Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Microchemical Journal 167 (2021) 106305

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Current state of diagnostic, screening and surveillance testing methods for COVID-19 from an analytical chemistry point of view

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ARTICLE INFO

Keywords:
COVID-19
SARS-CoV-2
RT-PCR, chest computerized tomography
Antigen and antibody tests

ABSTRACT

Since December 2019, we have been in the battlefield with a new threat to the humanity known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In this review, we describe the four main methods used for diagnosis, screening and/or surveillance of SARS-CoV-2: Real-time reverse transcription polymerase chain reaction (RT-PCR); chest computed tomography (CT); and different complementary alternatives developed in order to obtain rapid results, antigen and antibody detection. All of them compare the highlighting advantages and disadvantages from an analytical point of view. The gold standard method in terms of sensitivity and specificity is the RT-PCR. The different modifications propose to make it more rapid and applicable at point of care (POC) are also presented and discussed. CT images are limited to central hospitals. However, being combined with RT-PCR is the most robust and accurate way to confirm COVID-19 infection. Antibody tests, although unable to provide reliable results on the status of the infection, are suitable for carrying out maximum screening of the population in order to know the immune capacity. More recently, antigen tests, less sensitive than RT-PCR, have been authorized to determine in a quicker way whether the patient is infected at the time of analysis and without the need of specific instruments.

Abbreviations: 2019-nCoV, 2019 novel coronavirus; ACE2, Angiotensin-Converting Enzyme 2; AI, Artificial Intelligence; ALP, Alkaline Phosphatase; aM, Attomolar; ASOs, Antisense Oligonucleotides; AuNIs, Gold Nanoislands; AuNPs, Gold Nanoparticles; BSL, Biosecurity Level; CAP, College of American Pathologists; Cas, CRISPR Associate Protein; CCD, Charge-Coupled Device; CG, Colloidal Gold; CGIA, Colloidal Gold Immunochromatographic Assay; China CDC, Chinese Center for Disease Control and Prevention; CLIA, Chemiluminescence Enzyme Immunoassay; CLIA, Clinical Laboratory Improvement Amendments; COVID-19, Coronavirus disease-19; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; CT, Chest Computed Tomography; Ct, Cycle Threshold; DETECTR, SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter; DNA, Dexosyrosyribonucleic Acid; dNTPs, Nucleotides; ddPCR, Droplet digital PCR; dPCR, Digital PCR; E, Envelope protein; ELISA, Enzyme Linked Immunosorbent Assay; EMA, European Medicines Agency; EUA, Emergence Use Authorization; FDA, Food and Drug Administration; FET, Field-Effect Transistor; fM, Femtomolar; GeneBank, Genetic sequence data base of the National Institute of Health; GISAID, Global Initiative on Sharing All Influenza Data; ICTV, International Committee on Taxonomy of Viruses; IgA, Immunoglobulins A; IgG, Immunoglobulins G; IgM, Immunoglobulins M; IoMT, Internet of Medical Things; IoT, Internet of Things; LFIA, Lateral Flow Immunochromatographic Assays; LOC, Lab-on-a-Chip; LOD, Limit of detection; LSPR, Localized Surface Plasmon Resonance; M, Membrane protein; MERS-CoV, Middle East Respiratory Syndrome Coronavirus; MNP, Magnetic Nanoparticle; m-RNA, Messenger Ribonucleic Acid; MS, Mass spectrometry; N, Nucleocapsid protein; NER, Naked Eye Readout; Net, Neural Network; NGM, Next Generation Molecular; NGS, Next Generation Sequencing; NIH, National Institute of Health; nM, Nanomolar; NSPs, Nonstructural Proteins; ORF, Open Reading Frame; OSN, One Step Single-tube Nested; PDMS, Polydimethylsiloxane; pfu, Plaque-forming unit; pM, Picomolar; POC, Point of Care; PPT, Plasmonic Photothermal; QD, Quantum Dot; RBD, Receptor-binding domain; RdRp, RNA-Dependent RNA Polymerase; rN, Recombinant nucleocapsid protein antigen; RNA, Ribonucleic Acid; RNaseH, Ribonuclease H; rS, Recombinant Spike protein antigen; R_0, Basic reproductive number; RT, Reverse Transcriptase; RT-LAMP, Reverse Transcription Loop-Mediated Isothermal Amplification; RT-PCR, Real-Time Reverse Transcription Polymerase Chain Reaction; S, Spike protein; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SERS, Surface Enhanced Raman Spectroscopy; SHERLOCK, Specific High Sensitivity Enzymatic Reporter UnLOCKing; SiO2@Ag, Complete silver nanoparticle shell coated on silica core; ssRNA, Single-Stranded Positive-Sense RNA; STOPCovid, SHERLOCK Testing on One Pot; SVM, Support Vector Machine; US CDC, US Centers for Disease Control and Prevention; VOC, Variant of Concern; VTM, Viral Transport Medium; WGS, Whole Genome Sequencing; WHO, World Health Organization.

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https://doi.org/10.1016/j.microc.2021.106305
Received 10 February 2021; Received in revised form 12 April 2021; Accepted 14 April 2021
Available online 19 April 2021

1. Introduction

In December 2019, a seafood market in the Chinese city of Wuhan was suspected to be at the center of an outbreak of pneumonia of unknown origin. This pneumonia was later confirmed to be secondary to infection by a novel coronavirus originally named 2019 novel coronavirus (2019-nCoV). On the 11th of February 2020, the World Health Organization (WHO) named the disease COVID-19 and the International Committee on Taxonomy of Viruses (ICTV) called the virus "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) [1].

The main symptom of COVID-19 is fever, and the incubation period is estimated to be 2 to 14 days. Most patients infected with SARS-CoV-2 virus suffer respiratory failure. Other symptoms of viral infection include anosmia, sore throat, runny nose, malaise, painful breathing, wheezing and shortness of breath. Nausea and diarrhea have also been reported in COVID patients [2]. The symptoms are diverse and depending on the patient they can be mild or very serious causing complications that can lead to death. This, together with the great capacity of the virus to spread, has turned this epidemic into a threat to humanity in just a few months.

SARS-CoV-2 has the ability to infect more than three healthy people from one infected patient (R_0, basic reproductive number, >3), which gives it a high capacity for rapid spread [1,3]. The virus can spread through saliva as an aerosol when speaking, through droplets when breathing, or by contact with a contaminated surface [4,5]. The virus can also be transmitted by contact with infected organic matter such as feces where live viruses have been identified [6]. Currently the most worrying aspect is the detection of the virus transmission from asymptomatic people [7,8]. At present, >131 million people have been identified as infected and at least 2.8 millions of people have died from COVID-19 worldwide [9]. It is having a dramatic impact on the world economy and it is causing changes in the system for managing public health and our daily life in general [10].

SARS-CoV is an enveloped, single-stranded positive-sense RNA (ssRNA) virus classified in the group of the *Coronaviridae* family. It contains four major structural proteins in SARS-CoV-2 virus: E (envelope protein), M (membrane protein), N (nucleocapsid protein), and S (spike protein) (Fig. 1) [11]. According to previous reports, the M and E proteins are necessary for virus assembly. The S protein is critical for adhering to host cells, where the receptor-binding domain (RBD) of S protein mediates the interaction with angiotensin-converting enzyme 2 (ACE2). The most commonly used biomarkers for the detection of COVID-19 are the S and N protein antigens [8].

Early detection and diagnosis of COVID-19 is necessary to prevent the rapid spread of the virus and to isolate contacts [10]. Currently, the reverse transcription polymerase chain reaction (RT-PCR) test is the standard method for the detection of COVID-19 [12,13]. The test consists of analyzing the RNA of the virus presents in respiratory samples. The amplification of the genetic material of the virus makes the analysis last from a few hours to two days. The acquisition of chest computed tomography (CT) images is presented as an alternative and/or complementary method to RT-PCR to confirm or detect infected patients [1,10,14]. However, this technique requires large facilities that are only found in hospitals, which limits its use for the screening of the population. Other methods such as serologic tests [15–18] and viral throat swab testing [19] based on antibody or antigen detection make the screening possible. They have allowed carrying out the analyses outside a hospital or specialized laboratory, facilitating the massive screening of the population [20]. These analyses can provide rapid results that report relevant information about the infection. They are presented as an important advance to control the spread of the infection, to identify the infected people and to recruit people into clinical trials of treatments [16].

This ongoing global pandemic has motivated researchers to investigate new alternatives for the detection, diagnosis, treatment and vaccines of this virus. The fertility in the field is reflected by the frequency of relevant reviews published, e.g. within the beginning of the pandemic to August 2020. Table S1 (Supplementary material) presents a classification of the published reviews based on their main purposes: (i) reviews and minireviews about the main advances carried out in the diagnostic tests in the last months, together with a critical discussion about their utility [2,3,21–53]; (ii) information about the biosensors and the nanotechnology is presented as a useful tool to develop new strategies for the detection of the infection and also for its treatment [8,11,54–67]; (iii) different point of view about how the artificial intelligence (AI) can be presented as a versatile tool to control the pandemic and, even more interesting, in containing the spread of the virus [20,68,69]; (iv) current and update information about the structure and genomic of SARS-CoV-2 [70–79]; (v) the treatments that are being applied to fight the infection and the type of vaccines that are being developed [80–85]; and (vi) finally the Table S1 (Supplementary material) collects two reviews that cannot be classified in the previous groups. They provide interesting information about de workflow that should be followed for the detection of the infection, as well as information about natural products that could have a positive effect in the prevention and treatment of the infection [86,87].

The aim of this manuscript is to introduce from an analytical point of view the main coronavirus detection techniques. The gold standard method to detect SARS-CoV-2 is named RT-PCR. This method is described in detail, together with its strengths and weaknesses at each stage of the analysis. Different quicker alternatives proposed to carry out this analysis are presented and discussed. Other types of tests developed in order to obtain a different kind of information or to confirm better the infection of the virus are also reported. These tests are briefly described,

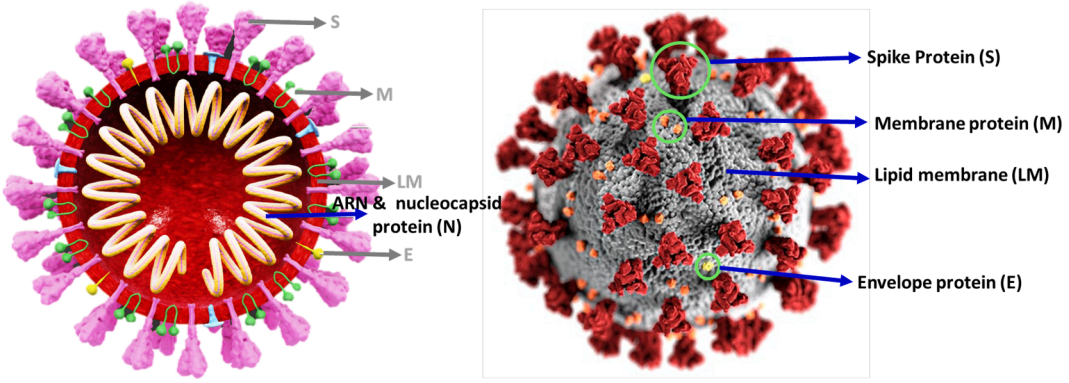


Fig. 1. SARS-CoV-2 structure. Virus from the Coronaviridae family. The S protein mediates attachment of the virus to cellular receptors and virus entry by fusion with cell membranes. Both M and E proteins are integral membrane proteins and form the minimum protein units for virus assembly. The N protein is an extensively phosphorylated, highly basic protein, which interacts with viral RNA and makes up the viral core and nucleocapsid.

and their advantages and disadvantages are highlighted. The different types of analyses are classified and explained in the frame of their utility.

