigenic Properties of SARS-CoV2 Viral Proteins**

Spike is considered the most antigenic protein of coronavirus and the major inducer of neutralizing antibodies which can block the virus attachment to the host cells. [25] Moreover, the S protein contains the important targets of cytotoxic lymphocytes. Its immunogenicity seems not to be affected by other viral structural proteins, being a major target for the development of vaccines against coronaviruses. A major mutation in the Spike area of SARS-CoV-2 (D614G) associated with virus transmissivity led to the development of a mutated recombinant S1 protein, (40591-V08H3), which could be exploited in biotechnologies as much as it demonstrates binding activity to ACE2. A recent study showed antigenic differences between spike proteins of SARS-CoV-2 and SARS-CoV differences that are related to amino acid divergence in the non-conserved regions.[26] Lee *et al.* noticed cross-reactivity within S protein antigen of SARS-CoV and SARS-CoV-2 (S and S1 subunit proteins) attributed to genetic homology of these viruses, which could have an impact on serologic tests. The N protein is a highly immunogenic phosphoprotein used as a marker in diagnostic assays, N antigen-based assays being more sensitive than S1 subunit-based tests. A high level of homology was found between N proteins of SARS-CoV and SARS-CoV-2. [27]

- **The Role of Serological Assays for Understanding the SARS-CoV2 Epidemiology**

Serological assays can be used for epidemiological purposes, providing data on transmission patterns, silent infections, disease progression, and identification of the origins of a virus. [28] All SARS-CoV-2 proteins will elicit antibody responses to some extent, but the spike protein is the major antigen that elicits neutralizing antibodies (NAbs), followed by the nucleocapsid protein. These data are a starting point for developing NAbs to block binding and fusion of SARS-CoV-2. [29]

The diagnosis of viral infection could be realized by detecting the presence of viral protein in clinical samples, using immunoassays. [30] Their effectiveness is associated with strong specificity and binding affinity between antigen and antibody and the basis of the test relies on the formation of an antigen-antibody complex which generates a measurable virus-specific immune signal. [31] The application of such types of binding assays as ELISAs and immunofluorescence assays (IFAs) for SARS-CoV-2 diagnosis could be very important and useful [28, 32].

At the moment there are FDA EUA approved antibody tests that target IgM and IgG. Most of them target the spike protein, nucleocapsid protein, or both of them. [33] On 25 November, COVID-SeroKlir Semi-Quantitative SARS-CoV-2 IgG Antibody Kit (Kantaro) was emergency authorized by FDA. Designed for serum and Li-Heparin plasma specimens, the test is a two-step assay consisting of a direct Enzyme-Linked Immunosorbent Assays (ELISA) performed against recombinant Receptor Binding Domain of SARS-CoV-2, followed for positive specimens, by a confirmatory semi-quantitative detection against full-length SARS-CoV-2 Spike protein. The producer's Clinical Agreement Study has demonstrated 99% specificity and 99% sensitivity 15 days post-symptom onset. IgG antibodies to SARS-CoV-2, generally detectable in two weeks after the initial infection do not have a well-characterized duration. Following seroconversion, the virus may be detected for a few weeks. [34]

A positive test result for any assays means that the patient has been infected with the virus and that it has induced an immune response regardless of whether the subject displayed symptoms or not. Because the immune response occurs about two to three weeks after the infection, serological assays cannot be used to diagnose current infections (Fig 1).

