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.

Talanta 225 (2021) 121898

Contents lists available at ScienceDirect

Talanta

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

Review

Commercialized diagnostic technologies to combat SARS-CoV2: Advantages and disadvantages

Briza Pérez-López [b], Mònica Mir [a,b,c,*]

[a] Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Monforte de Lemos 3-5, Pabellón 11, 28029, Madrid, Spain
[b] Nanobioengineering Group, Institute for Bioengineering of Catalonia (IBEC) Barcelona Institute of Science and Technology (BIST), 12 Baldiri Reixac 15-21, Barcelona, 08028, Spain
[c] Department of Electronics and Biomedical Engineering, Universitat de Barcelona, Martí I Franquès 1, 08028, Barcelona, Spain

ARTICLE INFO

Keywords:
Covid-19
SARS-CoV-2
In vitro diagnosis (IVD)
Reverse transcriptase polymerase chain reaction (RT-PCR)
Point of care (POC)
Lateral flow immunoassay

ABSTRACT

The current situation of the Covid-19 pandemic is indicated by a huge number of infections, high lethality, and rapid spread. These circumstances have stopped the activity of almost the entire world, affecting severely the global economy. A rapid diagnosis of the Covid-19 and a generalized testing protocol is essential to fight against the pandemic and to maintain health control in the population.

Principal biosensing and diagnostic technologies used to monitor the spread of the SARS-CoV-2 are based on specific genomic analysis and rapid immune tests, both with different technology platforms that include advantages and disadvantages. Most of the in vitro diagnosis companies are competing to be the first on validating under different regulations their technology for placing their platforms for Covid-19 detection as fast as possible in this big international market.

A comprehensive analysis of the commercialized technologies for the genomic based sensing and the antibody/antigen detection methods devoted to Covid-19 diagnosis is described in this review, which have been detailed and listed under different countries regulations. The effectiveness of the described technologies throughout the different stages of the disease and a critical comparison of the emerging technologies in the market to counterattack this pandemic have been discussed.

1. Introduction

The Covid-19 pandemic is an unprecedented global health crisis, impacting in 188 from the 200 countries in the planet with 14.7 million people infected and a total of 609,986 deaths in approximately 7 months, at June 21, 2020, [1]. Due to this pandemic, majority of the inhabitants of the planet have stopped their normal activity having to be confined at home, affecting severely the global economy.

Covid-19 is produced by the infection with the virus SARS-CoV-2. The symptoms of this disease ranging from mild symptoms; fever, chills, dry cough and difficulty breathing, to severe illness especially in the case of people with compromised immune systems, where the Covid-19 can cause severe respiratory problems, organ failure and even death [2].

2. The importance of an early diagnosis

In vitro diagnosis (IVD) and point of care (POC) technologies has played a crucial role in the Covid-19 pandemic for two important reasons. The first is because an early diagnosis of the infected people permits to cut the sooner the spread out of the diseases. In the case of a highly contagious virus, such as SARS-CoV-2, it expands fast and it is critical to find the infected people as soon as possible to isolate the focuses and the people in contact with those to quarantine them and decontaminate the affected area and thus reduce the widespread of the disease. The second reason is common in many other diseases, an early detection of the disease increases the possibilities of being cured and survive the disease. The numbers behind this pandemic corroborate this fact. The amount of infected people with SARS-CoV-2 versus the persons that have died due to this disease has some correlations with the amount of early detection test performed. Moreover, biosensors will be also a powerful tool when the curve of infection decrease to alert about new

* Corresponding author. Institute for Bioengineering of Catalonia (IBEC), Baldiri Reixac 15-21, Barcelona, 08028, Spain.
E-mail address: mmir@ibecbarcelona.eu (M. Mir).

https://doi.org/10.1016/j.talanta.2020.121898
Received 31 July 2020; Received in revised form 9 November 2020; Accepted 12 November 2020
Available online 3 December 2020

outbreaks of the epidemic for an early an effective clinical response.

For these reasons the World Health Organization (WHO) general director Tedros Adhanom Ghebreyesus, spoke about the impossibility of combating the virus if it is not detected "You cannot fight a fire blindfolded" and he recommended the wide use of Covid-19 diagnosis test; "We have a simple message for all countries: test, test, test." [3].

In order to study the veracity of the hypothesis that correlates the higher use of diagnosis tests with a lower impact by the pandemic, statistics in this regard are shown in Fig. 1. But it is important to highlight that while we are writing this paper the Covid-19 is evolving and the numbers related with the pandemic are changing over the days. Also, it is important to point out that there is not a direct comparison of these values between countries and just approximations can be done, since many other variables not considered in this study will influence on the number of infected people and the mortality such as; the ageing of the population, at which point of the disease trajectory were collected the values, the method of confinement applied in each country, the celerity on starting the confinement and the border closure, the transit with other countries, the population density, the incidence percentage of other pathologies in the population, the investment in National Health Service, the number of hospital beds per population, at which point of the disease incidence were started the used of the test, the reliability of the test used and the credibility of the reported values.

To reduce these variables only countries from the same continent with similar population density and evolution of the pandemic was used in the comparison. Considering this, in Fig. 1 is plotted the correlation between the fatality rate with the number of tests per population performed in European countries when the pandemic was approximately at the half of the first curve evolution, at April 21, 2020, [1]. A trend with fewer diagnosis tests (>22,500 test/population) can be observed in the countries with higher Fatality Rate (<10%), except in Italy where the pandemic begins first, validating the assumption of the reduction on the pandemic incidence with the increase in test of diagnosis.

The tests are not only a sensitive and selective analytical tool for diagnosis but an essential component in fighting the pandemic, where extensive testing is required in people with even mild symptoms to more quickly stop the spread of the pandemic. Consequently, all the affected countries hasten to buy these valuable tools for combating the virus.

3. The analyte of interest

SARS-CoV-2 is the virus that generates Covid-19 disease, and despite its high capacity of transmission and lethality rate, it has a small size of 60–140 nm diameter and a very simple structure [4]. SARS-CoV-2 is made up of a glycoprotein layer membrane that encapsulates a fragment of RNA. The virus genome just contains ~30,000 nucleotides to encode about 27 proteins in a single-stranded RNA. The lipid membrane contains 4 different type of proteins; spike surface glycoprotein (S), envelope protein (E), matrix protein (M), and nucleocapsid protein (N) that help the virus to bind to target cells through the host cell receptor and the membrane fusion [5]. The N-protein is the most abundant in the virus and it is the protein that our immune system usually detects. The N-protein rarely changes along the diseases and it has an easy access due to its external virus membrane presence, for these reasons is often used as a marker in diagnostic assays [6]. Considering the virus structure, its detection can be performed by two strategies: detecting the external specific proteins of the virus and analysing its specific genomic information. The viral dose detected by RNA is very high (10^4-10^8 copies/mL) in the pre-symptomatic and at the starting of symptoms. After 10 days of infection the viral dose is reduced more than 100 times [7]. Moreover, the serologic response of the patients against the virus can be analysed considering the presence of immunoglobulin (Ig) antibodies due to the immune response of the body confronting Covid-SAR-2. The first antibodies generated by the body are the IgM that takes about 4–7 days during the onset of the infection [8,9]. IgG antibodies takes longer to appear, about 10–14 day when patients start convalescence, but it brings a very relevant information. This type of antibody is generated by the body for preventing future infections with the same virus, and it can stay in the blood for year, showing the passage through the disease. So, the IgG detection offers an extra information comparing with the direct detection of the virus (RNA test and antigen test) and the IgM serologic detection, which is the fingerprint of the virus.

Thus, depending on the infection phase of the patient the analyte choose for the analysis may be at low dose or inexistent and it can be an important issue to consider for an appropriate diagnosis. Fig. 2 shows a scheme with the different phases of the disease and an approximation of the dose and period of each analyte present in Covid-19. In the bottom of the plot is tabulated the positive or negative presence of the analyte in each infection phase.

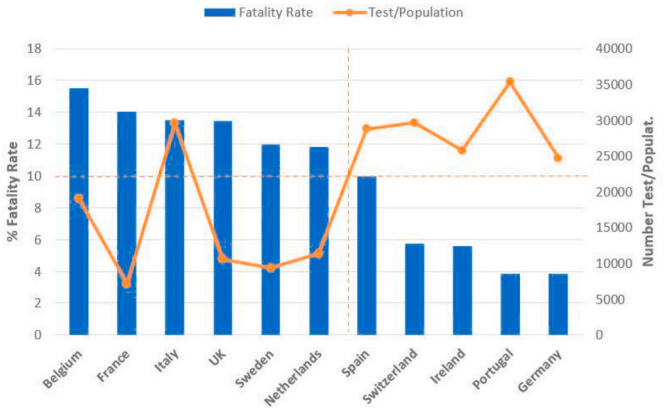

Fig. 1. Plot of the European countries with higher cases of infections around the beginning of pandemic, considering their fatality rate and the number of tests per population utilized (Valued from the April 26, 2020).