2. Critical review of analytical methods for detection, diagnosis and monitoring of COVID-19.

As discussed in the introduction, it is essential to detect the SARS-

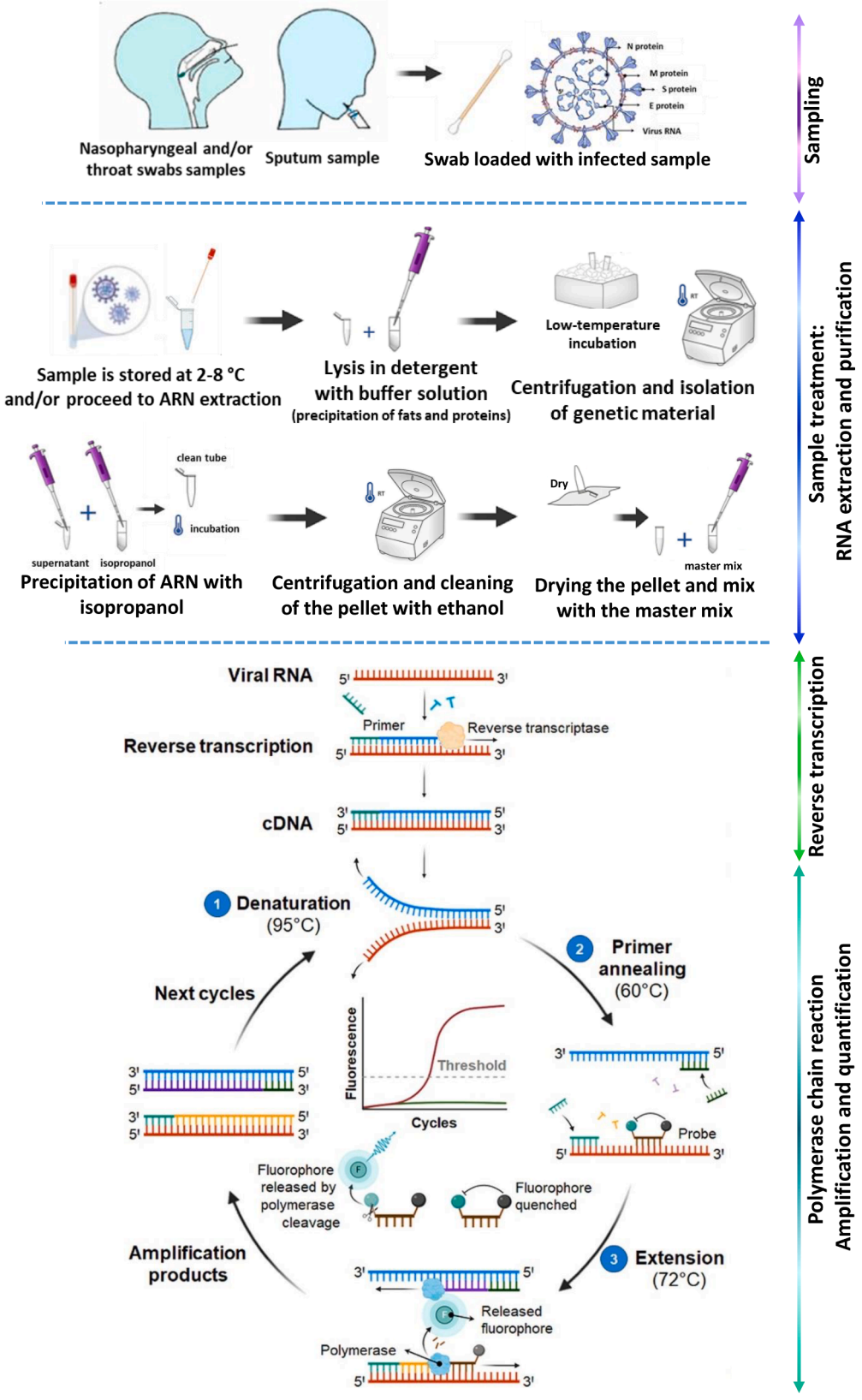


Fig. 2. Schematic diagram of sampling, extraction and purification of ARN, and molecular representation of RT-PCR.

CoV-2 at an early stage of the infection and immediately isolate infected patients. Currently, there are four different types of methods in clinical practices to diagnose or control COVID-19, which are summarized below [28].

2.1. Analysis for the detection and confirmation of COVID 19

At present, different types of analyses have been used to detect and diagnose the SARS-CoV-2 infection, to confirm the infection and/or to assess the immunity developed after SARS-CoV-2 infection. Thus, these analyses could be classified from different points of view. In this review, the analyses are classified according to their purposes as follows: (i) Analysis to detect the SARS-CoV-2 including molecular analysis and antigen tests; (ii) Analysis to confirm the infection by radiological imaging tests, including the X-Ray and CT; (iii) Finally, another independent section has been included to introduce bioanalytical tests which provide relevant information to assess the immunity after the infection. In this section, antibodies tests are explained.

2.1.1. Testing for the virus. Analysis to detect the SARS-CoV-2: Molecular analysis

Currently the gold standard method to detect SARS-CoV-2 is the RT-PCR test as indicated above. This method is based on detecting viral nucleic acids in respiratory specimen. The method has a high sensitivity and specificity, as long as the sampling has been carried out properly. The Fig. 2 shows a schematic diagram of the three different steps necessary to carry out RT-PCR analysis:

1. Sampling: The first step of the RT-PCR analysis is to obtain a sample with enough viral load. This step is crucial to avoid false negative results. The sampling of upper and lower respiratory specimens is the most widespread. In the context of COVID-19, different studies have proved that the most efficient sampling for the detection of the virus is the upper respiratory samples. The nasopharyngeal samples obtained by flocked swabs of Dracon or polyester are the most common. It is because the process is rapid, simple and safe compared with the processes needed to obtain other type of upper respiratory specimens. In the case of patients with productive cough, the lower respiratory sample is also indicated. In this type of patients, the most common used sample is the sputum, which has been described as the most accurate sample for the detection, following by the nasal swabs [88]. Once that the sample is in the swab, it should be kept into a sterile transport tube with 2–3 mL of viral transport medium (VTM) or Amies transport medium, or sterile saline. If the sample is not to be analyzed at that time, it must be stored at 2–8 °C at the latest 72 h after sampling. If the analysis needs to be delayed longer, the sample must be stored at −70 °C or below. In no case, it is not recommended heat treatment of the sample prior to RNA extraction [89].

In this step of the analysis, it is important to note that the viral load in the sample depends on the moment of the illness and on the anatomic place of sampling. Thus, at the beginning of the illness, 6–8 days, the virus is found in higher concentration in sputum > nasopharyngeal swabs samples > throat swabs samples. However, the concentration of the virus decreases in throat swabs samples after 8 days of illness, but the concentration remains detectable in sputum and nasopharyngeal swabs samples after 14 days [40,90]. The evolution of the virus is highly dependent on each patient. In consequence, the sampling process is one of the causes of the inaccuracy of the RT-PCR. Thus, it is recommended, that the RT-PCR of respiratory specimens would be combined with RT-PCR of blood, urine and fecal/anal samples during the illness to avoid false negative and to understand better the kinetic of the viral load [91,92].

2. Sample treatment: RNA extraction and purification: The patient's virus samples that arrive to the lab for testing are extremely infectious. Thus, the RNA extraction should be done in a biosafety cabinet in a biosecurity level 2 (BSL-2) or equivalent facility [89]. Note that viral cultures require necessarily working in a BSL-3 laboratory given the high transmissibility of SARS-CoV-2. Furthermore, in order to reduce the risk of infection in the lab during the analysis, the samples could be inactivated before the lab-test undergoes. The virus can be inactivated either at 75% alcohol, 56 °C incubation for 30 min, or 65 °C incubation for 10 min. Studies carried out by Wang et al. [93] proved that these inactivation methods had no significant effect on the subsequent detection of the COVID-19 by nucleic acid test. However, other studies reported that the heat inactivation of the virus could decrease the qualitative real-time RT-PCR detection rates of clinical samples with high cycle threshold values in COVID-19 [94]. This can give false-negative results in samples with poor viral loads [95]. The cycle threshold (Ct) value is the number of PCR amplification cycles necessary to yield a positive result in a diagnostic testing. The Ct value increases with decreasing the viral load. Thus a low Ct value indicates a high viral load. According to these studies, it is recommended to avoid the heat inactivation of the virus.

After the inactivation, the first stage of the lab-test is the isolation and purification of the RNA from the complex specimens. The classical procedure for RNA extraction and purification consists of an organic extraction to remove proteins and fats, followed by acid centrifugation to purify the RNA (Fig. 2). A lower efficiency in the extraction/purification could affect the replication process and cause false negative. This technique requires manual handling which can result in experimental errors and higher risk of cross-infections [96,97]. However, in clinical settings, the RNA extraction and purification step is mainly performed with commercial kits and automated high-throughput workstations which follow vendor-specific conditions. The use of these RNA purification kits could reduce the problems derivate from the manual handling. Several RNA purification kits are available for convenient, fast and effective isolation, where the extraction and purification of RNA is carried out in a microcentrifuge tube. However, although these kits reduce the risks mentioned above, they result in a significant increased cost. Furthermore, they are dependent on the supply chain and it could have as a result a bottleneck during the course of the pandemic, as has already happened. Different studies have been developed in order to propose new kits or strategies for the isolation/purification of RNA. These new proposals would be affordable, quick, efficient and independent on the industrial kits [96,97]. Thus, Smyrlaki et al. [97] propose to circumvent the RNA extraction process analyzing directly the inactivated sample. Their proposal could be a working option to avoid the extraction process but efforts to optimize the protocol are still needed before to implement this procedure in the laboratory testing of COVID-19. Other alternatives for the RNA extraction have been studied such as paramagnetic particle technology or silica-membrane based technology. Somvanshi et al. [96] suggest magnetic nanoparticles (MNP's) for the RNA extraction. In particular, they propose the use of zinc ferrite nanoparticles, which can be prepared in the lab and can be used to extract the viral RNA by an automatic process. The use of these nanoparticles avoids the heavy man-power, the lack of effective detection and the high risk of cross-infections. Although being this a promising alternative, more studies are required to employ the protocol in clinical diagnostics of COVID-19. The current situation demands a high number of RT-PCR analyses worldwide. Therefore, the best way to obtain reliable results in this stage of the analysis is to avoid heat inactivation and to use industrial kits for the extraction/purification of RNA. Thus, it is necessary to strengthen the supply chain of these kits to meet the required needs in times of pandemic, while other alternatives are investigated, and their efficiencies are tested. Another alternative capable of performing a larger number of RT-PCR analyses without relying on the supply chain is to optimize the working conditions of existing extraction kits in order to increase the efficiency of SARS-CoV-2 RNA extraction. Therefore, Ambrosi et al. [98] carried out a study to improve the protocol of four different extraction methods. The optimization process involves increasing the alcohol evaporation time (Fig. 2 drying step) to ensure its elimination, avoiding its interference in the RT-PCR analysis, and increasing the centrifugation or elution time to

improve the extraction of the RNA. As a result, the increase of the amount of RNA extracted due to the light changes of these parameters was proven. The study carried out by Ambrosi et al. [98] revealed that for a low concentration of ssRNA, the order of highest to lowest sensitivity for the different optimized extraction methods was: Qiamp DSP Virus Spin kit > Viral Nucleic Acid (DNA/RNA) Extraction Kit I >, Total RNA Purification Kit > EXTRAzol. In addition, the improvement of the sensitivity of the Viral Nucleic Acid (DNA/RNA) Extraction Kit I with the concentration of ssRNA was demonstrated. This study revealed that synthetic standards ssRNA for SARS-CoV-2 are needed to perform this type of in-house optimization. A known amount of these standards is used as a positive control to compare the efficiency of the different extraction protocols. Therefore, the optimization step of the standard extraction kits is presented as an alternative to avoid problems with the shortage of reliable kits. The optimization of RNA extraction methods could be performed in-house and would help to increase the extracted amount of virus RNA, which is crucial for the detection of patients with a very low viral load. This proposal requires the development of standards and reference materials of virus RNA for the proper optimization and validation of protocols.