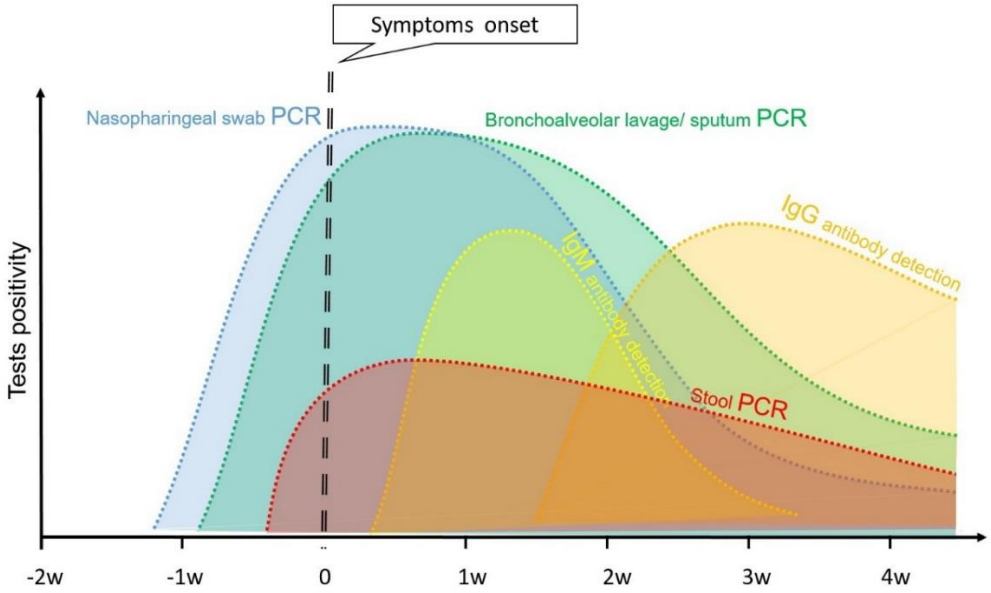

Fig 1. Serological and Molecular Approaches for COVID-19. Estimated time and intervals for SARS-CoV2 detection.

Early studies showed that generally in persons infected with SARS-CoV-2 the antibody response appears between day 10 and day 21 after infection. 40% of patients develop antibodies within a week and increase to 100% after 15 days. Mild cases develop a belated antibody response (four weeks or more) and a small number of cases do not present detectable levels of antibodies (i.e., IgM, IgG). After the disease onset, IgM and IgG antibodies to SARS-CoV-2 are detected between 6–15 days, with the median seroconversion time for total antibodies-11 days, IgM-12 days, and IgG-14 day.s [35-40]

SARS-CoV2 is a new virus and little is known about the longevity of the antibody response. However, for other coronaviruses, the antibody response wanes over time (range: 12 – 52 weeks from the onset of symptoms) and homologous re-infections have been encountered. [41] For all seasonal coronaviruses that infect humans, reinfection occurs generally after three years. [42] SARS-CoV, infected patients presented detectable IgG antibody levels for two (90%) and three years (50%). [43, 44] Xiao et al. showed that in 80% of cases, SARS-CoV-2 IgM and IgG antibody levels can persist over seven weeks. [45] Coronavirus 229E induces a persistent IgA level in the nasal mucosa (one-year post-infection) and further studies regarding SARS-Cov2 IgA antibodies in nasal mucosa are needed. [46] Quan-Xin et al. showed that asymptomatic patients present low levels of IgM (62.2% positive) while the symptomatic group had an increased level of 78.4% and IgG values were comparable for both groups (83.3% positive symptomatic, 81.1% asymptomatic group). After the convalescent phase, the IgG declines in 93.3% of the asymptomatic and 96.8% of the subjects that displayed symptoms. [47]

The immune response evaluation is also important for vaccine development which mainly targets the spike glycoprotein of the nucleocapsid protein.

Considering that in the case of SARS-CoV infection all surface proteins (S, M, E, and N) are involved in T cell responses, especially CD8+ cells, and that lymphopenia is a common trait of both SARS-CoV and SARS-CoV-2 infection, it would be useful to decipher T-cell response to viral proteins since it could provide important information on designing a vaccine. [48-50] The main question regarding protective immunity in SARS-CoV-2 infection remains if it is dependent on how strong the immune response has to be. In virus clearance a key role is played by CD8+ T cells associated with inflammation; a status observed frequently in COVID-19 patients. It was noted that in both mild and severe cases a decreased number of T lymphocytes, CD4+ and CD8+ T cells occurs being more heighten in acute cases. [51]

Another important question is if the protective immunity is achieved and for how long it lasts. Several studies have shown that the protective immunity time scale can last for 6 to 12 months. Also, for HCoV-OC43 and HCoV-HKU1 infections a protective immunity duration was estimated around 45 weeks. [52]

A major role in the neutralization of the SARS-CoV is played by IgGs. Their levels diminish after recovery and have their peak levels during the convalescent phase of the infection. [53] Convalescent plasma was already used to clear the virus in patients with severe COVID-19.