4. Genosensors for Covid-19 diagnosis

Most of the detection kits available for the diagnosis of Covid-19 are based on genomic analysis by means of reverse transcriptase polymerase chain reaction (RT-PCR) assays, which is the usual gold standard for virus testing. This technology detects the specific DNA mutation

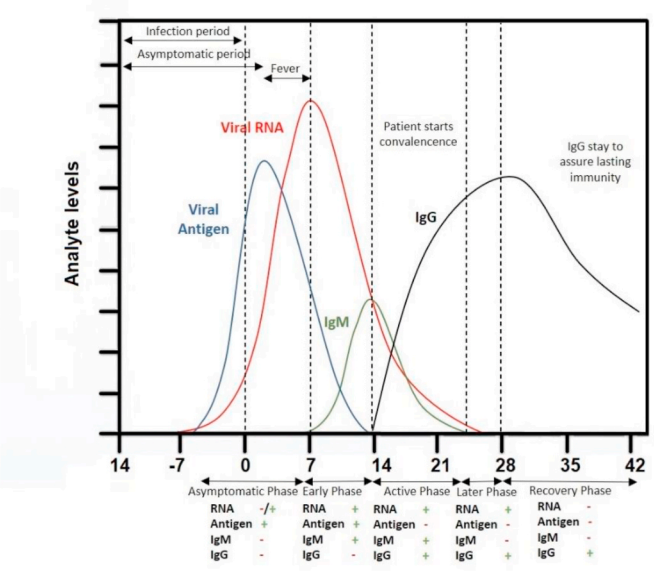

Fig. 2. Graph on temporal dynamics on the Infection disease [8,10] versus analyte dose [7,11,12].

sequences correlated with SARS-Cov-2. This technic relying on the transcription of the RNA extracted from the virus with reverse transcriptase enzyme to complementary DNA (cDNA) and then cDNA is exponentially amplificated with PCR. PCR is a common molecular biology tool invented in 1985 by Kary Mullis that permits the amplification of millions of DNA copies of a specific fragment of DNA. So, the presence of the oligonucleotide sequence of interest in the sample triggers its amplification, revealing the existence of the sequence in the sample.

Considering the global impact of the pandemic, in terms of COVID-19 diagnosis the increasing cumulative incidence of different coronavirus genotypes creates a great challenge for public health. Preferred targets or regions of interest (ROIs) in PCR detection include for SARSCoV-2; ORF1a/b, ORF1b-nsp14, non-structural RNA dependent RNA polymerase (RdRp), S, E, or N gene. The validity of a test is measured by its analytical and clinical sensitivity and specificity [13]. Clinical sensitivity measures how accurately a test identifies positive patients who are infected. A test with 95% sensitivity will identify 95% of patients who have the disease and produces false negatives in 5% of patients. A lower sensitivity test means higher false negative results.

At the starting of the pandemic some of the commercialized kits mismatching with SARS-CoV-2, bringing false negative. But when more countries and companies become involved, highly specific RT-PCR kits to diagnose Covid-19 where commercialized. Most of these kits where based on the SARS-CoV-2 sequences reported from the science community in the public database GISAID [14]. After the sequences publication in January, just one week was required to achieve the first validated RT-PCR kit for Covid-19 by Prof Christian Drosten's from the Charité Institute of Virology in Germany [15]. This protocol for RT-PCR with others were published by the WHO and it was used in many laboratories in countries around the world [16].

Depending on target selection can affect assay performance in specificity and sensitivity as well as cross reactivity due to conserved regions from another virus. For this reason, many approved molecular detection kits use multiplex RT-PCR to detect more than two target regions to enhance the detection selectivity and sensitivity of the kit [17]. Recent clinical evaluations reported by Nalla et al. [18] have demonstrated that the N1, N2, and E gene detection assays have better detection performance than the RdRP and N3 detection assays. More recently, Chan et al. [19] designed novel primers and probes of RdRp/Helicase (Hel) and S and N genes. These assays showed a higher detection sensitivity than the previously developed RdRp-P2 gene assay, being the assay that exhibited higher detection sensitivity than other gene detection assays. Comparative analysis of the detection of 273 specimens of 15 COVID-19 patients demonstrated a 43.6% positive rate for the RdRp/Hel detection assay, which was significantly higher than the RdRp-P2 gene assay (28.2%). Most of the RT-PCR kits target two to three virus mutations, mainly Orf1, RdRp, E-gene and N-gene, to increase the feasibility of the kit in case the virus mutates. A different strategy was followed by Fulgent Genetics, which uses their Next Generation Sequencing test to characterizes the entire viral genome. However, this technology is mainly focused on research to understand the virus' properties for drugs development [20].

It is also very critical to understand how the predictive value of the test varies with time from exposure and symptom onset to avoid false negative test results. The false-negative rate for SARS-CoV-2 RT-PCR testing is highly variable: highest within the first 5 days after exposure (up to 67%), and lowest on day 8 after exposure (21%). Clinicians should consider waiting 1–3 days after symptom onset to minimize the probability of a false-negative result. Thus, in response to the rapidly increasing number of confirmed and suspected cases of COVID-19 in many countries, is vital testing different clinical specimens for SARS-CoV-2 and optimize the performance of RT-PCR assay in order to increase the rate at which we are able to test. For example, Wölfel et al. [21] have reported a study where all collected samples (swabs) over the whole clinical course in all patients taken between day 1 and day 5 tested positive. The average virus RNA load was 0,67 copies/μL until day 5, and the maximum load was 711 copies/μL. Swab samples taken after day 5 had an average viral load of 0,34 copies/μL and a detection rate of 39.93%. The last swab sample that tested positive was taken on day 28 after the onset of symptoms. Another interesting study about the evolution of the virus load along the temporary evolution of the infection was performed by Miller and co-authors [22]. They identified 209 PCR-positive SARS-CoV-2 patients with 624 total PCR tests results and calculated daily sensitivity from date of symptom onset. Clinical sensitivity of PCR decreased with days post symptom onset with >90% clinical sensitivity during the first 5 days after symptom onset, 70%–71% from days 9–11, and 30% at day 21.

The sample extraction is also very relevant for a successful diagnosis, since it can influence on the sensitivity of the assay. At the starting of the pandemic, the guidelines from China recommended only the use of throat swabs [23]. But once the pandemic expanded, more research results were coming up with new information in this regard. These studies demonstrated that depending on the stage of the diseases and the patient complications, the sample source is extremely relevant to achieve enough viral load to assure sensitive and reliable assays [24]. Yang et al. reported that sputum collection was the most effective method followed by nasal swabs. The extraction methods that reported false negative was throat swabs sample extraction, mainly in the cases of patients with more than 15 days after the onset of illness. Additionally differences on sensitivity were also reported between bronchoalveolar lavage fluid (93%), sputum (72%), nasal swabs (63%), fibrobronchoscope brush biopsy (46%), pharyngeal swabs (32%), feces (29%), and blood (1%) [25]. The US Centers for Disease Control and Prevention (CDC) recommended in February the use of both; nasal and throat swabs for diagnostic testing using RT-PCR [26]. Also, it is important to consider the material of the swab for extracting the sample, since calcium alginate and wool swab as well as the wooden sticks may interfere the PCR test [27]. After sample extraction, it is recommended to introduce the used swabs inside viral transport media.

When the pandemic jumped to the American continent, the US CDC developed their own protocol for real time RT-PCR considering other genes and protocol than the published in WHO. However, quality control issues were found in February 2020 and they need to reanalyse their protocol and produce new kits [28]. This problem delayed the massive use of test at the starting of the pandemic in US and makes that US Food and Drug Administration (FDA) changes their policy and permitted other laboratories than US CDC, to perform and validate Covid-19 diagnosis [29]. At the end of March around 20 organizations got the FDA approval for their RT-PCR kits.

In addition to the widely commercialized RT-PCR kits, reverse transcription loop-mediated isothermal amplification (RT-LAMP) is also entering the COVID market. This is a cheaper alternative with open access test designs [30]. This technique amplifies in a single step at constant temperature, which is not necessary for a thermal cycler and takes shorter times. Another advantage over classical PCR, is the directly analysis from swabs without RNA isolation [31].

However, inaccurate RT-PCR and RT-LAMP results were reported when the viral load in the sample is low, due to insufficient sample load in extraction and/or RNA degradation during the sample handling process. This limitation in the sensitivity of RT-PCR generates false negatives that delay the diagnosis and a better treatment time window and lead to the premature medical discharge of infected patients. For this reason, a highly sensitive RNA detection method such as digital PCR (dPCR) was developed for the detection of COVID-19. This second PCR generation is based on splitting the sample into multiple independent PCR microchamber using microdroplets or solid partitions to reduce the number of RNA molecules in each PCR amplification, for more reliable fluorescence tag detection to achieve highly sensitive measurement. Reverse transcription dPCR reported 10-fold lower limit of detection and better accuracy than RT-PCR in samples with low viral load, offering a COVID-19 diagnosis with less false positive and negatives [32]. Thus,

dPCR is an excellent tool to combat the spread of a pandemic with rapid detection of the infected patient at an early stage, but this complex new technology can lead to erroneous results in the hands of inexperienced users [33].