3. Reverse transcription, amplification and quantification: After extraction and purification, the RNA is mixed with a master mix consisting of: a buffer, reverse transcriptase (RT) enzyme, nucleotides (dNTPs), reverse primers, direct primers, probe and DNA polymerase. The homogenized reaction mixture is loaded onto the PCR plate and introduced into a thermal cycler where different steps take place (Fig. 2): (i) Reverse transcription forming RNA/DNA hybrids (Fig. 2, cDNA). (ii) Denaturation, breaking the formed hybrids using high temperatures (95 °C) and inactivating the reverse transcription. (iii) Annealing with the forward primer and with the probe, which occurs when the temperature decreases at 60 °C, this temperature being dependent on the length and composition of the primer. (iv) Extension step, in which a new complementary strand is synthesized, being the temperature of this step depending on the DNA polymerase used. Once the process is completed, a new double-stranded DNA target is obtained. To amplify this genetic material, it is necessary to repeat cyclically the steps (ii-iv). In general, 30–45 cycles of PCR are needed to detect the virus. Amplification is the limiting factor to transform the standard method into a rapid test, but it is thanks to this process that RT-PCR has such a good sensitivity. The virus genome is detected by a fluorescence signal. A probe is used for this purpose. The probe is a strand [20,25,45,99] with a fluorophore at the 5′ end and a quencher at the 3′ end. During step (iv) the fluorophore fluoresces by excitation after release by polymerase cleavage (Fig. 2) and the signal is detected by a charge-coupled device-CCD camera. The detected fluorescence intensity is proportional to the amount of viral DNA synthesized after each cycle.

During the steps of this procedure different changes and alternatives have been proposed in order to increase the sensitivity and selectivity of the RT-PCR. For the master mix, different combinations of primers and probes have been developed and applied for the SARS-CoV-2 detection [40,100,101]. These primer-probe sets are found in the market as kits with different commercial names. These commercial diagnostic tests have been approved by the WHO and the Food and Drug Administration (FDA) for emergency use authorization (EUA) in the current situation. The approval of these tests is based on the clinical sensitivity and specificity independently tested. Other pre-commercial kits are authorized for research use only.

From January 2019, the first genome sequence of SARS-CoV-2 was added to the GenBank (genetic sequence database of the National Institute of Health, NIH) sequence repository. Since then, a large number of different genome sequences of the virus have been included on the "Global Initiative on Sharing All Influenza Data" (GISAID) (>845,000 sequences are shared at March 22, 2021 [102]), and GenBank by researchers across the globe. Thus, since the genome of the new coronavirus was sequenced, different genetic targets have been available for the development of genome amplification systems. SARS-CoV-2 has a single-stranded positive sense RNA genome that is ~30,000 nucleotides in length, larger than many others RNA virus other than the coronavirus family. The genome encodes 27 proteins [40], most of which are nonstructural proteins (NSPs), which also plays a crucial role during the replication cycle. They are encoded by two open reading frame genes (ORF1a and ORF1b) including RNA-dependent RNA polymerase (RdRp), also known as Hsp12. Four of them are structural proteins. One is surrounded by the virus RNA and is identified as the nucleocapsid protein (N). The other three structural proteins are embedded in the enveloped itself: being (S) the spike surface glycoprotein, the less conserved protein compared to the other three; (E) the small envelope protein and (M) the matrix protein (Fig. 1). The genetic material of the virus contains information to build the viral proteins needed to replicate the virus inside human cells. The aim of RT-PCR is to identify genetic sequences of the virus in patient samples by amplification of target sequences in the RdRp gene, the E gene and the N gene. The choice of the target gene depends on the primers and the probe sequences [103,104]. The sensitivity and detection capacity of SARS-CoV-2 varies depending on the amplified gene. In general, most of the RT-PCR tests use some of the following genes for the amplification: the N gene, which is involved in the instruction for making the nucleocapside protein [40], the E gene or the RNA-dependent RNA polymerase RpRd genes [103,105]. The last two types of genes have high sensitivity for detection in comparison to the first one. The study of Reina and Suarez [105] proved that the sensitivity of the RT-PCR test based on the amplification of the gene E decreases in samples taken during the last period of the infection, what represents mostly patients with resolving infection. However, the sensitivity in the detection of the N gene increases in these samples. These authors suggest that there is a possible progressive disappearance of the different genes due to the destruction of the virus. Thus, the monitoring of the kinetics of the different genes could be useful for a better understanding of the clinical evolution of patients.

Some consideration regarding the quantification process followed in the RT-PCR should be taken into account in order to confirm a positive result. The amplification of the viral genome is repeated until a certain level of fluorescence is surpassed and then, the presence of the virus is confirmed. The number of cycles needed to reach this fluorescence level is also monitored in order to estimate the severity of the infection. Thus, the fewer the cycles, the more severe the viral infection is. For the quantification, the determination of the fluorescence signal threshold is required. It is calculated by the standard deviation of the average baseline fluorescence of cycles 3–15. The cycle threshold (Ct) is determined by the number of PCR cycles needed to report a detectable fluorescence signal higher than the fluorescence signal threshold. Thus, lower Ct value means greater RNA viral load. According to China CDC (Chinese Center for Disease Control and Prevention) [106], Ct values < 37 can be reported as positive results, Ct values > 40 are considered clinically negative and Ct values within 37–40 should be considered as dubious and the test should be repeated [106]. However, some studies have shown that the virus culture is unfeasible in those cases with Ct > 33 [107,108] and others inform that the culture of the virus failed in samples with Ct > 30 [109]. Salvatore et al. [110] have proved that the lowest Ct values take place shortly after symptom onset and correlate significantly with the time elapsed since the onset of symptoms. Furthermore, a study conducted by the College of American Pathologists (CAP) with 700 laboratories has shown that Ct values can vary depending on the method used for the analysis. Thus, this study reported that the inter-laboratory median of Ct values within laboratories was higher than 14 cycles and the intra-laboratory median was 3 cycles [111]. Hence, it is very difficult to establish a Ct value above which the infectivity of the patient can be ruled out due to: (i) the low reproducibility of the Ct values between laboratories; and (ii) the different evolution of the virus depending on multiple factors during infection. Virological studies do not reveal conclusive results in this respect either. Wolfel et al. [112] concluded that the virus can only be cultured from respiratory samples during the first week of symptoms, but not after day

8, in spite of persisting high virus loads as determined by quantitative RT-PCR. The US Centers for Disease Control and Prevention (US CDC) in a study with various age groups of adults and with different grades of disease severity concluded that the virus could not be cultured>10 days after the onset of symptoms [113]. Consequently, the US CDC recommends a symptom-based decision for returning from isolation, rejecting the exclusively test-based strategy, unless a decision is taken before the 10 days of isolation. [114]

An important issue with the real-time RT-PCR test is the risk of obtaining false-negative and false-positive results. In order to get reliable results and to improve the sensitivity, selectivity and efficiency of the RT-PCR diagnostic some strategies have been suggested [40,45,99]. Thus, currently, in the master mix, is common to add an internal control (IC), which consists of an unrelated RNA sequence, together with a specific fluorescent-labeled probe in order to identify experimental errors. Furthermore, duplex and multiplex real-time RT-PCR tests have been developed. These types of RT-PCR are able to simultaneously detect two or more sequences. These tests can detect simultaneously the presence of RdRp and N genes or the presence of ORF1ab, N, and S genes. In general, it is an interesting advance because the kinetic of the virus provokes changes in the structural proteins during the infection. Therefore, sequences for the detection of the E gene (primers and probes) could be used as screening tool and the sequence of RdRp or the N gene could be used simultaneously to confirm the infection.

The nucleocapsid gene for protein N is stable and its sequences are maintained in time, thus being a promising target for its detection. On the other hand, point mutations in the RdRp gene are well documented, contributing this fact to disrupt test methods and antiviral treatments, e.g. Remdesivir. In recent months, new variants of concerns (VOC) of SARS-CoV-2 have been identified that have emerged in countries such as United Kingdom, South Africa, Japan or Brazil, rising alarms. As a matter of fact, mutations in the S gene are more likely to occur, since they give the virus a genetic advantage in terms of transmissibility, morbidity/mortality or vaccine escape. Confirmation of infection with a specific VOC requires either carrying out the WGS (Whole Genome Sequencing) or sequencing the S gene in whole or in part.

Currently, there are above 430 molecular assays for COVID-19 diagnostic. However, most of them have not been validated by the healthcare authorities to be authorized for emergency use. Information about the approved molecular assays and the values of the main analytical parameters have been currently published in a review of Afzal [100]. The most important analytical parameters that should be taken into account to select a molecular assay for COVID-19 diagnostic are the sensitivity expressed as positive percent agreement (Table 1), and the limit of detection (LOD) at early stage of the infection avoiding false-negative results. However, due to the dramatic situation arising from the pandemic, the protocols established for the authorization of the molecular assays have become more flexible in order to have enough tests to be able to carry out large-scale analyses. The relaxing of the protocols has allowed obtaining molecular assays for fast detection of the virus and/or the disease. It is increasingly vital to control the sources of infection and help patients to prevent the illness progression. However, failure to make a representative validation of methods, due to the lack of time, may provoke that molecular assays -without enough reliable performance data- appear in the market. In particular, the sensitivity of the molecular assays is guaranteed by the own sensitivity of the RT-PCR analysis. Despite the high sensitivity of the RT-PCR analysis some of the proposed molecular assays present a high number of false negative. It could be caused by a low specificity of the primers and probes used in the kits. Multiple target genes could be used as primers and probes to avoid or reduce invalid results. Several combinations of primers and probes have been proposed in different kits, but with different results [100]. The moment of the infection, the evolution of the virus genome during the course of the infection and the specific primer and probe used in the RT-PCR also can produce false-positive. Thus, the S-protein gene remains even when the virus is inactive. A patient who has overcome the infection can still be positive if the target primers and probes are specific for the detection of the S genes. In this context, RT-PCR tests that combine different gene sequences (two or more) are highly recommended to improve the sensitivity of the analysis [115].

Table 1
Comparison of the analytical parameters (sensitivity and specificity) of the different proposals for the standard RT-PCR in order to reduce the time of analysis and/or improve the sensitivity of the method.

Proposal	n	Sensitivity	Specificity	Ref
RT-PCR in one step				
OSN-qRT-PCR	181	100%	91%	[13]
Isothermal amplification (RT-LAMP)				
RT-LAMP (multiple primers)	260	91%	99%	[21]
iLACO assay for COVID-19 (single primer)	248	90%	99%	[120]
Mismatch-tolerant LAMP assay	24	100%	100%	[121]
RT-LAMP combined with CRISPR/Cas12a				
DETECTR	83	95%	100%	[125]
CRISPR/Cas12a-NER	31	100%	100%	[126]
SHERLOCK (STOPCovid)	17	100%	100%	[127]
Others				
SimplexaTM COVID-19	278	100%	96%	[128]

Note: n, number of clinical samples; sensitivity and specificity percentages calculated with the data published in the literature as follows.

$$Sensitivity = \frac{TP}{TP+FN} \times 100;\ Specificity = \frac{TN}{TN+FP} \times 100$$

being: TP, true positive; TN, true negative; FN, false negative and FP, false positive according to the standard RT-PCR.