One important fact is that producing neutralizing antibodies is relatively expensive and difficult. However, this type of therapy can enhance immunity and help the pathogen clearance. [54]

T Cell Assays

In an early 2020 study, Meckiff et al. showed that CD4+ T cells obtained from blood samples collected before the coronavirus pandemic are both human coronavirus (HCoV) and SARS-CoV-2-reactive. Moreover, these cells express activation markers (CD154, CD69, CD39, CD137 (4-1BB), CD279 (PD-1), and HLA-RA), along with cytotoxicity linked factors (PRF1, GZMB, GZMH, GNLY, and NKG7). SARS-CoV-2-reactive T cells express fewer IFN-γ and IL2, comparing with influenza reactive T cells, which may indicate a weaker response of TH1 cells. [55]

Regardless of Covid-19 severity, Giménez et al. found that IFN-γ CD8+ T cells develop at the same rates and are detected relatively late after the onset of symptoms. [56]

In contrast with the study of Meckiff et al., using ex vivo ELISpot assay, Ogbe et al. demonstrated a remarkable absence of SARS-CoV-2 T cell-specific responses in most of the healthy unexposed subjects. Interestingly, the same healthy subjects showed responses to the S1 and S2 subunits of spike protein in a 7-day CellTrace® Violet (CTV, Life Technologies) proliferation assay, probably because of a retained cross-reactive central memory response to the spike protein of seasonal coronaviruses. [57]

The same group showed that T cell responses to SARS-CoV-2 infection increases in magnitude around 4 weeks after the onset of symptom. [55]

Rapid Antigen Test

- **The Importance of Rapid Antigen Tests in Fast Time Tracking and Low-cost Detection**

Over the decades, the progress made by these diagnostic methods was well proved to distinguish between active infection and a vaccine-induced antibody response. [58] Moreover, detecting both antigen and/or antibody may reduce the diagnostic window given by an early diagnosis of viral infection. [59] Throughout the acute stage of infection with SARS-CoV-2, antigen detection tests can detect the virus's presence, being an additional option to RT-PCR-based testing because of their fast turnaround time and easier use of the technique. In this pandemic context, the development of rapid tests for SARS-CoV-2 diagnosis is one of the most necessary actions in COVID-19 disease's emergency control.

Rapid tests are defined as qualitative or semi-quantitative diagnostic tools, that do not involve automated mechanisms, following IVDs technical specifications. [60] The rapid test could be used as a triage method along with symptoms and epidemiology. and this may diminish the pressure on laboratories based on molecular techniques (RT-PCR) and also be used for near-patient testing.

- **Rapid Antigen Tests Sensibility and Specificity**

At this moment, one of the main problems of commercial rapid tests based on antigen and antibody immunoassays is the high probability for cross-reactivity to proteins common to other types of coronavirus [32]. Moreover, these assays are susceptible to obstruct the results due to the similar structures of the reagents with other agents or their concentration, leading to false-negative or false-positive results. [61, 62]

Non-automatic interpretation of the results, such as visual interpretation, is less sensitive than automatic readers, which provides a lower LOD (limit of detection) and an increased reproducibility. [63] However, this type of reader requires an increased operator's manipulation and availability wherever the test is carried out. Besides, one of the advantages is represented by the stability of antigens as compared with the RNA's.