4.1. Kits for RNA analysis for Covid-19 diagnosis

Most of the Covid-19 genomic methods of analysis available in the market where based on SARS-CoV-2 specific mutations detection with real time RT-PCR. This technique is the most spread for PCR amplification detection and it is based on the addition of a fluorescence dye in the PCR reagents mixture, which intercalates in the DNA doble strand, lighting the formation of the amplified DNA that can be monitored kinetically in real time. The continuous monitoring of this fluorophore elucidates an ascendant curve when the sequence of interest is present in the PCR tube, due to its amplification. This technique permits the quantification of tiny amount of DNA in the sample. In the case of SARS-CoV-2 analysis a limit of detection with RT-PCR of RdRP, E genes and N gene was reported to be of 3.6, 3.9 and 8.3 copies per reaction respectively [34]. All the steps required for real time RT-PCR are shown in Fig. 3, where advantages and disadvantages of this technique are discussed.

The equipment required to run this technique is commonly use in genomic clinical analysis laboratories around the word and the fabrication of the kit just revert on the mixture of the required reagents in a tube, which make very simples and fast its fabrication and commercialization.

Different companies have been rushed to bring to the market their real time RT-PCR kits for SARS-CoV-2. In Table 1 the main companies commercialising this type of kits are listed, comparing the genes analysed, the sample collection procedure, the regulatory acceptance, the sensitivity, and the analysis time. As you can appreciate by the large number of companies, there is a strong competence in the market for this type of in vitro diagnostic PCR test. These kits can run with most of the real time PCR equipment's commercialized such as; Roche Light Cycler® 480, Qiagen Rotor-Gene® Q 5plex HRM, Applied Biosystems® 7500 Real-Time PCR system, Bio-RAD CFX96TM Real-Time PCR Detection system, among others.

The first companies commercialising real time RT-PCR kits were from the country where started the pandemic; China. BGI was the first Chinese company selling the Real-Time Fluorescent RT-PCR Kit for detection SARS-CoV-2, under the name of SARS-2019-nCoV kit. Although, Chinese companies started earlier the PCR kits validation race, BGI did not achieve the CE mark until de March 2, 2020, the FDA for US approval the March 27, 2020 and the Australia's Register of Therapeutic Goods (ARTG) on April 10, 2020 [35].

The firsts companies reaching the CE-marking for SARS-CoV-2 detection with real time RT-PCR, with little difference on time, was the French company Primerdesign the February 17, 2020 with the Genesig® Coronavirus (Covid-19) 1.0 Real-Time PCR Assay [36] and the US company Co-Diagnostics the 24th February with the Logix Smart Coronavirus Covid-19 test. The FDA mark for the Primerdesign needed to wait until the March 20, 2020 and it was not until April 3rd that the US company did [37]. Comparing with big companies in PCR tests such as Roche, they did not get the CE mark until the 13th of March, almost 3 month later than Primedesign and in case of pandemic the celerity is important.

Also in this type of kits is important the celerity in the assay, being Anitoa Systems, LLC, SD Biosensor and Sansure Biotech the ones that reported shorter time of analysis of about 30 min, when the usual time for a PCR is between 2 and 3 h [38].

But even lower COVID-19 RNA analysis times are achieved with RT-LAMP based kits. One-step isothermal amplification reduces analysis time and cost, being reported just 15 min for the COVID positive cases with the AQ-TOP™ COVID-19 Rapid series commercialized by Seasun Biomaterials, which was approved by FDA in October 2020 [39].

A study comparing basic analytical and clinical performance of selected RT-PCR kits from seven different manufacturers (Altona Diagnostics, Seegene, BGI, PrimerDesign, KH Medical, CerTest Biotec and R-Biopharm AG) was developed by van Kasteren et al. [40]. They used serial dilutions of viral RNA to establish PCR efficiency which was ≥96% for all assays and the estimated LOD95 varied within tests a 6-fold range. They also reported that using clinical samples observe variations in detection rate between kits (3.8–23 copies/mL). The positive identification rate for the various RT-PCR kits varied from 10 to 13 out of 13 samples, with performing best (13/13) R-Biopharm AG, followed by BGI, KH Medical, and Seegene (12/13), CerTest BioTec (11/13), and Altona Diagnostics and PrimerDesign (10/13).

However, for sample patients with low virus doses the sensitivity of amplification with RT-PCR and RT-LAMP is an issue, reporting false positives and negatives. To overcome this problem, some companies have developed and commercialized the highly sensitive dPCR technology for COVID-19 diagnosis. The first to reach the market was Gnomegen LLC in April 2020 with the COVID-19 RT-Digital PCR Detection Kit. This Kit is able to detect 8 copies of viral RNA per reaction (570 copies/ml) with 95% of the replicates positive with 100% specificity in a short time; 35 min [41]. Similar sensitivity was achieved by the kit commercialized in Juny 2020 by PreciGenome LLC; FastPlex Triplex SARS-CoV-2 detection kit [42]. But Bio-Rad Laboratories was able to reduce even more the analytic sensitivity for RNA COVID-19 detection, achieving 260 copies/mL with the SARS-CoV-2 ddPCR Kit for use on Bio-Rad QX200 or QXDx AutoDG Droplet Digital PCR Systems [43].

PCR is for decades a reliable and very widespread technique, present

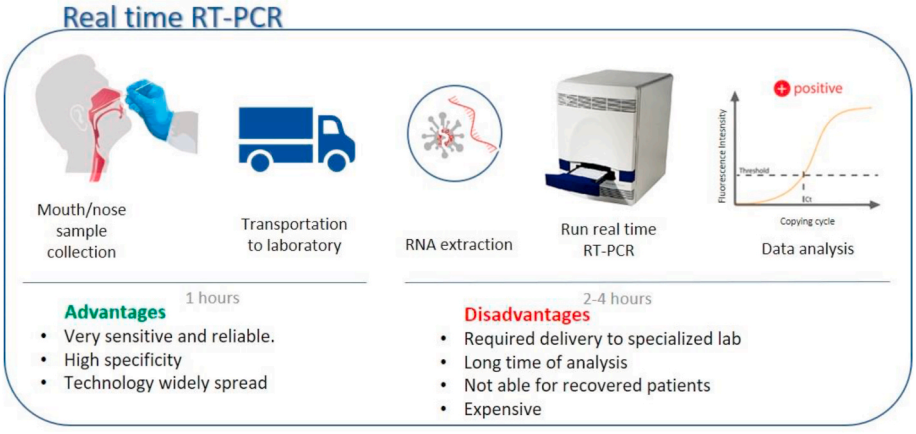

Fig. 3. Schematic representation of the steps required for Covid-19 diagnosis by means of real time RT-PCR. Picture of the equipment ABI 7500 Fast RT-PCR.

Table 1
Commercially available real time RT-PCR kits for a rapid coronavirus infection diagnosis in humans (LOD: Limit of Detection, NR = Not reported).