However, changes produced in the genetic sequencing of the virus (appearance of new strains) and its evolution during the infection represent a new challenge for the manufacturers of molecular tests. It is very important to update the shared database of the virus genome with the new identified genomic sequence [102] to be able to produce molecular tests with enough sensitivity of the oligonucleotide primers and probes avoiding false negatives. Other source of false negative can be produced by error in the sampling or in the handling, storage, transfer, purification, or processing due to the lack of laboratory practice or personnel skills [88]. Thus, to avoid these errors, the RT-PCR should be carried out by highly authorized, experienced and qualified staff in this kind of sampling and analysis.

A large number of studies can be found in the literature concerning about the analytical parameters of RT-PCR test. For instance, in a study carried out with 1014 patients (601 COVID-19 positive and 413 healthy patients), the sensitivity and specificity of the RT-PCR detection was estimated to be within 0.715–0.849 and 0.933–1.000 respectively [21]. However, these ranges could be improved depending on multiple factors mentioned above.

Thus, RT-PCR is the gold standard for its high sensitivity and specificity. However, at present, the RT-PCR method cannot be performed directly at POC because specific installations, specialized instrumentation and personnel, and considerable analysis time are required for that purpose. The need for early and safe detection of infected patients has motivated researchers to develop different alternatives. In order to reduce the time required for the molecular analysis, and to minimize the requirements of the RT-PCR analysis, several proposals can be found in the literature.

2.1.1.1. Alternative molecular tests to classical RT-PCR analysis for more rapid detection of SARS-CoV-2. Different studies have been carried out in order to reduce test turnaround times. Somvanshi et al. [96] propose a more rapid and efficient method to extract the virus RNA from the sample based on magnetic nanoparticles (MNP's). This procedure may significantly lessen the operation period and necessities for the RT-PCR. However, more studies are required to apply this protocol to clinical diagnostics of COVID-19. Vincent et al. [116] suggest making shorter PCR thermal cycles. This proposal consists of a photonic platform for RT-

PCR that integrates a rapid-cycle fiber-optic PCR chamber with feedback-controlled laser heating, online fluorometric detection of PCR products, and all-optical thermometry using an optical readout from color centers of diamond nanoparticles. This approach can radically shorten the PCR thermal cycle relative to standard PCR systems. This alternative reduces the time of analysis but, it does not allow to perform the analysis in retail pharmacies, workplaces, or even developed at home use. More studies concerning about the analytical parameters of this proposal should be carried out.

Some of the most promising approaches to reduce the requirements of the classical RT-PCR analysis found in the literature are presented underneath. These proposals have been classified depending on the modification applied on the classical RT-PCR. Thus, four different groups are presented: (i) RT-PCR in one step, (ii) Isothermal Amplification termed RT-LAMP (Reverse Transcription PCR Loop-mediated isothermal AMPlification) (iii) RT-LAMP combined with CRISP-Cas 12 or Cas 13 (Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR associate protein) detection methods, specially used for analytical purpose and (iv) other approaches. These proposals should be compared to the standard method in order to evaluate their diagnostic capacity from an analytical point of view. Table 1 shows the analytical parameters (sensitivity and specificity) obtained in different studies per each alternative proposed.

RT-PCR in one step. Regarding the number of steps needed to perform the RT-PCR, two variants are currently available commercially. These two variants can be classified as RT-PCR in one step or RT-PCR in two steps. In the one-step assay, the reverse transcription and the amplification are carried out in the same tube with the same buffer solution. In the two-steps assay, the reverse transcription is carried out in one tube with a specific buffer solution and the amplification is carried out in another tube with a different buffer solution. One-step RT-PCR assay is rapid, robustness, and appropriate for high performance analysis. It reduces the contact with the sample avoiding contamination and handling errors. However, this assay leads to lower target amplicon generation because of the difficulty to optimize the conditions for the transcription and amplifications simultaneously. Two-step assay is more sensitive and offers low detection limit. However, it is more time-consuming and requires optimizing the conditions for each reaction independently [40,99]. Wang et al. [13] compared a new *one step single-tube nested (OSN-qRT-PCR) RT-PCR* with the RT-PCR test and the digital PCR. The results of this study revealed that the sensitivity of one step single-tube nested RT-PCR is 10 fold higher than for the RT-PCR kit showing better suitability for the detection of SARS-CoV-2 in patients with low viral load. However, it provides some false positive (Table 1) and the time required for this analysis is not sufficiently reduced being 2 h vs RT-PCR test (1.5 h) and vs digital PCR (3–4 h). Furthermore, the efficiency throughout the period of infection has not been studied yet.

Isothermal amplification (RT-LAMP). PCR remains to be the gold standard in the molecular diagnostic of COVID-19. However, it requires an expensive sophisticated thermal cycler that is not ideal for POC application. *RT-LAMP* is another proposal that reduces the time (30–45 min) and the cost of the analysis, having a large diagnostic capacity. Moreover, it can be applied without previous RNA extraction. It consists basically of carrying out the amplification step at a constant temperature of 60–65 °C, without demanding the use of a thermal cycler. However, this alternative is not a quantitative test. Some studies have been carried out to try to improve the read out of the RT-LAMP assays using sensors integrated in smartphones [117,118], colorimetric detection [119] fluorescence detection or gel electrophoresis [54]. This alternative is less specific than the classical RT-PCR which can provoke higher false positive results, and it is also less sensitive. Suitable LAMP primers and probes have been designed in order to improve the specificity of the RT-LAMP test for the detection of the SARS-CoV-2 [54,120]. The accuracy of the test is acceptable, and the sensitivity of this diagnostic test is reasonable [54] (Table 1). However, these analytical performances depend on the primers used. For example, in a study with 260 patients (213 healthy patients and 47 COVID-19 positive), in which a RT-LAMP method was applied using primers for the ORF1ab, E, and N genes the sensitivity and the specificity obtained was 91.4% and of 99.50% respectively [21]. In the case of *iLACO assay,* where only primers for ORF1ab gene were used, the time of analysis is reduced but the sensitivity is also negatively affected (Table 1). It decreases when the viral load is low, generating more false negative results [120]. Other variants have been developed in order to improve the efficiency and the sensitivity of the RT-LAMP test. One of them is the *Mismatch-tolerant LAMP assay,* which consists of adding the corresponding high-fidelity DNA polymerase in the master mix. The results obtained with this assay showed 100% consistency with the results obtained by RT-PCR method. However, more results with clinical samples should be obtained in order to get first the analytical performance and to report then the proper information (Table 1) [121]. Other proposed assay is the *Penn-RAMP* assay, which consists of adding recombinase polymerase amplification during the amplification process. The analytical parameters of this method seem promising. El-Tholoth et al. [122] proved that this method is 10 times more sensitive than LAMP and PCR when processing purified nucleic acids, and 100 times more sensitive than LAMP and RT-PCR with minimally processed samples. However, it has not been proved for COVID-19 clinical samples yet [54,122]. Another study carried out by Huang et al. [123] proposes the combination of one-step reverse transcription and RT-LAMP with colorimetric detection. The results were consistent with those obtained by conventional RT-PCR. However, though the test is sensitive and easily applied for screening at public domain, regional hospitals and medical centers in rural areas, the results based on changes of color are pH dependent. Thus, these results are significantly affected by the quantity of the buffer used in the test, being necessary the optimization of the RNA buffer concentration to improve the accuracy of the RT-LAMP methods based on colorimetric detection.

Schmid-Burgk et al. [124] proposed a method named *Barcoded RT-LAMP (LAMP-Seq) for COVID-19.* It consists of labeling the specific sample for identification with a compressed barcode space of 10 nucleotides. The total amount of samples is divided in different pools of samples. Twelve-cycles of PCR tests with specific barcoding primers were carried out for each pool of samples in order to amplify the virus DNA and to label each individual batch of samples with a unique barcode sequence. Then, the products are sequenced, and the association between the barcodes and the correct viral sequence is determined computationally, identifying the positive patients. The authors have assessed the protocol by interpreting the barcoding system as a modified Bloomfilter, a probabilistic data structure that identifies elements in a group. The results of this study conclude that this procedure increase significantly the capacity of detection and reduce the cost of the analysis. However, there is not any clinical data available, and the analytical parameters were computationally estimated by the authors [54].

RT-LAMP combined with CRISPR-Cas12 or Cas 13 detection methods. Another promising alternative proposed in the literature are based on the combination of the RT-LAMP and the CRISPR-Cas12 or -Cas 13 systems, being able to rapidly and precisely detect viral nucleic acid even at the attomolar (aM) concentration level providing results within one hour with a setup time $<$15 min.

One of the alternatives to use proposes the use of a Cas12 enzyme after an RT-LAMP procedure to detect a specific E gene and N gene sequence in the amplified virus RNA and once complexed, indiscriminately cleave nearby structures by a reporter molecule confirming the detection of the virus. Different tests based on this alternative have been developed and evaluated in the literature.

Broughton et al. [125] determined the analytical parameters of a molecular test for SARS-CoV-2 on the basis of *DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter).* This test presents the advantages of single nucleotide target specificity, integration with accessible and easy-to-use reporting formats such as lateral flow strips and no requirement for complex laboratory infrastructure. The results showed 95% positive predictive agreement and 100% negative

predictive agreement (Table 1). Clinical validation of this assay is ongoing in a Clinical Laboratory Improvement Amendments (CLIA) certified microbiology laboratory.

Wang et al. [126] proposed a rapid detection assay with a naked eye readout (NER) named *CRISPR/Cas12a-NER*. They proposed this assay as a diagnostic method suitable for a local hospital or community testing. The results reported by the authors confirmed a good consistency with the results obtained with RT-PCR assay. They reported that this assay can detect as few as 10 copies of the virus gene in 45 min without any special instrument. The intensity of the fluorescence signal shall be compared to a control to confirm a positive result and the analytical parameters should be obtained with more clinical samples. However, the authors suggested this alternative for the portable, simple, sensitive, and specific detection of the COVID-19, providing a simple and reliable on-site diagnostic method suitable for a local hospital or community testing.

Another alternative for the detection of this kind of assay is the *SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing)*. This proposal consists of turning to the classically two-step SHERLOCK into a single-step reaction by integrating the isothermal amplification step with the CRISPR-mediated detection step, establishing a common reaction capable of supporting both steps [127]. The STOPCovid (SHERLOCK testing on one pot) proposed by Joung et al. [127] has minimal sample processing step and does not require complex instrumentation, can be performed by lay users, making it deployable in low-resource clinics, retail pharmacies, workplaces, and even developed for at-home use. The proposal seems promising but still needs to be tested with real samples and validated by external parties to be proposed as an alternative to current methods.

CREST (CAS 13a-based, Rugged, Equitable, Scalable Testing) amplifies and detect the N genes protein for SARS-CoV-2 and overcomes the main problems related to its current detection. It may be used in POC platforms and is a low cost assay in comparison to classical lateral flow assay, because antibody conjugates for the detection are not required. Its LOD is similar to the CRISPR assay previously indicated.