According to a non-governmental organization, FIND, the European Centre for Disease Prevention and Control has reported 32 rapid antigen tests, that conform with EU legislation and may be accessible internationally, excepting EU (Table 1). [64]

Table 1. Diagnostic Tests for which Conformité Européenne -marking Is Claimed

Kit's Name	Manufacturer
2019-nCoV Colloidal Gold Ag	Shenzen Bioeasy
2019-nCoV Fluorescence Ag Rapid Antigen	Shenzen Bioeasy
AccuQuik Covid-19 Antigen Rapid Test Kit	Advacare Pharma
BIOCREDIT COVID-19 Ag	RapiGEN Inc.
Biocredit Covid-19 Ag	Rapigen Inc. (South Korea)
Coronavirus Ag Rapid Test Cassette (Swab)	Confirm BioSciences
Coronavirus Antigen Rapid Test Kit	JOYSBIO Biotechnology
COVID-19 Ag Respi-Strip	Coris BioConcept
Covid-19 Antigen Nasal Swab Test Kit	Atlas Link Technology Co., Ltd
COVID-19 Antigen Rapid Test (Colloidal Gold)	MEDAKIT LTD
COVID-19 Antigen Rapid Test Cassette	Btnx Inc. (Canada)
COVID-19 Antigen Rapid Test Device	Liming Bio-Products Co., Ltd
Covid-19 Antigen Rapid Test Device	Assure Tech. (Hangzhou) Co. Ltd. (China)
COVID-19 Antigen Test Kit (Rare Earth Nano Fluorescence Immunochromatography)	AmonMed Biotechnology Co., Ltd
COVID-19 Viral Antigen Test Kit (Colloidal Gold Immunochromatography)	Beijing Abace Biology Co., Ltd
COVID-19 Viral Antigen Test Kit (ELISA)	Beijing Abace Biology Co., Ltd
Dynamiker SARS-CoV-2 Ag Rapid Test	Dynamiker Biotechnology (Tianjin) Co., Ltd
Kewei COVID-19 Antigen Rapid Test Kit (Colloidal Gold)	Beijing Kewei Clinical Diagnostic Reagent Inc.
Kewei COVID-19 Antigen Rapid Test Kit (Fluorescence)	Beijing Kewei Clinical Diagnostic Reagent Inc.

NowCheck COVID-19 Ag Test	BIONOTE Co., LTD
Panbio Covid-19 Ag Rapid Test Device	Abbott Rapid Diagnostics Jena Gmbh (Germany)
PCL COVID19 Ag Rapid FIA	PCL Inc.
PerfectPOC Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit	Jiangsu Bioperfectus Technologies Co. Ltd
QuickNavi-COVID19 Ag	Denka Co., Ltd.
SARS-Cov-2 Antigen Fluorescence Rapid Detection Kit	Beijing Savant Biotechnology Co., Ltd
SARS-CoV-2 Antigen Rapid Test Kit (DTS922)	Creative Diagnostics
Sgti-Flex Covid-19 Ag	Sugentech, Inc. (South Korea)
STANDARD F COVID-19 Ag FIA	SD BIOSENSOR, Inc.
STANDARD Q COVID-19 Ag Test	SD BIOSENSOR, Inc.
The BinaxNOW™ COVID-19 Ag Card	Abbott Diagnostics Scarborough, Inc.
VTRUST COVID-19 Antigen Rapid Test	TaiDoc Technology Corporation
WANTAI SARS-COV-2 Antigen Rapid Test	Beijing Wantai Biological Farmacy Enterprise

Regarding the accuracy of medical tests, two types of measures are required: sensitivity and specificity, which are important in reducing errors. [65] While a sensitive test will correctly identify people with the disease, a specific test will accurately identify people without it (Table 2).

Table 2. The Model of Calculation for Sensitivity and Specificity for SARS-CoV-2 Diagnostic Test

Test Result	**Condition Present***	**Condition Absent***
Positive	True Positive (a)	False Positive (b)
Negative	False Negative (c)	True Negative (d)

*As determined by the gold standard diagnostic test Sensitivity is calculated as a/(a+c). Specificity is calculated as d/(b+d).