Company (Country)	Kit Name	Regulation (Validation Date)	Collection	Gene	LOD	Analysis Time (min.)
Pathomics Health (China)	Real-Time Fluorescent RTPCR Kit	EUA US FDA (Mar 27, 2020)	Throat swab	NR	91,2%	120
Primerdesign, of Novacyt (France)	Genesig Real-Time PCR COVID19	EUA US FDA (Mar 20, 2020)	NR	NR	96%	210
Co-Diagnostics (USA)	Logix Smart Coronavirus COVID19	EUA US FDA (Apr 3, 2020)	NR	NR	100%	120
1drop (Republic of Korea)	1copy™ COVID-19 qPCR multi Kit	CE Mark	Pharyngeal swab	RdRp, E E	4 copies/ reaction	130
3DMed (China)	ANDiS® SARS-CoV-2 RT-qPCR	EUA US FDA	Respiratory	ORF1ab, N, E	5copies/ reaction	<180
Biomaxima (Poland)	ARS-CoV-2 Real Time PCR LAB	CE Mark for IVD	Respiratory	ORF1ab, N	≥10 RNA copies	62
Co-Diagnostics (China)	Logix Smart™ 2019-nCoV qPCR test	EUA US FDA (Apr 3, 2020)	Respiratory	RdRp	9.35×10^3 copies/mL	63–90
ELITech Group (Republic of Korea)	GeneFinderTM COVID-19 Plus RealAmp Kit	EUA US FDA (April 18, 2020)	Respiratory	RdRp, E and N	10 copies/ reaction	150
Roche Diagnostics (Switzerland)	Roche cobas SARS-CoV-2 Test	HSA (March 19, 2020)	Pharyngeal swab	RdRp, E	NR	4800
PrimerDesign (United Kingdom)	Genesig 2019-nCoV	EUA US FDA FDA (Mar 20, 2020)	Nasopharyngeal swab	ORF1ab, E	<100 copies +	210
Sansure Biotech (China)	Sansure Biotec 2019-nCoV	EUA US FDA	Pharyngeal swab	ORF1ab, N	200 copies/ mL	<30
SD Biosensor (Republic of Korea)	Std M nCoV Real Time Kit	EUA US FDA (April 23, 2020)	Nasopharyngeal swabs throat swab	ORF1ab RdRp, E	1-10 copies	<30
LifeRiver Bio-Tech (United States)	Liferiver Real Time Multiplex RT-PCR	Commercially available	Bronchoalveolar lavage, Sputum	ORF1ab, N, E	1×10^3 copies/mL	NR
Gencurix (Korea)	GenePro COVID-19 Detection Test + v2	Received CE Mark for IVD	Sputum, Nasopharyngeal swab	ORF1ab, E	1 copy/μl	<90
AssayGenie (UK)	COVID-19 CE IVD qPCR assay	CE Mark	Nasal swabs, Nasopharyngeal swabs, sputum, bronchial washes	ORF1ab and N	200 copies/ mL	90
Beijing Applied Biological (China)	Multiple Real-Time PCR Kit	EUA US FDA	Nasopharyngeal swabs	ORF1ab, N, E	200 copies/ mL	NR
BioFire Defense (UK)	BIOFIRE® COVID-19 test	EUA US FDA	Nasopharyngeal swabs	ORF1ab and ORF8	3.310^2 copies/mL	45
Getein Biotech (China)	Novel Coronavirus Real Time PCR Kit	Received CE Mark for IVD	NasopharyngealOropharyngeals bronchoalveoral lavage	ORF1ab, RdRp	1000 copies/ mL	NR
Kogene (Korea)	PowerChek™ Real Time PCR	EUA Korean CDC (Feb 4, 2020)	Nasopharyngeal swabs and throat swab	RdRp, E	NR	NR
Altona Diagnostics (Germany)	RealStar® SARS-CoV-2 RT-PCR Kit 1.0	EUA US FDA (April 22, 2020)	NR	E, S	1-10 copies	NR
AB Analitica (Italy)	REALQUALITY RQ-2019-nCoV	CE Mark	Respiratory specimens	RdRP, E	NR	NR
BGI Genomics (Denmark & China)	Fluorescent RT-PCR Kit, Target: ORf1ab	EUA US FDA (Mar 27, 2020)	Respiratory swabs, sputum, bronchoalveolar lavage	ORF1ab	100 copies/ mL	180
PerkinElmer (UK)	PerkinElmer® New Coronavirus NA	EUA US FDA (Mar 24, 2020)	Oropharyngealnasoph, aryngeal swabs	ORF1ab, N	20 copies/ mL	105
BioMérieux (France)	SARS-COV-2 R-GENE® & BIOFIRE® FILMARRAY®	EUA US FDA (Mar 24, 2020)	Respiratory specimen	ORF1ab, ORF8	100%	~45
CTK Biotech (US)	Aridia COVID-19 Real-Time PCR test	Australia's ARTG (April 24, 2020)	Nasal, Sputum	ORF1ab, N	500 copies/ mL	NR
Certest Biotec SL (Spain)	VIASURE® Real-Time PCR Detection Kits	Australia's ARTG (March 21, 2020)	Respiratory specimens	ORF1ab, N	>10 copies	120
Genomica (Spain)	qCOVID-19	CE Mark (Mar 6, 2020)	NR	NR	100%	<120
Liming Bio (China)	SrongStep®Novel Coronavirus Multiplex rtPCR	CE Mark	Respiratory specimens	ORF1ab, E, N	NR	NR
KH Medical (Korea)	RADI COVID-19/COVID-19 Triple Detection (CE-IVD)	CE Mark	Respiratory specimens	S, RdRP	0.66 copies/ μl	NR
Seegene (US)	Allplex™ 2019-nCoV Assay	EUA US FDA (April 21, 2020)	Sputum, Nasopharyngea, Bronchoalveolar lavage, Throat	RdRP, N, E	NR	NR
Advanced Molecular Diagnostics (UK)	Zena Max-SARS-CoV-2 RT-PCR detection	CE Mark	Respiratory specimens	NR	1copie/25 μl	NR
Qiagen (Germany)	QIAstat-Dx Respiratory SARS -CoV-2	EUA US FDA (Mar 30, 2020)	Retrospective nasopharyngeal swabs	RdRP, E	500 copies/ mL	NR
NeuMoDx (US)	NeuMoDx SARS-CoV-2 Assay	EUA US FDA (Mar 30, 2020)	Nasopharyngealoropharyngeal and nasal swab	Nsp2, N	NR	80
Luminex Corp. (US)	NxTAG CoV Extended Panel	EUA US FDA	Respiratory	ORF1ab, N, E	NR	~240
Thermo Fisher Scientific (US)	TaqPath™ COVID-19 Combo Kit	EUA US FDA (Mar 13, 2020)	Nasopharyngeal swab, bronchoalveolar	ORF1ab, N, S	95%	40
Genomica (Spain)	CLART® COVID-19	CE Mark (Mar 6, 2020)	NR	NR	>96%	<300

in most of the clinical and research laboratories all around, demonstrating excellent selectivity and sensitivity. However, this technique requires a laboratory for being performed, since expensive equipment's, qualified clinical laboratory personnel and a clean and controlled environment is needed to avoid contaminations. So, this technique cannot be run in the point of use and requires a time consuming and less cost-effective.

Transportation to clinical laboratories, or in the case of this pandemic to certain research Institutes certified for supporting hospital, such as the Orfeu project [44]. Moreover, the RT-PCR technic as itself requires around 3 h and considering the shipping to the lab, the analysis result is not obtained before 24 h. The samples delivery must be collected, transported, and stored using appropriate procedures and conditions to assure reliable result, which increase the analysis time and cost.

4.2. ELONA kits for Covid-19 diagnosis

Enzyme-linked oligonucleotide assay (ELONA) is another technology for analysing and quantifying the resulted DNA amplified by PCR. This technology is based on the traditional Enzyme-linked immunosorbent assay (ELISA) format. In the case of ELONA, oligonucleotide-based receptors containing the complementary sequence of interest for SARS-CoV-2 detection are covalently immobilized on a substrate, usually on polycarbonate well plates. Then, the DNA-modified substrate is left to react with the product of the PCR. If the Covid-19 sequences are present in the sample those reacts with the immobilized probe and the hybridization is elucidates by the enzyme label attached to the hybridized sequence. The signal upon the hybridization of the target is detected by spectrophotometry with and ELISA reader.

The company Genomica SAU in Spain received the CE mark the 6th of March for the commercialization of the CLART Covid-19 based on ELONA technology, reporting 96% of sensitivity and 98% of selectivity [45].

However, as happen in the real time PCR, ELONA technique also requires specialized equipment (PCR and ELISA reader) and skilled personnel to carry out the test. Therefore, it is necessary shipping the samples to a clinal laboratory. Moreover, comparing with real time PCR, ELONA requires extra steps becoming more time consuming (about 5h per test), but this platform can test 96 sample at a time and uses equipment's cheaper that the real time PCR.

4.3. DNA based POCs fully automatized for Covid-19 diagnosis

The main inconvenient of the previous described techniques, real time PCR, LAMP, dPCR and ELONA is the necessity of a full laboratory to carry out the analysis, being required the delivery and the processing of the samples out of the places where it is required the results of the analysis, enhancing the cost and time for the test. Variables very relevant in a pandemic, where the time cost lives, and the elevated number of tests required for a country have a very negative impact in their economy.

For these outbreak situations, it is crucial to have a medical diagnostic equipment that could analyse the sample in the point of use, in this case near the patient. This equipment should be use friendly and should not need complex steps. Just introducing the sample into the equipment, it should proceed all the necessary steps and just pressing a button the result should come out, with reliable results, obtained at short time and in a cost-effective way. It may sound chimera, but fortunately all these needs are already answer with the point of care (POC) diagnosis devices that encompass all these important advantages. This technology applied on DNA analysis integrates sample treatment (just in some cases), the amplification of DNA and the detection of the sequence of interest by a microarray of genosensors. All these steps are integrated in a single cartridge by means of microfluidic channels. All the required steps are fully automatized and those operates with a portable electronic equipment that incorporated the data analysis with an easy to use software (Samiksha, 2017). The steps required to run out a Covid-19 analysis with a DNA based-POC are shown in Fig. 4.

These automatized sequencing platforms were typically used for diagnosis of many disease such as cancer screening, but several companies have adapted their technology for SARS-CoV-2 analysis. However, comparing the amount of companies commercialising PCR kits there are very little examples of POCs based on genomic analysis that were launched on the market.

The VitaPCR COV Covid ID-19 assay (CE marked) is a microfluidic automatized real time RT-PCR POC that has emerged from the collaboration of Credo Diagnostics Biomedical Pte. Ltd. from Singapore and the Italian company A. Menarini Diagnostics. This technology can reach the analysis response in 20 min but measuring just one sample at a time [46].

Abbott's ID NOWTM platform has achieved the validation for its commercialization in US and Australia. This equipment coming through collaboration between Mesa Biotech and Abbott Diagnostics. It is amazing the fast response reported by this equipment; 13 min in total and just 5 min for the positive results. This fast DNA amplification time is reached with isothermal amplification methods, where is not required the temperature ramps for heating and cooling, needed in the traditional PCR for DNA melting, primers annealing, and enzymatic polymerization of the DNA [47].

The Sherlock CRISPR SARS-CoV-2 commercialized by Sherlock BioSciences, Inc uses the SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) technology, which is based on combination of LAMP amplification and clustered regularly interspaced short palindromic repeats (CRISPR) mediated detection, able to analyse SARS-CoV-2 in 1 h with a limit of detection of 100 copies of viral genome input [48].

Cepheid has commercialized in US a mechanized molecular test for the qualitative detection of SARS-CoV-2 by real time RT-PCR, as their other competitors. But this equipment takes longer than the above described technologies to give a response; 45 min, which is an excellent time comparing previous described methods but slow compared with the DNA based POCs. Another limitation of Xpert Xpress is the single sample at a time that runs this equipment [49,50].