Others approaches. Another different approach based on another type of modifications of the standard RT-PCR method are at hand in the literature. Among them, *Simplexa COVID-19* is a promising alternative. It is a direct assay and does not require a separate extraction of RNA to perform the analysis. It is established with an all-in-one reagent mix. Furthermore, this assay reduces the time of analysis because it is based on a direct amplification of the SARS-CoV-2 RNA by using a direct amplification disc combined with LIAISON® MDX instrument and LIAISON® MDX Studio Software (DiaSorin). Bordi et al. [128] evaluated the analytical performances of this assay (Table 1). The comparison to the classical RT-PCR showed an almost perfect agreement in the results of both assays. The lower LOD for both S and ORF1ab genes was the same and cross-reactive analysis confirmed a 100% of clinical sensitivity of the assay. The high sensitivity and the specificity of this assay indicate that it is promising for laboratory diagnosis, enabling high speed detection in just over one hour, which is significantly faster than the up to five hours currently required by conventional extraction followed by amplification technologies.

All the alternatives proposed at the moment for a rapid RT-PCR analysis, reduce the time of analysis and simplify the analytical procedures. However, few nucleic acid amplification tests have been authorized for maximum screening at POC. Nevertheless, other analytical methods based on bioanalysis have evolved more rapidly and have been authorized to be used at POC. This is the case of antigen tests.

2.1.2. Detection of viral proteins. Analysis to detect the SARS-CoV-2: Antigen analysis

Antigen tests are immunoassays based on the detection of the internal protein of the nucleocapsid (N) of SARS-CoV-2, which is an ideal target for viral-antigen based detection or on the S1 and S2 subunits of the spike (S) protein found on the outer surface of the virus. The detection of these viral proteins is carried out through the use of specific antibodies that bind to the S or N proteins, allowing the capture of the complete virus or its fragments (broken virions or infected cell debris), respectively.

Antigen tests are based on the detection of SARS-CoV-2 proteins present in respiratory specimens. As has happened in the case of tests based on the amplification of nucleic acids, antigen tests have been developed to be performed at laboratory level and to be carried out at POC, the latter being known as rapid tests [129]. Thus, antigen tests are presented as a multipurpose tool to respond to the COVID-19 pandemic [130]. Antigens are considerably more stable than RNA, which makes them less susceptible to degradation during transport and storage. Furthermore, antigen tests are affordable, most of them need about 15 min to return results and can be used at the POC. They have a high specificity reporting few false positives, but their number may increase when the antigen test is used in populations where few people are infected. In general, the sensitivity of antigen tests is lower than the sensitivity of nucleic acid amplification tests. Antigen tests do not amplify the information found in the samples tested, unlike nucleic acid amplification tests. Consequently, the amount of antigens present in the sample is sometimes lower than the OLDs of the test. This can lead to a false negative result due to a lack of sensitivity of the test. This would not occur in the case of using a nucleic acid amplification test. However, the WHO and US CDC highly recommended the use of the antigen test in locations with a high incidence of infected people. Antigen tests can be used rapidly, and they are cost-effective. This would allow the identification and confirmation of infected people and thus control the spread of the virus. In these situations, where rapid results are essential to stop the spread of the virus, the use of antigen tests is justified, although these tests have a lower sensitivity than the nucleic acid amplification tests [130]. Thus, antigens tests that indicate a negative result on a suspected patient should be contrasted with a counter-analysis. The confirmatory test must be carried out as soon as possible, and not longer than 48 h after the antigen test. If the time between tests is more than 48 h the confirmatory test should be considered as a separate test. The results of the confirmatory test would be definitive for the diagnosis of the COVID-19 according with US CDC [130]. Therefore, antigen tests must have advantages over nucleic acid amplification tests to justify their use in place of the latter. Their use is justified because they allow rapid, simple and economical repetition of the analysis and they are also suitable for combination with other types of analysis, such as RT-PCR, to confirm doubtful cases. Furthermore, studies have proved that the rapid antigen tests work better when the viral load is high (5 days after the onset of symptoms). Thus, they are sensitive enough for cases with low RT-PCR cycle threshold (Ct < 25) [114].

Antigen tests have been widely used to detect infection with viruses other than SARS-CoV-2. The development of more cost-effective and high throughput test systems will be important for preventing viral spread and monitoring infection levels in COVID-19 patients [131]. Table 2 [131–136] shows some of the analytical procedures proposed in the literature for that purpose and the advantages and disadvantages of the antigen tests evaluated from an analytical point of view.

The antigen tests could be designed for quantitative or qualitative analysis. Some of the antigen tests, mainly the quantitative ones, have been designed to be used in certain locations such as laboratories or hospitals. However, other antigen tests have been designed to be used in a simple way, by personnel without specific training, as if it was a rapid pregnancy test. The quantitative tests need more time for the analysis, about 30 min, but they can inform about the antigen levels at the moment of the analysis and it could be used to distinguish between the early and late phases of the COVID-19 clinical course [131]. Most of the available tests authorized by FDA or WHO imply the use of immunochromatographic tests or fluorescence immunoassays. The studies shown in Table 2 reveals that the sensitivity of these tests increase when the sample are collected from patients within the first 5–7 days of symptom onset, when respiratory secretions have high viral loads.

Table 2
Comparison of different types of antigen tests on emphasizing their strengths and weaknesses.

Name of test	Analytical procedure	Advantage/ disadvantage	Ref
LUMIPULSE SARS-CoV-2 (Fujirebio, Inc., Tokyo, Japan)	Chemiluminescence enzyme immunoassay (CLEIA) applying anti-SARS-CoV-2 Ag monoclonal antibody-coated magnetic particle solution and alkaline phosphatase-conjugated anti-SARS-CoV-2 Ag monoclonal antibody. Detection using LUMIPULSE G600II automated immunoassay analyzer. The signal reaction is proportional to the amount of SARS-CoV-2 Ag. Quantitative detection.	The results allowing monitoring viral clearance in hospitalized patients. It present 100% of concordance with RT-PCR for high and moderated viral load samples. However, scattered correlation patterns were observed in samples with low viral loads, which were collected from patients at later periods after symptom onset. The results are obtained after 30 min and need instrumentation.	[131,132]
SOFIA SARS Antigen FIA (Quidel Corporation, San Diego, CA, USA) and STANDARD F COVID-19 Ag FIA" (SD Biosensor Inc., Gyeonggido, Republic of Korea)	Immunofluorescence-based lateral flow technology. Automated analyzer: SOFIA 2, Quidel Corporation and F2400, SD Biosensor Inc. Qualitative detection.	Results show that they have an excellent sensitivity to detect the virus in samples with (Ct values $\leq$ 25), which are found in pre-symptomatic (1–3 days before symptom onset) and early symptomatic COVID-19 cases (5–7 days after symptom onset). In vitro experiments showed no viral growth from samples with Cts $>$ 24 or taken $>$ 8 days after symptom onset. However, the exact threshold of contagiousness is unknown at the moment. Additional studies with larger numbers of samples are needed. They need instrumentation.	[133]
FIC assay developed in house to specifically detect the NP antigen of SARS-CoV-2	Immunofluorescence assay consists of a nitrocellulose membrane with fluorescent microparticles-labeled SARS-CoV-2 NP-specific antibody (mouse anti-SARS-CoV-2 NP M1). This antibody was produced by vaccination of mice with long peptides containing the SARS-CoV-2 NP-specific epitopes. A double-antibody sandwich is formed, and a fluorescent signal is detected if the sample contain the virus. The fluorescent results are read by an immunofluorescence analyzer. Qualitative detection.	The results show high specificity and relative high sensitivity in the early phase of infection. However, the study does not include individuals without symptoms. It need instrumentation.	[134]
PanbioTM COVID-19 Ag Rapid Test (Abbott; Lake Country, IL, U. S.A)	Immunochromatographic assay consists of a membrane-based which detects the nucleocapsid protein of SARS-CoV-2 in nasopharyngeal samples. Single lot of later flow analysis (LFA) testing devices were used: lot 41ADF011A. Qualitative detection.	Results in 15 min. Study carried out extensively to determine the sensitivity of the LFA. The test has a sensitivity higher than 73% when samples from patients with symptoms for less than seven days are analysed. It does not need instrumentation. However, the test gave false negative results in patients with low viral load which may occur very early in the infection before viral replication peaks, or in a late stage of infection when replication has decreased after.	[135]
COVID-19 Ag Respi-Strip (Coris BioConcept, Gembloux, Bel-gium)	Immunochromatographic test consists of dipstick based on a membrane technology with colloidal gold nanoparticles using monoclonal antibodies directed against SARS-CoV-2 highly conserved nucleoprotein antigen. Qualitative detection.	Results in 15 min However, sensitivity and specificity of 57.6 and 99.5%, respectively with an accuracy of 82.6%.	[136]

However, the tests based on fluorescence assays present high specificity and can reach remarkably high sensitivity, if they are applied to samples from early phases of infection or with high viral loads in comparison to immunochromatographic tests [133]. In the case of immunochromatographic tests, the detection system consists of the use of a colored or fluorescent indicator that allows the change to be detected visually, as the case of the use of colloidal gold, for example. Other more sensitive detection alternatives have been developed. However, they require a reading device to interpret the results.

Most antigen-detection rapid diagnosis tests for COVID-19 are based on a lateral flow immunoassay approach making use of a double-antibody sandwich fluorescence detection system. It usually consists of a plastic holder with two holes, one for sample and other for buffer. When the sample is applied to the testing strip, it moves along the strip by capillary effect. When the sample reaches the section of the strip where fluorescent-labeled antibodies are fixed, these antibodies bind to the nucleocapsid proteins present in the sample forming antibody-antigen complexes. These complexes continue to move on the strip

until they reach what is called the test line. The test line contains immobilized virus-specific antibodies. Thus, if the sample contains virus antigens, these will bind to the antibodies and the test line will change color indicating a positive result. If the antigen is not present in the sample, then the test line will not change color. However, the sample will continue to advance along the strip and reach the control line. This line will change color indicating that the test has been performed correctly. The control line contains labeled antibodies that bind to non-antigen targets in the sample like streptococcus C antigen that are normally found in the microbiome of the pharynx. The antigen test is read as either positive with test line and control line visible, negative if only a control line is visible or invalid with only a test line appearing or no line appearing at all. There are also digital devices that show positive, negative or invalid results without the operator having to interpret the results off the strips.

The antigen test kits contain all the materials necessary to carry out the analysis including the swab to take the sample. The timer is not included in the kits. The instructions for the kits explain how the results should be read and interpreted. An important factor in antigen analysis is the time needed to read the results. The accuracy of the analysis depends on this factor. The time needed to correctly read the result is also indicated in the manufacturer's instructions. A longer or shorter time than the indicated can lead to false positive or false negative results.

The analytical method follows to carry out the antigen analysis could be divided in three different steps. Firstly, the sample should be collected. The sampling procedure is similar to that described in the section of RT-PCR (section 2.1.1). Once that the sample is in the swab it should be analyzed as soon as possible and the temperature during this time should be controlled. The use of VTM during storage and transportation of the sample is not recommended because could dilute the sample and may decrease the sensitivity of the test. However, currently there are antigen tests developed to analyze samples diluted in VTM. If the sample is found in a viral transport medium the analyst should confirm that the test is applicable of this type of sample [129]. The next step consists in the extraction and labelling of the nucleocapsid protein (N) of the virus if it is present in the collected sample. Finally, the last step is the identification and the quantification if the analysis is quantitative. The most common procedures to carry out these steps are exposed in Table 2.

WHO and FDA have authorized the antigen tests for screening and diagnostic of patients suspected of COVID-19 within five days of symptom onset. These institutions have developed a guide to help in order to use these tests properly. This guide explains how antigen tests should be used in asymptomatic people, or how to proceed to determine whether an infected person is still infected. However, the "gold standard" for clinical diagnostic detection of SARS-CoV-2 remains nucleic acid amplification test, as is the case with RT-PCR. Therefore, it may be more appropriate to perform an antigen test or a nucleic acid amplification test depending either on the particular situation. However, it should not be forgotten that occasionally the antigen test may need confirmation of a counter-analysis. To easily visualize the similarities and differences between these two types of tests, Table 3 summarizes the analytical features and shows the advantages and disadvantages between RT-PCR and antigen test, among others.