Even if RT-PCR molecular test is treated as the gold standard in viral detection (specificity 96%), in the real world, the accuracy may suffer a decrease considering that the testing condition and the methods are not perfect. Therefore, a decrease of sensitivity from 100% to a range from 80% to 66%, suggests that one in three infected people will have a false-negative result. PCR and serological tests are not ideal, but the information given by them is very important to patients, public health experts, and the medical team. On the other hand, in infected patient's screening, antigen tests have lower accuracy than antibody and PCR tests, but they are great in new cases' detection

Quidel Corporation has received the first EUA antigen assay authorization for the Sofia 2 SARS Antigen FIA test. Moreover, a small adaptation of the protocol could increase the sensitivity to nearly 90%, when the sample doesn't need dilution. [66] On contrary, one study shows that a clinical sensitivity of 31% could be estimated, which makes to miss 7 from 10 infected patients. [67]

In September, Roche developed a new **SARS-CoV-2 rapid antigen** test which is a rapid chromatographic immunoassay, which detects a specific antigen of SARS-CoV-2, using samples from the nasopharynx with a 15 minutes time of response. Based on a study that included 426 samples, the test has a sensitivity of 96.52% and a specificity of 99.68%.

NADAL® COVID-19 Antigen Rapid Test is a lateral flow chromatographic immunoassay that detects the virus directly by checking for the presence of viral nucleoprotein (N) antigens of the SARS-CoV-2 virus because of its abundance and sufficiently specific for SARS-CoV-2. The test uses human naso- and oropharyngeal specimens. The diagnosis presents 97.56 % sensitivity (ct value: 20 – 30), and >99.9 % specificity, which means that positive results from antigen tests are very precise. However, as there is always a residual risk of a false-negative result when in doubt, a PCR test should be carried out to confirm negative results. The choice between antibody, antigen, and PCR tests is not an either/or decision. Rather, the best tactic is a combined application. Also, the test has no cross-reactivity with human pathogenic coronaviruses (like hCoV-229E, -HKU1, -NL63, and -OC43) nor influenza viruses (like influenza A/B). [68]

For all this type of rapid antigen tests, FDA recommends that negative results should be confirmed by a molecular method to confirm the result, if necessary, for patient management.

Results and Discussion

The point-of-care technology is essential for a rapid diagnosis and includes medical tests characterized by simplicity, being easily performed at the bedside. ID NOW™ is one of the most important platforms from the United States that facilitates productive patient management in hospitals, urgent clinics, or physician offices, using molecular point-of-care technology. At this moment, the ID NOW™ COVID-19 assay, which is targeting the coronavirus RdRp Gene, is available under the FDA EUA. The assay is characterized by high-quality molecular positive results in as little as 5 minutes, negative results in 13 minutes, and the possibility of storage at room temperature. [69]

Next-Generation Sequencing (NGS) technologies give an important, novel, and effective avenue in viral screening and detection of novel viruses from clinical and environmental samples, without previous information about the agents. [70]

Illumina produced a new detection kit, Nextera™ Flex for Enrichment Library Preparation kit combined with viral pathogens targeting panels. This provides researchers to obtain genomic data, confirming the presence of CoV and advance analyses such as genotyping and variant analysis. Moreover, this is the only kit that has the potential to discriminate between SARS-CoV-2 and other 40 types of respiratory viruses. [71] Since the COVID-19 pandemic continues, it is significant to define which immune responses are important for protection. T cell response assays (like EliSpot) along with an antigens panel can respond to the questions about the role of T cells – induced by SARS-CoV-2 or by vaccines - in immune protection in the future. [57]

Based on previous experience with SARS-CoV's detection using nucleic acid hybridization new test can be developed using microarray assays. For SARS-CoV the mechanism behind relying on reverse transcription of viral RNA and the labeling of generated cDNA with specific probes. If labeled cDNAs hybridize after contact with solid-phase oligonucleotides fixed onto microarray trays surfaces, they will remain bound, indicating the presence of viral nucleic acid [72. Moreover, the microarray essay can detect up to 24 SNPs related to SARS-CoV's mutations in the spike (S) gene with 100% accuracy. [73]

In the evolution of the COVID-19 pandemic, detecting the emergent strains of SARS-CoV-2 is one of the most necessary actions and microarray assays can deliver those multiple coronavirus strain's rapid detections with similar RT-PCR sensitivity, as an answer to mutational variation. [74]

Conclusion

The evolution of molecular routine testing procedures and rapid tests for respiratory infections screening will be the next challenge for infection disease management.

Author's Contribution

All the authors have equally contributed for manuscript preparation.

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