The ePlex SARS-CoV-2 was the first DNA based POC to obtain FDA grants emergency use authorization the March 19, 2020. The ePLEX technology is the most original equipment, considering the POCs described above. Meanwhile the other platforms were based on the same transduction than the real time PCRs using fluorescence labelling. The ePLEX system is based on a multiplex array with electrochemical readout. This type of electrochemical technology is usually cheaper than its optical homologous. But the main disadvantage of this unique technology is the long-time of analysis, about 2h, that is comparable with the traditional real time PCRs [51].

The RNA POC analyser with higher throughput analysis is the developed by Mammoth Biosciences in collaboration with Millipore Sigma and Hamilton Company. The Mammoth's DETECTR BOOST™ platform, based on CRISPR COVID-19 RNA assay, was designed for minimal user interaction with automated liquid handling and it can run 1500 tests per 8-h [52].

Finally, the most cost-effective proposal is the equipment launched by Detectachem Inc. in September 2020. This platform used LAMP technology with a colorimetric read out that permits a qualitative analysis of the results with naked eyes, saving the cost of the detector, transducer and software. The company also bring the possibility of mobile app detection to handle patient data and GPS mapping. The MobileDetect Bio BCC19 (MD-Bio BCC19) Test Kit is able to run up to 96 tests in 30 min [53].

In Table 2 are listed the commercialized DNA base POC for Covid-19 diagnosis. The table compare the genes analysed, the sample collection procedure, the regulatory acceptance, the sensitivity, and the analysis time.

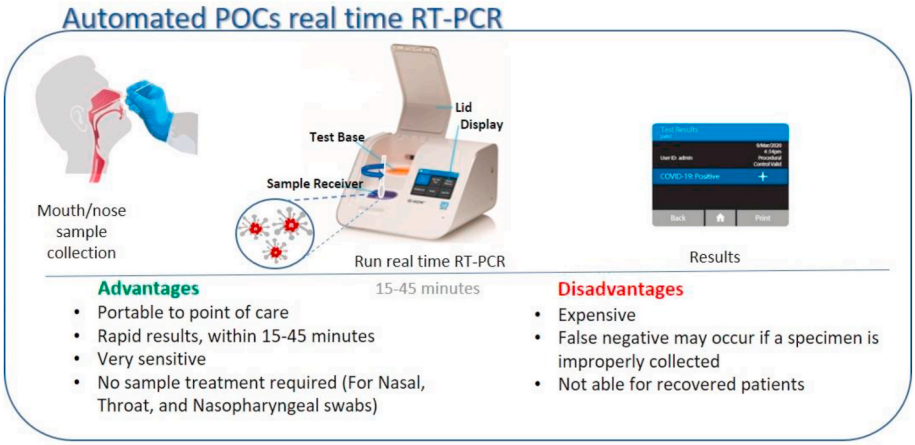

Fig. 4. Schematic representation of Covid −19 diagnosis by means automated POC real time RT-PCR. Picture of the equipment Abbott ID NOW™ Covid-19.

Table 2
Commercially available DNA base POC for a rapid SARS-COV-2 infection diagnosis in humans.

Company (Country)	Kit Name	Regulation (Validation Date)	Collection	Gene	LOD	Anal. Time (minutes)
Credo Diagnostics (Singapore)	VitaPCR COVID-19 assay	CE Mark (Mar 17, 2020)	NR	ORF1b-nsp14 Orfl abtRdRp	NR	~20
GenMark Diagnostics (US)	ePlex SARS-CoV-2	EUA US FDA (Mar 19, 2020)	RNA swab nasopharyngeal	NR	NR	<120
Cepheid (US)	Xpert® Xpress SARS-CoV-2	EUA US FDA (Mar 03, 2020)	RNA swab nasal nasopharyngeal,	E and N2 genes	0,25 copies/µl	45
Abbott (US)	Abbott ID NOW COVID	EUA US FDA (Mar 27, 2020)	RNA swab nasopharyngeal	RdRp & N genes	100%	5–13
MicrosensDX (UK)	Rapi Prep COVID-19	Awaiting CE mark	RNA (throat, nasal, sputum, feces)	NR	NR	<25
Mammoth Bioscience (US)	SARS-COV2 DETECTR (LAMP)	EUA from US FDA (August 31, 2020)	RNA swab nasopharyngeal oropharyngeal	E and N genes	12 copies/ µl	<45
Biomeme (US)	SARS-CoV-2 Test Go-strips	EUA from US FDA (August 11, 2020)	RNA swab nasopharyngeal	Orf1ab & S genes	NR	NR
Fluxergy (Germany)	COVID- 19 Test at Point-of-Care	CE Mark	RNA swab nasal	NR	NR	0
Sherlock BioSciences (US)	Sherlock CRISPR SARS-CoV-2	EUA from US FDA (May 06, 2020)	RNA swab nasopharyngeal oropharyngeal	ORF1ab, N	6,75 copies/µl	40
Detectachem Inc. (US)	MobileDetect Bio BCC19 Coronavirus	EUA from US FDA (September 1, 2020)	RNA swab nasopharyngeal, oropharyngeal	E and N genes	75 copies/ µl	30

5. Immunosensors for Covid-19 diagnosis

Other than the genomic virus material for Covid-19 diagnosis, also the proteins in the virus membrane are excellent tools for its detection. These proteins on the shell of the virus work as antigens that will be recognized by specific monoclonal antibodies attached to the immunosensor. This type of immune tests is commonly referred as Antigen tests. The most usual antibodies utilized for this purpose are the ones against N-protein and the S1 or S2-protein present in the SARS-CoV-2 virus membrane. Moreover, there is another type of immunosensor not based on the virus structure but on the natural immune response of the infected persons. The people who have developed the disease produce antibody to counterattack the action of the virus. In this serologic test the immunosensor works unlike the previous described system. In this case, the sensor surface is modified with the antigen (N and S proteins from the virus) for the detection of the produced antibodies in the blood of the patient. This type of sensors is known as serologic or antibody test. Detection of different antibodies can distinguish between IgM, IgG and/or IgA and thus give information on the phase of infection (early/current vs later stage/previous infection). IgA and IgM antibody are the first type of antibodies that release the immune system (3–6 days after infection). Several studies have reported that the IgA response in the early stage of the disease seems to be more pronounced than IgM [54–56]. But in contrast to IgM and IgG, IgA is more likely to be found in mucosal membranes including saliva, gastrointestinal tract and especially the respiratory epithelium than in plasma [54]. Meanwhile IgG antibody occurs after about 10–14 day of infection and remain in the blood for years, to accelerate the activation of the B-cell in case of new infections. The clinical value of antibodies largely depends on the understanding of host antibody responses during infection. Given that SARS-CoV-2 is a newly emerging virus, the antibody response in patients with COVID-19 remains unknown.

Serological assays are not well suited to detect acute infections. Immunosensors are less sensitive and produces more false negative results than DNA-based analysis. The membrane proteins from the virus mutates more often that the fragment of the RNA sequence used for diagnosis, being more likely to bring false negative if the virus evolves. However, serologic immunosensor has a very relevant advantage over genomic diagnosis. These platforms can detect the virus even when this has been removed from the patient after several years. Moreover, this test identifies patients with strong antibody responses, who are potential donors of plasma for and to study antibody responses for the protection from SARS-CoV-2 [57].

The sensitivity of the immunosensor depends on the stage of the disease in which the measurement is taken. Since it has been shown that in the first week of infection there is a higher viral load that decreases

throughout the disease, reducing the chances of be detected [8]. Thus, low sensitivity tests may have trouble detecting the virus after weeks of infection. In fact, this point has made that only some of these virus antigen tests are commercialized. Since they require a high viral count to function effectively, the majority of immunosensors on the market are based on serological detection.

The amount of analyte in the case of the serologic immune test is juts the contrary; at the first weeks of the disease the immune response has produced low load of antibodies in blood for being detected, which start to increases from the 10th day of infection. Another inconvenience of the serologic immune test is the potential cross-reactivity with antibodies produced by the body against similar coronaviruses [58].

There are different immunosensors technologies commercially available for Covid-19 diagnosis; the traditional ELISA platforms, the most cost-effective lateral flow immunoassays and the most evolved microfluidic POC immunosensors.

5.1. ELISA for Covid-19 diagnosis

ELISA is an old generalized biochemical test, present in most clinical laboratories, developed by Engvall and Perlmann in 1971, [59]. As we already introduced with ELONA, this type of test is performed in wells plate format, where is immobilized the specific antigen against the antibodies developed by the patient due to the Covid-19 infection. In ELISA is usually performed a dual test to detect IgM and IgG antibodies in the serum or plasma of the patients. The antibodies present in the sample interacts with the functionalised plate with the spike protein domain S1 and N from the virus and the interaction is elucidated with an anti IgG or anti IgM antibody labelled, that are attached with the antibodies present in the well. Horseradish peroxidase enzyme is used as label and it reacts with 3,3′,5,5′-tetramethylbenzidine added in the well, inducing the change to an intense blue colour in the well. The change on colour is detected by spectrophotometry with an ELISA reader (Fig. 5) [22,57].