2.1.3. Testing for the disease. Analysis to confirm the diagnosis: X-Ray and computed tomography

Imaging techniques can be used for the diagnosis of patients suspected to have COVID-19 infection, but a laboratory test based on RT-PCR is required to confirm the disease. In this respect, both methods are complementary and together they provide an accurate diagnosis. Chest imaging was one of the main clinical diagnostic tools for COVID-19 detection, particularly at the beginning of the pandemic due to the shortage of tests [10]. The two main imaging techniques for COVID-19 diagnosis are:

Chest X-ray may show multiple and progressive patchy opacities in both lungs as disease progresses [1,137]. These opacities have a "ground glass pattern" and linear opacities may also be observed in association with ground glass opacity. Eventually, in severe cases, lung markings completely disappear due to the whiteness consolidation [1]. Pneumothorax and pleural effusion have also been reported in severe cases [138]. However, initial chest X-ray may be negative, but patients may later develop clinical or radiological signs of COVID-19 pneumonia.

Chest computed tomography (CT) uses multiple X-ray images taken from different angles around the chest that are processed by a computer to produce tomographic (cross-sectional) images of the lung [8]. CT plays a crucial role in the early diagnosis of COVID-19 as it can determine the nature and extent of lesions and can detect subtle changes in the lungs that are usually not visible on chest X-ray. The characterization of lung lesions on CT images is based on different parameters including their distribution, number, density, pattern and presence of concomitant signs [1,139].

Fig. 3 and Table 4 show a chest X-ray and a chest CT, respectively, of a COVID-19 patient [1,10]. The CT findings of patients with COVID-19

Table 3
Summary of analytical features and advantages and disadvantages between RT-PCR, CT, antigen and antibody tests.

	RT-PCR test	Chest computed tomography	Antigen Tests	Antibody test
Intended Use	Detect current infection	Detect current infection	Detect current infection	Detect previous infections
Analyte Detected	Viral Ribonucleic Acid (RNA)	–	Viral Antigens	Viral immunoglobulins M and G
Specimen Type(s)	Nasal, Nasopharyngeal, Sputum, Saliva	Lung images	Nasal, Nasopharyngeal	Serological
Sensitivity	Varies by test, but generally high	High	Moderate	Moderate-Low (LFIA < ELISA < CLIA) Combined IgG/IgM test is the better choice
Specificity	High	Medium	Medium-High	Medium-High
Test Complexity	Varies by Test	Require expert radiologists	Relatively Easy to Use	Very easy
Authorized for Use at the Point-of-Care	Most are not, some are	No	Most are, some are not	Yes
Turnaround Time	Several hours to days for laboratory tests; less than an hour for point-of-care tests	Less than an hour	From 15 min to less than an hour	From 10 to 15 min (LFIA), 30 min (CLIA), to 1.5–2.5 h (ELISA)
Cost	Moderate	High	Low	Low
Advantages	High sensitivity and specificity	High sensitivity	Simple operation, low cost, high-throughput	Quickly results, simple operation, low cost.
Disadvantages	Sophisticated equipment, experienced operators	Very expensive and cannot be carried out massively. Not useful in asymptomatic, pre-symptomatic patients or in patients with mild symptoms without pneumonia	Produces false negatives depending on the viral load.	Low sensitivity during early infection. Widely in their accuracy.

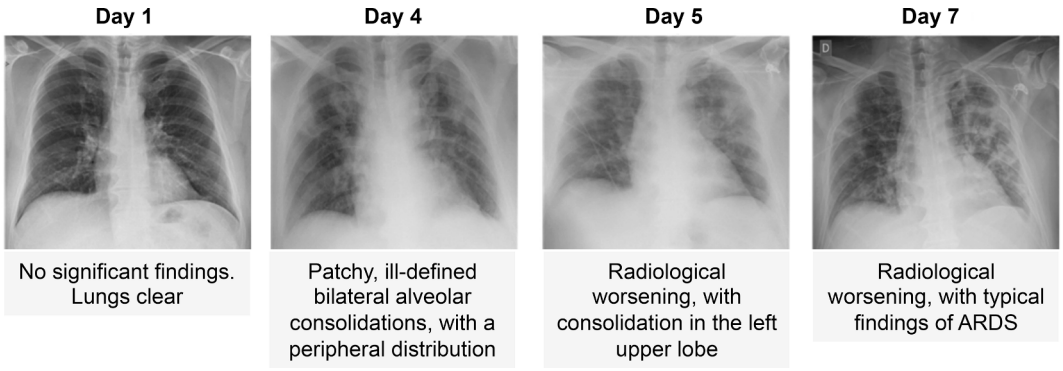
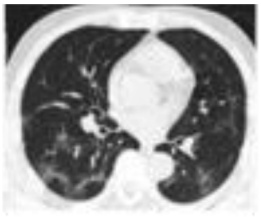
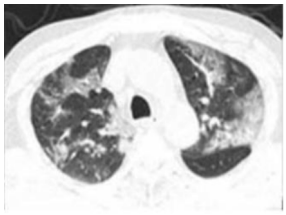
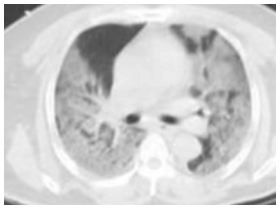
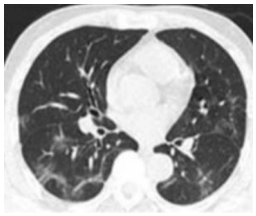


Fig. 3. Chest X-ray images of a 50-year-old COVID-19 patient with pneumonia over a week (taken from [10] with permission).

Table 4
CT manifestations of different stages of COVID-19.

Stage	Manifestations	CT Image
Early	Single or multiple scattered patchy ground glass opacities, predominantly in the middle and lower lungs and along the bronchovascular bundles. A crazy-paving pattern, secondary to intralobular and interlobular septal thickening can be seen.	
Advanced	Increased extent and density of bilateral lung parenchymal opacities.	
Severe	Diffuse consolidation of the lung parechyma with uneven density, air, bronchi and bronchial dilation.	
Dissipation	Areas of ground glass opacity and consolidation have nearly completely resolved, leaving some residual curvilinear areas of density.	

include multiple, peripheral, opacities with a "ground glass" pattern in both lungs and areas of consolidation with subpleural distribution and along the bronchovascular bundles. A scattered ground-glass attenuation with superimposed interlobular septal thickening and intralobular lines ("crazy paving" pattern) can also be seen on CT scans. Air filled bronchi (air bronchogram) are usually associated with consolidation and bronchial wall thickening. A less common finding is the thickening of the adjacent or interlobar pleura with some degree of pleural effusion [1]. CT findings of COVID-19 can be categorized in four temporal stages: early, advanced, severe, and dissipation according to the extent of the lesions (Table 4) [1].

The main advantage of CT is its sensitivity in the early diagnosis of COVID-19. In fact, patients with negative RT-PCR may present with characteristic CT features [1]. Usually, CT images are normal in days 0–2 of the infection, but after the onset of symptoms CT findings are visible over a long period [140], particularly at day 10 after the onset of symptoms [10,141]. Fang et al. [142] studied 51 patients with COVID-19 symptoms and found that chest CT had higher sensitivity (98%) than RT-PCR (71%). However, the small number of patients included in the study means that its results should be interpreted with caution. Nevertheless, another study involving>1000 patients reached similar conclusions [28,143]. CT examinations are however very expensive and cannot be carried out massively. In addition, CT cannot identify the specific virus related to the lung lesions and it is not useful in asymptomatic, pre-symptomatic patients or in patients with mild symptoms without pneumonia [144].

Recently, many researchers have proposed the use of AI in medical imaging for the automatic diagnosis of COVID-19 as AI may identify imaging pattern changes that are not easily amenable to human identification. In this respect, the use of deep learning algorithms could enhance the assessment and extraction of imaging features potentially increasing the radiologist's ability to diagnose the disease or even to reach a diagnosis without the need of the radiologist to look at the images [10,145]. Wang et al. [146] used a neural network design (COVID-Net) for COVID-19 detection and obtained 83.5% accuracy to classify patients as normal or having no pneumonia, bacterial pneumonia, and COVID-19 pneumonia. The deep learning model developed by Ioannis et al. [147] applied to 224 confirmed COVID-19 images provided 98.75% and 93.48% success rates for the diagnosis of patients with bacterial and viral pneumonia, respectively. Narin et al. [148] found that the neural network-based model ResNet50 had a 98% accuracy using chest X-ray images for the detection of COVID-19. Sethy et al. [149] stated that the ResNet50 model with support vector machine (SVM) classifier provided the best performance. Most recently, Ozturk et al. [10] found that the DarkNet model provided a 98.08% accuracy for the diagnosis of COVID vs. no-findings and 87.02% accuracy for pneumonia vs. COVID vs. no-findings. Recent studies have also developed deep learning-based models using CT images [150–152].

The large number of AI algorithms makes complicated to decide which one to use particularly because no particular AI approach seems to be better for COVID-19 diagnosis than the others. In addition, the different AI approaches are very difficult to compare because of the multiple evaluation criteria [20,153,154]. For this reason, Albahri et al. [20] have reviewed the research efforts for applying AI to imaging techniques for the detection of COVID-19.

2.2. *Other bioanalysis used for the control of the spread of COVID-19*

Antibody tests, referred to as a serology tests by researchers and clinicians, are used for surveillance and epidemiological research and to estimate how many people have already been infected with the SARS-CoV-2 virus, while antigen and RT-PCR are used to detect acute infection. Antibody tests do not provide information about the state of the

infection at the moment of the test but can help determine the proportion of a population previously infected and provide information about individuals that may be immune and potentially protected. It is highly recommended to use these tests in public health settings to estimate the immunity of the population, information needed to guide the response to the pandemic and to determine how the virus is spreading through the population over time. Lastly, repeated testing of infected people will help to determine the duration of immunity.

2.2.1. Human immune response proteins serology tests: Antibody response

Antibody tests are usually based on specific binding between antigen and antibody, similar to the detection scheme used for antigen detection. Protein S (S1 and S2 subunits) and especially the RBD, which is highly antigenic, is generally used to capture the antibody, though N protein has also been used occasionally. Serology tests look for the presence of antibodies to SARS-CoV-2 in the infected host organism, and the assays can detect immunoglobulins A, M and G (IgA, IgM and IgG) usually in blood, plasma or serum samples. These tests should not be used to diagnose an active coronavirus infection. IgM is the largest immunoglobulin and is the first that can be detected, usually around 5–10 days after the onset of symptoms. IgG is the most common antibody found in the body and will appear later, but it will be abundantly expressed. IgG may remain detectable for months or years. Seroconversion occurs within the first 3 weeks, usually 9–11 days after the onset of symptoms [15]. IgA is detected in mucous secretions within 6–8 days, but little is known about IgA response in the blood [27].

Although several days are required to develop immune response, serology tests are especially important for detecting previous infections in people who had few or no symptoms to control the spread of the virus [8,155–157]. However, the expression of IgM and IgG antibodies is higher in patients with severe symptoms [16,17,21]. In addition, antibody sampling is easier because antibodies are more stable than RNAs. In negative RT-PCR assays but with strong epidemiological evidence of infection, paired serum samples obtained during the acute (first week) and the convalescent (2 to 4 weeks) stages could help in the diagnosis of the disease [27,158].