ELISA assay requires specialized personal to carry out the test, since many different manual steps are needed, being a long process (about 4 h). So, it is necessary to deliver the sample to specialized laboratories, which makes such diagnosis more time consuming and expensive. Fig. 5 shows all the strep required for this type of analysis. However, ELISA-based testing enables to process many samples in parallel [55]. Alternatively, multiplexed testing enables detection of immunoglobulin binding to more than one antigen within a single tube, well, plate or slide. Multiplexed tests include but are not limited to microsphere immunoassays (MIAs) [60] and fluorescent protein microarrays [61]. The ELISA test uses the S proteins expressed in mammalian cells, finding a strong reactivity for all immunoglobulin G3 (IgG3), IgM and IgA.

Although ELISA technique is widely used in laboratories, few companies have been focused in the development of these type of kits for Covid-19 diagnosis, probably because of the inconveniences of antibody versus genomic analysis combined with the requirement of shipping the samples to an authorized laboratory. In Table 3 are summarized the companies commercialising ELISA kits for Covid-19 diagnosis. The table compare the genes analysed, the sample collection procedure, the regulatory acceptance, the sensitivity, and the analysis time.

The first companies putting on the market this type of kits were of course Chinese at the starting of the pandemic. At the end of January, the company Livzon got the National Medical Products Administration (NMPA) for commercialization just in China, but it was the Beijing Wantai Biological Pharmacy company the one achieved first the CE mark for commercialization in Europe. Then one European and two US companies played too the ELISA Covid game. It is important to remark the high sensitivity achieved from EUROIMMUN, a PerkinElmer, Inc. company.

5.2. Lateral flow immunoassays POCs for Covid-19 diagnosis

Lateral flow immunochromatographic assays or immunostrip are the most cost-effective sensors for SARS-CoV-2 detection, since the sensor response is read with the naked eye and a transducer is not needed for recording, and the main material used in this technology is cellulose. Thus, no expensive materials and bulky equipment's are required. This technology is based on the same principal as ELISA. But in this case, the support for the reaction, instead of a well plate, is a piece of paper. Also, comparing with ELISA, immunostrip do not requires complex manipulation, since all the steps are included inside the strip. This chromatographic paper contains the required reagents impregned in the paper. This platform has earned the name of rapid test, since just 10 min are needed to run the immunostrip analysis. Two different type of immunostrip configuration were fabricated for Covid-19 diagnosis, antigen test (detecting the virus) and antibody test (detecting the immune response). Most of the rapid test were based on antibody tests, due to the limitations on sensitivity that presents the antigen tests. This type of technology is available in two formats, dipstick and the most usual with the strip encapsulated in a cassette.

The steps and the configuration in an immunostrip for serologic analysis of Covid-19 are the next; In the conjugation pad, near the area where the sample is introduced, are adsorbed N-proteins labelled with colloidal gold (CG) and rabbit IgG antibody conjugated with CG as control purpose. N-protein is contained in the SARS-CoV-2 virus structure and it is the main protein that recognise our immune system. At the end of the strip are patterned three different lines of antibodies; the first with anti-human IgM antibodies, the second with anti-human IgG antibodies and the last one with anti-rabbit antibody as a control. To run the test is just required very little amount of blood sample, around a drop of blood, that is inserted in the sample pad of the cassette. To flow down

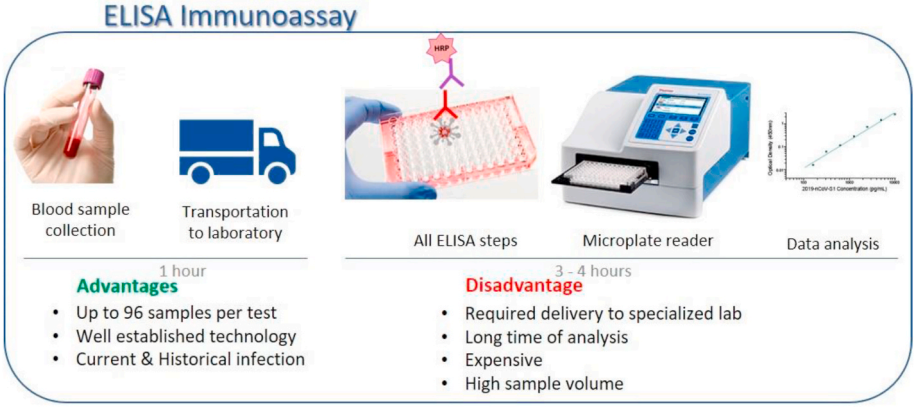

Fig. 5. Schematic representation of an indirect ELISA for antibodies detection against coronavirus. Picture of the ELISA kit from EDI Epitope Diagnostics Company. Picture of the microplate reader from Thermo Fisher Scientific.

Table 3
Commercially available ELISA tests for a rapid coronavirus infection diagnosis in humans.

Company (Country)	Kit Name	Regulation (Validation Date)	Collection	Specificity	Sensitivity	Analysis Time (min)
Euroimmun (Perkin Elmer) (Germany)	Anti-SARS-CoV-2 ELISA	CE-marked (March 25, 2020)	Blood	Serologic (IgA, IgG)	IgG – 99% IgA –90%	120
Beijing Wantai Biological (China)	Wantai SARS-CoV-2 Ab ELISA	CE-IVD marked Australia's ARTG (March 27, 2020)	serum, plasma or whole blood	Serologic IgG	96.6%	NR
Epitope Diagnostics, (United States)	EDI Novel Coronavirus COVID-19 IgG ELISA kit	CE-IVD marked	Human serum	Serologic (IgM, IgG)	51 units/ mL	80
Livzon (China)	Diagnostics kit for IgM/IgG to covid	China's NMPA (Jan 28, 2020)	Whole blood sample	Serologic (IgM, IgG)	NR	NR
Mount Sinai Laboratory (US)	COVID-19 ELISA IgG Antibody test	EUA US FDA (April 15, 2020)	Serum and plasma	Serologic (IgG)	92%	NR
Thermo Fisher Scientific (USA)	OmniPATH COVID-19 Total Antibody ELISA Test	EUA US FDA (October 02, 2020)	Serum and plasma	Serologic (IgM, IgG)	NR	NR
ZEUS Scientific, Inc. (US)	ZEUS ELISA SARS-CoV-2 IgG Test System	EUA US FDA (October 06, 2020)	Serum and plasma	Serologic (IgG)	NR	NR
University of Arizona Genetics Core for Clinical Services (US)	COVID-19 ELISA pan-Ig Antibody Test	EUA US FDA (August 31, 2020)	Serum and plasma	Serologic (IgM, IgG)	NR	NR
Bio-Rad Laboratories, Inc.	Platelia SARS-CoV-2 Total Ab assay	EUA US FDA (29 Abril 2020)	Serum and plasma	Serologic (IgM, IgG)	NR	NR

the sample is necessary some microliter of phosphate buffer saline and the capillary action helps on flowing the molecules in the sample along the strip. This is an important advantage over similar POCs that need more expensive microfluidic chips and pumping for moving the flow. When the sample enter in contact with the N-protein-CG, if the IgG and IgM antibodies are present (due to the Covid-19 infection), interacts with the proteins and flow together to the first antibody line, which attracts the IgM antibodies present in the sample. All the human IgM antibodies in the sample are attached, but just the one linked to the N-protein, brings a shiny red line due to the CG label. Similar thing happens when the sample reaches the second line where the IgG antibodies are entrapped. Finally, the sample attains the last control line where the rabbit IgG antibody conjugated with CG and demonstrates the well performance of the strip flow when this antibody link with the immobilized antibodies in the third line. The data analysis is also very simple; the third line should be always red after the test and depending on the IgG and IgM antibodies against Covid-19 present in the sample, the first and/or the second line will be red. Fig. 6 shows in detail the structure of the immunostrip and all the process for the Covid-19 diagnosis with this technology.

In the case of antigen based immunostrip the disposition of the reagents is a bit different. Since in this case the virus is detected. Then in this case, anti- N-protein antibodies CG labelled are mixed with the rabbit IgG antibody-CG (control) in the conjugation pad. Just two test lines are required; the first with anti-N-protein antibodies to construct a sandwich if the virus is present in the sample, generating a red line due to the presence of the CG. The second line with rabbit IgG antibody serves as control and turns red when interacts with the rabbit IgG antibody-CG. In the case of antigen test the sample is nasopharyngeal secretions, instead of using blood as in the serologic test.

The rapid immunostrip is the cheapest, fastest, and easiest option to combat this pandemic. In addition, this test can be used near patients

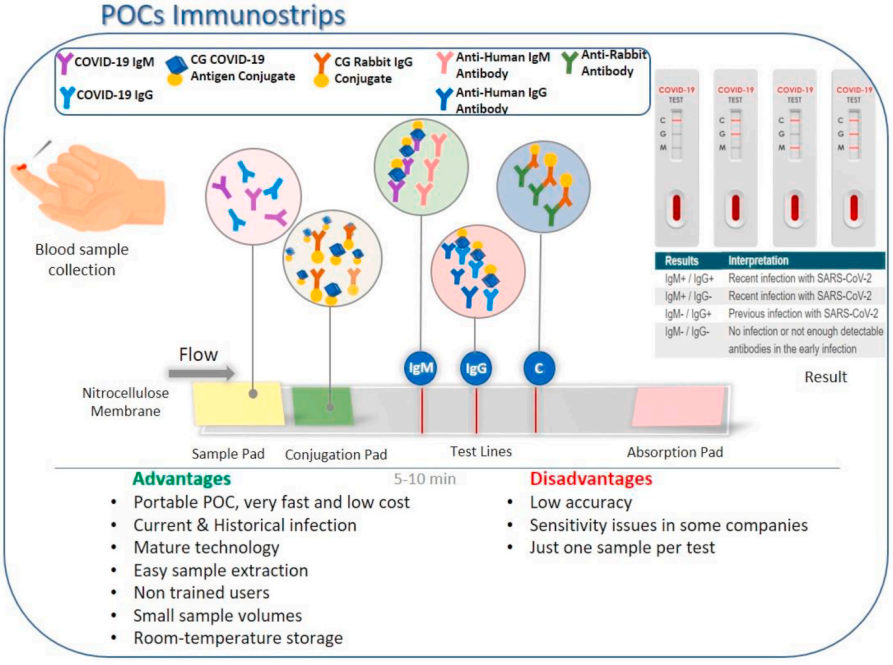

Fig. 6. Schematic representation of lateral flow Immunocromatography test based on the detection of antibodies against SARS-CoV-2. Picture of the Immunostrips from CliniSciences Company.

and it is not required specific training, so, it can be used not only at the point of care, but also in pharmacies, at home, at work. Although is highly recommended to use it under the supervision of medical staff. The main weakness of this type of rapid test, comparing with previous technologies, is mainly the sensitivity. In general, the IgG detection bring better sensitivity than IgM, being the antigen test the ones with lower sensitivity. The company reporting lower sensitivity in the serology immune-test is Chembio Diagnostics with their fingerstick test for Covid-19, which reported sensitivities of 50% for IgM and 100% IgG [62]. The other serologic commercialized tests have an average of 85%

Table 4
Commercially available rapid-immunostrip test for Covid-19 infection diagnosis in humans.

Company (Country)	Kit Name	Regulation (Validation Date)	Collection	Type of test	Sensitivity	Time (min.)
Wondfo (China)	Wondfo SARS-CoV-2 Antibody Test	China's NMPA. CE (March 2020) Australian's ARTG (March 25, 2020)	Whole Blood	Serological (IgM, IgG)	NR	15
Rapid Test Methods (Ireland)	COVID-19 IgM/IgG Lateral-Flow Kit	CE Mark	Whole Blood	Serological (IgM, IgG)	95%	10
Era Biology (China)	Virusee COV1D-19 IgG/IgM Lateral Flow	CE Mark	Whole Blood	Serological (IgM, IgG)	NR	10
AssayGenie (UK)	Acro Biotech COVID-19 Rapid POC	CE Mark	Serum, plasma or whole blood	Serological (IgM, IgG)	100%(IgG), 85% (IgM)	15
CTK Biotech (USA)	OnSite COVID-19 IgG/IgM Rapid Test	Australian's ARTG (March 19, 2020)	Whole Blood	Serological (IgM, IgG)	96,9%	10
Shenzen Bioeasy(China)	Covid-19 IgG/IgM GICA Lateral Flow	CE Mark	Serum, plasma or whole blood	Serological (IgM, IgG)	NR	10–15
Able Diagnostics (USA)	VivaDiag COVID-19 IgM/IgG Rapid Test	CE Mark	Serum, plasma or whole blood	Serological (IgM, IgG)	95,1%	15
Chembio Diagnostics and LumiraDx (USA)	DPP COVID-19 IgM/IgG Test	EUA US FDA (Apr 14, 2020)	Whole Blood	Serological (IgM, IgG)	IgM: 50% IgG: 100%	15
Edinburgh Genetics (UK)	COVID-19 Colloidal Gold Immunoassay Testing Kit	CE-IVD, China-FDA	Serum, plasma or whole blood	Serological (IgM, IgG)	99.31%	10
Innovita Biological Technology (China)	2019-nCoV Antibody Test (colloidal gold)	China's NMPA. Australian's ARTG CE-IVD approved.	Serum, plasma or whole blood	Serological (IgM, IgG)	87.3%	NR
Everest Links Pte (Singapore)	VivaDiag™ COVID-19 IgM/IgG Rapid Test	Australian's ARTG (Mar 26, 2020)	Serum, plasma or whole blood	Serological (IgM, IgG)	NR	NR
BioMedomics/Jiangsu Medomics (USA)	COVID-19 IgM/IgG Rapid Test Lateral flow	China's NMPA CE Mark (Mar 8, 2020)	Serum, plasma or whole blood	Serological (IgM, IgG)	88.66%	NR
Mologic (UK)	Lateral flow immunoassay for SARS-CoV-2.	CE mark.	Whole blood	Serological (IgM, IgG antibodies)	99% 243	NR
Hangzhou Biotec (China)	2019-nCoV IgG/IgM Rapid Test Cassette	CE Mark Australia's ARTG.	Serum, plasma or whole blood	Serological (IgM, IgG)	IgM 87% IgG 100.0%	10
Pharmact AG49 (Germany)	CoV-2 Rapid Test	CE Mark	Whole blood	Serological (IgM, IgG)	NR	20
Zhejiang Orient Gene Biotech (China)	COVID-19 IgG/IgM Rapid Test	China's NMPA CE Mark. EUA US FDA Australia's ARTG (April 1, 2020)	Serum, plasma or whole blood	Serological (IgM, IgG)	IgM 87.9% IgG 97.2%	NR
Cellex (USA)	qSARS-CoV-2 IgG/IgM Rapid Test	EUA US FDA (Apr 1, 2020) Australia's ARTG (Mar 1, 2020)	Serum, plasma or whole blood	Serological (IgM, IgG antibodies)	93.8%	NR
Mobidiag (Finland)	Anti-SARS-CoV-2 Rapid Test	CE-IVD CE-IVD marked	Serum, plasma or whole blood	Serological (IgM, IgG)	96.2%	<15
Qingdao Hightop (China)	SARS-CoV-2 IgM/lgG Antibody Rapid	Australian's ARTG (March 31, 2020)	Serum, plasma or whole blood	Serological (IgM, IgG	IgM (97%) IgG (97.5%)	15
SDBiosensor (Korea)	STANDARD Q COVID-19 IgM/IgG Duo	CE-IVD marked	Serum, plasma or whole blood	Serological (IgM, IgG)	81.8%	10
Beijing Wantai (China)	Wantai SARS-CoV-2 Ab Rapid Test Kit	CE-IVD marked. Australian's ARTG (Mar 27, 2020)	Nasopharyngeal swab	Antigen	95.2%	NR
SD Biosensor (Korea)	STANDARD Q COVID-19 Ag Test	CE-IVD marked	Nasopharyngeal swab	Antigen	NR	30
Coris BioConcept (Belgium)	COVID-19 Ag Respi-Strip	CE-IVD marked	Nasopharyngeal swab	Antigen	60%	15
Shenzen Bioeasy (China)	2019-nCoV Fluorescence Ag Rapid Antigen	CE-IVD marked	Nasal swab/sputum	Antigen	NR	10
RapiGEN (Korea)	BIOCREDIT COVID-19 Ag	CE-IVD marked	Nasopharyngeal swab	Antigen	98%	5–8
Nirmidas Biotech, Inc. (US)	Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit	EUA US FDA (September 29, 2020)	Nasopharyngeal swab	Serological (IgM, IgG)	NR	NR
Sugentech, Inc. (Korea)	SGTi-flex COVID-19 IgG	EUA US FDA (September 03, 2020)	Nasopharyngeal swab	Serological (IgG)	NR	NR
Biocan Diagnostics Inc. (Canada)	Tell Me Fast Novel Coronavirus (COVID-19)	EUA US FDA (August 25, 2020)	Nasopharyngeal swab	Serological (IgM, IgG)	NR	NR
Xiamen Biotime Biotechnology (China)	BIOTIME SARS-CoV-2 IgG/IgM Rapid Qualitative Test	EUA US FDA (July 07, 2020)	Nasopharyngeal swab	Serological (IgM, IgG)	NR	NR
Access Bio, Inc. (US)	CareStart COVID-19 IgM/IgG	EUA US FDA (July 24, 2020)	Nasopharyngeal swab	Serological (IgM, IgG)	NR	NR

sensitivity for IgM and 97% for IgG. On the other hand, the few antigen tests commercial available show an average of 84% sensitivity, being the less efficient the Coris BioConcept company with 60% of sensitivity [63]. The sensitivity of the rapid antigen test kits is mainly unclear when saliva samples are used but also in some test when nasopharyngeal swab specimens are used [64]. However, the performance of the commercialized rapid test has been improved along this pandemic, showing some companies excellent sensitivity (97–99%) as CTK Biotech, Edinburgh Genetics, Mologic, RapiGen among other, which demonstrated similar performance as ELISA and fully automatised immunoassays [65]. Table 4 summarizes the companies commercialising rapid-immunostrip tests for Covid-19 diagnosis. The table compare the types of commercialized tests antigen and serologic tests, the detected analyte, the sample collection, the regulatory acceptance, the sensitivity, and the analysis time.