Over the months of pandemic, more than 190 serology tests have been approved by the regulatory agencies for the detection binding antibodies [21]. Tests that detect binding antibodies fall into two broad categories: laboratory tests and POC tests. The former use enzyme linked immunosorbent assays (ELISA) [159] or chemiluminescence immunoassays (CLIA) [160] methods for detection, which are reliable but for some assays may require trained technicians and specialized instruments and require hours or even days [161]. POC tests are lateral flow immunochromatographic assays (LFIA), which are faster (10–30 min) and easier to use. Some of these tests using whole blood can be performed on blood samples obtained by fingerstick, but they offer only qualitative information and in some cases their accuracy is limited

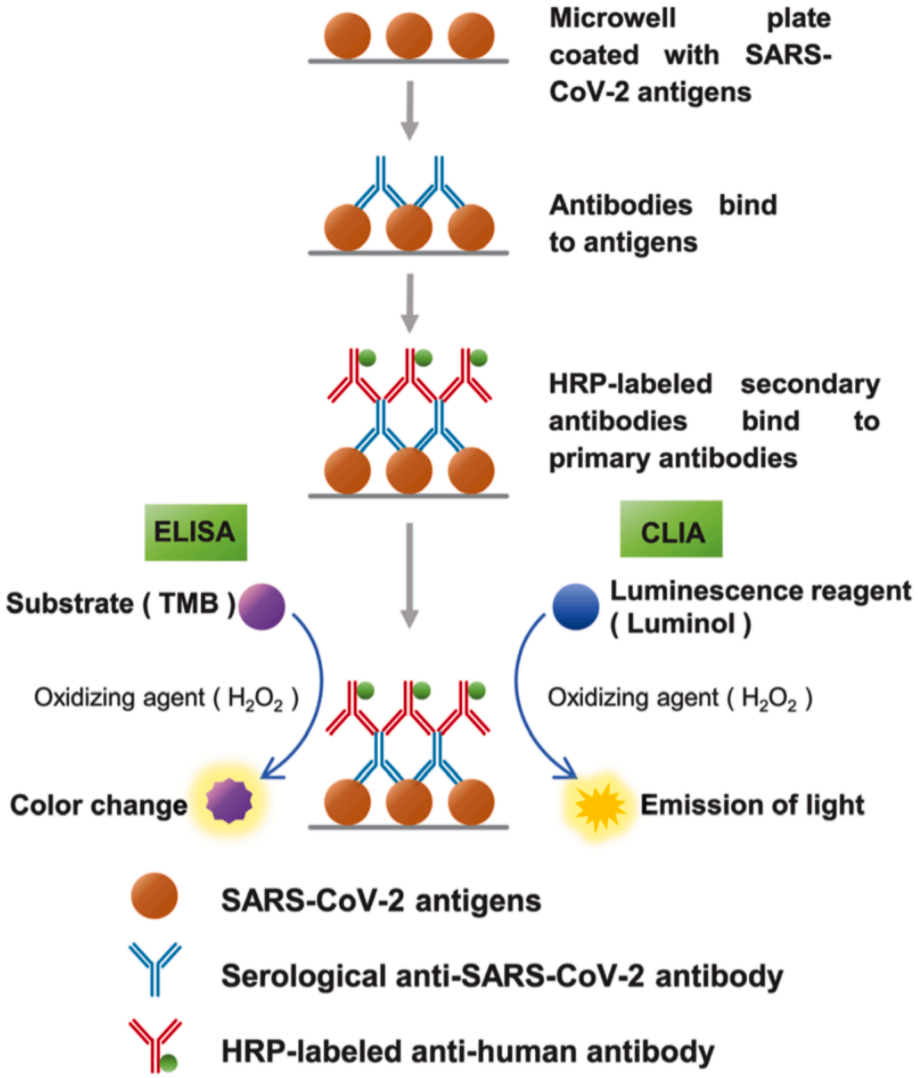


Fig. 4. Schematic diagram of ELISA and CLIA for COVID-19 IgM/IgG antibodies (taken from [21] with permission).

[40,157,161].

Serology tests usually detect antibodies against the recombinant nucleocapsid protein (rN) and spike protein (rS) antigens [162]. The ELISA test is performed by immobilizing the recombinant protein to the bottom of a multiwell plate, and then the diluted serum is added to capture its specific antibody. Antibody binding detection usually involves the addition of a secondary antibody conjugated to an enzyme and subsequent incubation. After a wash step and addition of an enzyme-catalyzed substrate, a color change indicates the presence of the antibody (Fig. 4). The advantages of ELISA are its easy operation and low cost, and a high throughput which can be obtained with an automated workstation [21].

Generally, authors have observed that both IgM and IgG can be detected after 5 days of illness onset using ELISA in $>$ 80% of positive patients, being almost 100% during days 10 to 18 [18,25,159,163].

CLIAs are also used for quantitative antibody detection and have high sensitivity and specificity. CLIA is similar to ELISA, though the antibody bound to the substrate is detected by luminescence (Fig. 4). More specifically, CLIAs usually apply an enzyme-catalyzed substrate, such as luminol, whereas ELISAs usually employ 3,3′,5,5′-tetramethylbenzidine. In addition, CLIA shows higher sensitivity than ELISA and the laboratory process is fully automated so that hundreds of samples can be screened per day [164]. Overall, shortest times for IgM and IgG detection after symptom onset are 1.5 and 2 days, reaching 100% detection rates between 8 and 14 days after symptom onset [165,166].

LFIA is considered a POC diagnostic tool because it can be used in the field due to its portability, affordability and rapid diagnostic capacity [21,167]. The antigens are immobilized onto the middle of a strip, usually a nitrocellulose membrane. The liquid samples are applied to the strip and if SARS-CoV-2 antibodies are present they react with labelled antibodies in the strip (usually labelled with colloidal gold [CG] or quantum dot [QD] nanoparticles) and bind to the immobilized antigens. The immobilized antibody-detection antibody complex will result in the appearance of a colored band on the strip due to the accumulation of CG or QD particles (Fig. 5) [21,45]. When LFIA uses CG nanoparticles, which is also known as colloidal gold immunochromatographic assay (CGIA), IgM and IgG can be simultaneously detected in blood within 15 min [8]. In comparison to other antibody tests, LFIA is more attractive for large seroprevalence studies but shows lower sensitivity [168,169]. Kontou et al. [167] compared IgM and IgG tests based on ELISA, CLIA and LFIA using a *meta*-analysis of diagnostic pooling sensitivities and specificities. These results showed that the serum testing using ELISA and CLIA were more reliable, while the LFIA results should be considered with caution. However, this last showed the advantage that can be performed at home without the need of any expert. In the same study, Kontou et al. [167] also demonstrated that those tests using the S antigen are more sensitive than N antigen-based tests, and that combined IgG/IgM test seems to be a better choice in terms of sensitivity than measuring either antibody alone.

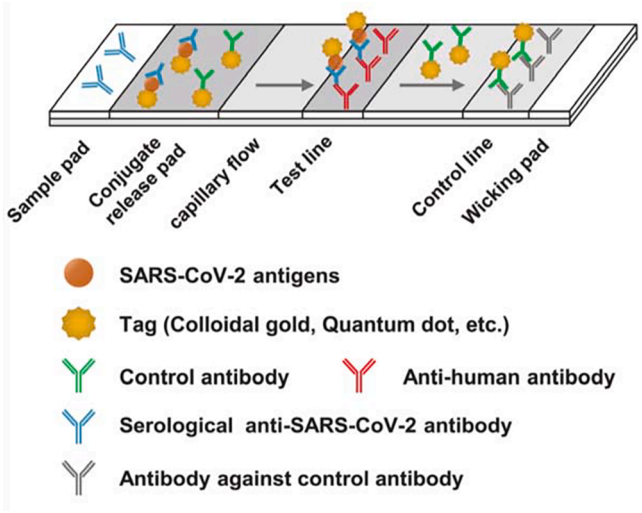


Fig. 5. Schematic diagram of the LFIA for COVID-19 IgM/IgG antibodies (taken from [21] with permission).

Overall, test accuracy varies for diagnostics from different manufacturers. It is commonly measured in terms of sensitivity (production of positive results) and specificity (production of negative results). Table 3 summarizes the differences (analyte, specimen, sensitivity, specificity, cost, time…) of the four detection methods from an analytical point of view, highlighting their advantages and disadvantages. For more details concerning antibody tests for COVI-19, the reference [170] should be consulted.

False negatives from the currently available antibody tests have also been reported usually related to a low concentration of antibodies present in the fluid samples; the presence of homologous proteins; and lack of sensitivity of the detection instrument. This strongly motivates researchers in the search of more sensitive tools, which will be approached in second part of the next section showing some significant examples drawn from the field of biosensors.

3. Some recent advances and emerging trends in analytical methods for COVID-19

The detection of minimal amounts of viral proteins constitutes one of the challenges of current analytical chemistry, requiring the application of ultrasensitive techniques since proteins, unlike viral nucleic acids, are not susceptible of being directly amplified. Both mass spectrometry (MS) and proteomic techniques [171–173] are called to play an important role in this direction, as well as in the characterization and quantitative measurement of viral proteins. MS detects in a robust way the N, S, and M, SARS-CoV-2 structural proteins.

The importance of nanotechnology in the field of direct or indirect COVID-19 detection has been highlighted repeatedly throughout this manuscript [3,8,11,21,24,25,54–67,96,116,157]. Nanotechnology's contribution to the analytical chemistry of COVID-19 has a promising future [174–178]. It also plays a useful role in improving personal protective equipment [179] since many nanoparticles exhibit powerful antiviral properties. Nanomaterials have nanoscale physical, chemical, biological, mechanical and electrical features [11,25,40], which cannot be found in the micro and macro scale domain. This is due to their high surface area, very small size, great stability and versatile chemistry of the modified and functionalized material's surface.

Pfizer-BioNTech and Moderna, the pioneers among COVID-19 vaccine developers, use nanoparticles [85,177] in its mRNA (messenger Ribonucleic Acid) vaccine delivery. Concerted globally effort has paved the route [180] into vaccine development, in a record time. FDA has authorized, for emergency use, the two mentioned vaccines as well as Johnson & Johnson's Janssen single dose viral vector COVID-19 vaccine. In addition to these three, the European Medicines Agency (EMA) has also approved the use of AstraZeneca viral vector vaccine. It is hoped that the efficacy of vaccines will provide sufficient control [181] over the disease and reverse social life to normality soon or, at least, little by little.

There are some opportunities for improvement in the development of new RT-PCR platforms [182–185], covering better tolerance of matrix effects and compatibility with simpler or minimal sample, e.g. dPCR or ddPCR (droplet digital PCR). Primers, probes and reagents are the same, but the bulk reaction solution is partitioned into a myriad of nanoliterized microdroplets yielding more precise measurements in comparison to traditional PCR. The employment of microfluidic devices known as Lab-on-a-Chip (LOC) combined with POC, may lead to instruments [64,157,186] providing better healthcare solutions for the general population, in terms of cost-benefits, without having to resort to train professionals and complex tools to interpret the results. The

development of systems with these characteristics that allow the detection of SARS-CoV-2 in biological fluids at concentrations of the order of femtomolar (fM), picomolar (pM) or nanomolar (nM) would undoubtedly constitute a great achievement. Saliva (properly treated if necessary) is the most widely used fluid for diagnosis carried out with most microfluidic studies.

The importance of emerging techniques in detecting COVID-19 has been highlighted in a number of recent reviews [38,48,50,52,53,64,187–191]. These emerging techniques are of vital importance for COVID-19 detection, monitorization, diagnose, screening and surveillance. A number of approaches used either alone or in combination with others may be the subject of mention: (i) single stranded nucleic acid oligonucleotide bioinspired receptor molecule aptamer-based detection; (ii) molecular imprinting technology-based detection; (iii) protein parallel high-throughput multifunctional microarray tools; (iv) agglutination tests; (v) highly sensitivity and specificity paper-based devices multiple DNA targets detection; (vi) simultaneous targets multiplexed LFIA recognition; (vii) new biomarkers approaches [22,61,192], e.g. breath analysis; (viii) decentralization setting, e.g. wearable sensors; (ix) robots based sample test and other robotic applications to detection, screening and diagnose. Some of these issues need to be the subject of further research and attention, but so far, they have a promising future in order to move from the laboratory scale to the real situation of commercialization. Next Generation Sequencing (NGS), such as amplicon based mutagenic NGS whole-viral genome profiling, allows the screening of a large number of samples.

On the other hand, the use of pseudovirus expressing the S protein of SARS-CoV-2 instead of the virus itself is also a great opportunity to simplify and accelerate the development of assays [38] for viral proteins. The integration of technologies to smartphone mobile devices [30,31,40,117,118,193] provides new opportunities in different areas. In the field of medical diagnostics, they represent a paradigm shift [194] that allows the provision of mobile health care and personalized medicine. Both AI and IoT/IoMT (Internet of Things/ Internet of Medical Things) [11,64,178,195–199] may be combined with, e.g. immunosensing, to prevent and manage COVID-19, giving fast and inexpensive increasing specific and sensitive approaches to research the proper networking through the management of database, sharing, and analytics. Novel systems to remotely detect COVID-19 based on AI and IoT/IoMT and the combination of several smart medical sensors (pulse, thermal and blood sensors) are under scrutiny and study. These "on line" diagnostic platforms systems collect invaluable data from patients in a remote way [184,199,200], uploading them to the Cloud. Thus, the physicians have access to them in real time. We are walking in the right direction towards the revolutionary paradigm of precision medicine.

Currently, there is an urgent need to perform extensive diagnostic tests (easier, cheaper and more sensitive and accessible) in order to avoid the spread of the virus and decreasing the occurrence of unreported cases (i.e., asymptomatic cases). In this regard, as can be seen in Table S1 (Supplementary material), a great effort has been made recently developing potential biosensors for COVID-19 that increase the sensitivity of the methods devised. A biosensor device includes a biomolecular recognition element to confer selectivity and a signal transduction element [8,24,57,192,201–205] to allow quantitative or semi-quantitative analysis. The interaction between the analyte molecules and the biorecognition element (eg a layer of bioreceptor molecules) in a generic receptor causes the transduction of a measurable physical–chemical change, such as current flow, heat transfer, mass change or refractive index, or more sophisticated measurable properties. The captured signals are later amplified and processed in a suitable way to proceed with the data analysis. Among the most common biosensors are the following: electrochemical biosensor, field-effect transistor (FET)-based biosensor, localized surface plasmon resonance (LSPR)-based biosensors, surface enhanced and Raman scattering-based biosensor.

Electrochemical biosensors have been profusely used in the detection of viral RNA, proteins, small molecular antibodies, and whole virus particles. The target can be recognized using an antigen–antibody reaction, hybridization of RNA, DNA, or peptide nucleic acids or aptamers that bind to the target with high affinity and specificity. The biological interaction of these processes is translated into electrical signals, measuring then electrochemical properties such as capacitance, charge accumulation, conductance, current, impedance or potential changes [26,201,206,207]. Mavrikou et al. [207] set up a membrane-engineered kidney cell, which modifies with the SARS-CoV-2 SpikeS1 antibody, with the aim of detecting the SARS-CoV-2 S1 antigen. Changes in the membrane potential are produced when interacting the antibody with the target protein. The set is manufactured on eight gold screen-printed electrode, being covered with a layer of polydimethylsiloxane (PDMS) with eight wells. The sensor achieves an excellent sensitivity. For the detection of SARS-2 in saliva, Fabian et al. [208] focuses on both S and N protein, preparing magnetic beads in combination with carbon black-based screen-printed electrodes. The technique includes: 1) precoating of magnetic particles, 2) solid support magnetic particle immunoassay with alkaline phosphatase (ALP) -labeled reporter antibody, and 3) electrochemical finish; 1-naphthol, resulting from enzymatic cleavage of 1-naphthyl phosphate by ALP, is analyzed by differential pulse voltammetry technique, using carbon black-modified screen-printed electrodes. Among the main advantages of the approaches are the short analysis time (<30 min), straightforward sampling (untreated saliva) and handling of use (printed sensor and portable potentiostat). Furthermore, in comparison to RT-PCR, this kind of biosensors can present a competitive cost per analysis. Sampling and sample handling are critical points for POCT devices; manual operations, even minimal, pose serious inconveniences for their acceptance.

In another attempt, Seo et al. [209] use an anti-spike antibody binding to SARS-CoV-2 virus particles to make up graphene-based FET biosensors. The FET is covered with a saline buffer to keep an efficient gating effect, which uses the electrical signal transduction process. The aqueous solution-gated FET system detects SARS-CoV-2 on the basis of channel surface potential changes and the corresponding effects produced on the electrical response. These FET biosensors can respond to 16 pfu/mL (plaque-forming unit/mL) of virus particles in phosphate-buffered saline within 10 min. Furthermore, the biosensor clearly discriminates between patient and healthy people samples.

AuNPs attached to the antibodies, biomolecules, proteins, and aptamers can also increase the LSPR and SERS (surface enhanced Raman spectroscopy) signals, and the energy transfer between the fluorophore and AuNPs. A number of nanomaterial-based biosensors allows, actually, the detection of SARS-CoV-2 and related viruses [3,24,25]. For example, Qiu et al. [210] applied a dual-functional plasmonic biosensor utilizing plasmonic photothermal (PPT) effect and LSPR sensing transduction for the detection of various viral sequences genes from SARS-CoV-2. The aforementioned device is able to distinguish between SARS-CoV and SARS-CoV-2 viruses. The sensor shows a LOD of 0.22 pM.

Most recently Liu et al. [211] develope a surface-enhanced Raman scattering-based lateral flow immunoassay (SERS- LFIA) to detect IgM and IgG simultaneously. To fabricate SERS, tags are modified with dual-layers of Raman dye, Silica core is completely coated with silver shell (SiO_2@Ag), which shows superb SERS signal, decent monodispersity, and excellent stability. The anti-human antibodies immobilized capture SiO_2@Ag- spike S protein anti-SARS-CoV-2 IgM/IgG complexes. The proposed technique presents a good performance under clinical environment and it is cost-effective.

Colorimetric biosensors have also found an use to detect SARS-CoV-2. These biosensors show interesting properties such as rapid response, accuracy and cost-effectiveness. Moitra et al. [212] use thiol-modified antisense oligonucleotides (ASOs) capped AuNPs (Au-ASOs) for the specific detection of N- gene from nasal swab. The test allows detecting SARS-CoV-2 infection within 10 min. The detection mechanism involves the agglomeration of Au-ASOs architecture with target SARS-CoV-2 RNA, leading to red-shift. The cleavage of RNA strands from RNA-DNA hybrid is done by the addition of RNaseH (Ribonuclease H), a

precipitate is finally obtained in the solution due to the additional agglomeration of AuNPs, being visible to naked-eye. The biosensor shows a LOD of 0.18 ng/mL for SARS-CoV-2 RNA and no cross-reactivity being apparent with MERS-CoV viral RNA.

Altogether, it is evident that up to now neither methods are ideal approaches for COVID-19 diagnose, but they should complement each other [205] in a reasonable way. Overall, the main advantages of biosensors are the cost-benefits relationships, reduced sample size and good reproducibility, as well as a rapid detection and high sensitivity.

4. Concluding remarks

The gold standard method to detect SARS-CoV-2 is the RT-PCR test which is the method with higher sensitivity and specificity and consists of detecting viral nucleic acids in respiratory specimen. However, its limitation to be applied at POC has motivated the development of other tests based on nucleic acid amplification. To improve the sensitivity of these tests, multiples target primers (E, N and RdRp genes) specifically created for isothermal amplification should be simultaneously identified in a single test. These multiplex targeted assays avoid the potential risk of susceptibility due to unknown genetic variability, monitoring genomic variation. It is necessary to update databanks with information about the genetic diversity of the SARS-CoV-2, given the changing nature of the virus and the emergence of new strains. This information should be worldwide open access. CRISP-based nucleic acid detection shows a great potential in developing next generation molecular (NGM) diagnostic approaches as specific, sensitive, and reliable techniques which may be established and applied to the COVID-19 diagnosis. The future for the screening detection of COVID-19 stands for nucleic acid electrical detection on the basis of lab-on-chip CRISPR-Cas based platforms given the fM levels achieved in its detection.

The SARS-CoV-2 can also be detected by the antigen tests which have been authorized to determine if the patient is infected at the time of analysis. Although these tests are less sensitive than RT-PCR, they are quicker and can be carried out without the need of specific instruments. The last advance in this kind of tests has improved the sensitivity of the results if the test is carried out in a specific period of the infection (5 days after symptoms onset).

COVID-19 can also be confirmed through CT scan of suspicious patients, being its sensitivity in early diagnosis one of the main advantages. However, CT images are limited to larger central hospitals. Hence, the application of radiological imaging techniques coupled to the advanced AI is emerging as a helpful tool for the automatic diagnosis of COVID-19 through the use of imaging pattern changes that are not easily amenable to human identification.

The need for fast, transportable, low-cost, easy-to-use, decentralized, and high-frequency POC platforms for early identification and diagnosis has increased as the pandemic has progressed. In the same way, the impact of asymptomatic transmission has been evident. Patients can thus easily self-isolate or quarantine themselves. Antibody tests are suitable tools for carrying out maximum screening of the population and knowing the immune capacity developed after a period of exposure to the virus. They are also crucial for the identification of asymptomatic infections even though they are unable to provide reliable results on the status of the infection at the time of testing. Tests that detect binding antibodies fall into two broad categories, laboratory tests (ELISA or CLIA) and POC tests (LFIA). LFIA tests are easier to use and provide the results quickly (10–30 min), but they offer only qualitative information, and their accuracy is not always sufficient. False negative results are also reported as a consequence of the low concentration of antibodies and the lack of sensitivity. This situation has led to a strong motivation for researchers to develop potential biosensors, being used on this respect those based on, e.g. AuNPs, AuNIs (gold nanoislands) and graphene.

A number of emerging novel techniques, some of which have been mentioned in the previous section, has a promising future for the detection of SARS-CoV-2. IoT/ IoMT has been used to support rapid diagnosis of COVID 19 screening through POC including blockchain integration and 5G high bandwidth cellular technology. It can be said that it is a challenge the integration of nanotechnology with wearables and smartphones, making the diagnostic handy by developing smart biosensors. However, more studies for the comparison between newly developed methods in terms of sensitivity, reproducibility, reliability, and robustness are still needed worldwide. Accurate validation of the performance characteristics of the methods is of utmost importance when the health of mankind is at stake. Analytical chemists must join forces to also contribute -together with another multidisciplinary scientists' colleagues- to solving the problems derived from COVID-19 and future pandemics, helping to prevent numerous deaths and economic disasters and mitigate the resultant social consequences.

Research funding

This work was supported by the University of Seville through the III Plan Propio de Docencia.

Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to express their gratitude to Ms. Irene García Sáez and Dr. Purificación García Sáez for their help in the grammatical correction of the manuscript. They also thank Dra. María del Mar Orta for her helpful comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2021.106305.

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