Although the great advantages of immunostrip, these rapid tests have given rise to controversy in the news. The first complains were about the difficulties to access to these rapid tests. In February 2020, when the pandemic starts to spread in Europe, the different governments rush to buy these cherished tools. But most of the companies that commercialized this type of technology (listed in Table 4) were from China, with limited commercialization abroad due to regulatory issues. Although, this companies move quickly to expand their markets, these rapid tests did not get the CE mark until March. Even so, these tests were not easy to purchase on all the European countries, since there was limitation of stocks and there were no distributors selling on all countries.

But the most important complains arose at the time when the hospitals started to use these rapid test, since high amounts of false negatives and the lack of sensitivity were observed with these tests. This issue rises great concern and the WHO and the European Centre for Disease Prevention and Control (ECDC) in collaboration with reference laboratories were performing validation studies of certain tests. Although the test has the CE mark, it has been pinpointed incomplete technical sheets and fraudulent documentation [66].

Some of the problems came due to the low sensitivity obtained with the antigen test. This type of test that directly detects the virus, brings false negative due to the low load virus after weeks of infection, being difficult to correctly diagnose the infection with this type of test. For this reason, it is important to take into consideration the infection disease stage and the analytes present in this stage of the infection (Fig. 2) to choose the most suitable analysis technique.

5.3. *Fully automatized immunoassay for Covid-19 diagnosis*

As in the case of DNA detection, immunosensors have progress towards fully automatized equipment's that can process the sample and analyse with almost a single click. In these equipment's the ELISA plate and/or the immunostrip are substituted by robotic equipment that carry out all the manual steps of previous technics in an automatised way by microfluidics that contains all the required steps and reagents integrated in the device to perform the sample treatment, antigen or antibody detection and data analysis. All the processes of flow pumping, read-out and data processing are run by the help of fully automatized electronics. At the starting of pandemic, few companies were offering this kind of equipment, since it takes time to develop them. But in few months, despite stiff competition with the fast, easy to use and low-cost point of care immunostrip, the supply of this expensive and bulky automated technology has grown, competing through its high throughput and short time of analysis capability.

Siemens healthcare diagnostics was the first company FDA EUA-authorized, the February 7, 2020, for the commercialization of a fully automatized immunoassay test. Siemens presented different solutions for COVID diagnosis; the Dimension Vista SARS-CoV-2 Total antibody assay, Atellica IM SARS-CoV-2 IgG (COV2G) and ADVIA Centaur SARS-CoV-2 IgG (COV2G). This technology is based on chemiluminescent immunoassay in a sandwich configuration against S1 and N proteins of the virus by means of Luminescent Oxygen Channeling Immunoassay (LOCI technology). The illumination at 680 nm of the formed sandwich generates singlet oxygen on the sensor surface that diffuses to the chemiluminescent dye of the antibody, triggering a signal. The most powerful equipment from Siemens can provide a response in 10 min with a high throughput analysis of 440 analyses per hour [67].

A few days later, the 19th February, the Chinese company Snibe Diagnostic was the first company to obtain the CE mark for supplying an automatized rapid immunoassay test, [68]. Snibe's Maglumi 2019-nCoV is also based on chemiluminescence immunoassay detection, using 10 μL sample volume of serum or plasma. This equipment can run 180 test/run with a total analysis time of 12 min. Magnetic microbeads are used in the microfluidic cartridge for separation and concentration of the analyte. Maglumi's detection uses an enzyme label free chemiluminescent, based on an organic molecule; N- (4-aminobutyl) -N-ethylisoluminol (ABEI) that is more stable over time and less affected by the storage conditions. But it is required the addition of NaOH and H_2O_2, for getting a chemiluminescence response.

In April 2020, three companies introduced new equipment for COVID diagnosis with a fully automated immunoassay. The VITROS® ECi/ECiQ Immunodiagnostic Systems commercialized by Ortho Clinical Diagnostics in US can run the Anti-SARS-CoV-2 Total Reagent Pack to combat Covid-19. This technology is also based on chemiluminescence read out and process 150 samples per hour, less that previous equipment, since needs 18 min more to fulfil the analysis than Maglumi. The company reported a sensitivity of 83,3% (n = 36) and selectivity of 100% (n = 400) [69]. Abbott presented also a chemiluminescence technology for immune Covid-19 test, first available in US and then marked CE for Europe selling. It is based on the Abbot's patented Chemiflex technology that run with the ARCHITECT i1000SR and i2000SR. This equipment's can run 100–200 samples per hour, taking from 30 to 43 min the analysis depending on the sample pre-treatment. As previous, the sample volume required is just 10 μL [70]. Within a few days of each other, it was also US approved another chemiluminescence equipment commercialized by DiaSorin Inc under the name of LIAISON SARS-CoV-2 S1/S2 IgG in combination with the LIAISON Control SARS-CoV-2 S1/S2 IgG on the LIAISON XL analyser. This technology run in two stages, first S1 and S2 antigens coated on the well interact with the SARS-CoV-2 antibodies present in the sample of the patient and then this interaction is elucidated with isoluminol-antibody conjugate that bind with SARS-CoV-2. LIAISON XL analyser has a throughput of 170 results/hour in 35 min [71,72].

Months later, FDA approved three devices with alike magnetic beads separation and chemiluminescence read out, similar to Snibe's technology. The first, the 2nd of May from Roche Diagnostics under the name of Elecsys Anti-SARS-CoV-2. This kit can be analysed with different equipment; Cobas e411, e602 and e811, which are able to get a response in 18 min and run from 85 to 300 test per hour [73]. The August 17, 2020 passed the FDA requirements for commercialization the IgM antibodies against SARSCoV-2 from the Diazyne Company that runs with DZ-lite 3000 Plus Chemiluminescence Analyzer. This equipment is able to run 180 test/hour taking just 17 min for the first result [74]. It had to wait until September 2020 to see a portable chemiluminescence automatised immunoassay commercialized. Although it is based on a separation and detection technology similar to the previous ones, the required fluidics has been miniaturised in a microfluidic cartridge. Most chemiluminescent based immune assays are bulky device (150 × 76 × 150 cm approximately), meanwhile the Sophonix MS-Fast is an affordable benchtop portable device (50 × 50 × 30 cm approximately). Biochek Inc. commercialized the BioCheck SARS-CoV-2 IgM/IgG Antibody Test Kit analysed in the portable Sophonix MS-Fast Automated Chemiluminescent Immunoassay Analyzing System. This technology can run in 30 min 8 samples, which has lower performance than competitors, but in much smaller device dimensions, to be able to be used next to the patient [75].

Another portable POC automated immunoassay for COVID diagnosis approved by FDA at the end of September is the COVID-19 Ag in combination with the FREND™ system, commercialized by NanoEntek. This benchtop technology uses the same configuration than the lateral flow immunoassay described in previous section but integrated in a microfluidic cartridge rather than on cellulose and with a semiquantitative fluorescence reader. As the rapid test, this technology can just run one sample, in a short time of analysis (3–4 min). The company claims high accuracy in comparison with lateral flow; 94.12% of Positive Percent Agreement and 100% of Negative Percent Agreement) similar to rapid test at lower price, as a claim to purchase a more expensive technology [76]. Similar chromatographic immunoassay automatised was commercialized by Becton, Dickinson and Company with the BD Veritor™ System for Rapid Detection of SARS-CoV-2, which have similar limitation than FREND system with longer analysis time; 15 min [77].

Last but not least, a completely different technology proposed by Scottish company Quotient, which got the CE Marking the May 1, 2020, but must wait until September 25, 2020 for the FDA authorization. The MosaiQ COVID-19 Antibody Microarray is a pre-printed single use solid-phase microarrays with 132 probes per microarray of SARS-CoV-2 antigens and controls to analyse by colorimetric detection any antibodies present in the specimen by means of gold-conjugated secondary antibody, combined with washing steps. This technology can test 3000 microarrays in 24 h, providing results every 24 s with only 5 μl of serum or plasma. The company reports excellent sensitivity and specificity of 100% and 99.8% respectively. The main disadvantage of this technology is the high volume and price of the equipment, like the chemiluminescence equipment presented at the beginning of this section. Removing this technology from the points of care, being necessary to send the sample to a clinical laboratory [78].

In serologic analysis the fully automatised immunoassays based on chemiluminescence analysis and the MosaiQ platform are the most complex and expensive technology for this purpose, but it offers a high throughput analysist in relative short time, very necessary when thousands of samples need to be tested every day to stop the spread of the pandemic. The sensitivity and selectivity reported by the companies that market different serological analysis technologies are comparable, being the rapid test the ones that present more variability at this point, tending to lower values. To bring more light in this point, a comparative analysis of three serological technologies from different companies was studied under the same conditions by GeurtsvanKessel et al. [65]. They compared three rapid tests (Cellex IgM/IgG, InTec IgM/IgG, and Orient gene/Healgen IgM/IgG), four ELISA Kits (Wantai Ig total ELISA, Wantai IgM ELISA, Euroimmun IgG ELISA and Euroimmun IgA ELISA) and the chemiluminescent assay from Diasorin. Wantai ELISA IgG was the best performing test overall with a specificity of 96–100% and a sensitivity of 99%. The test with the least specificity is two rapid tests; Intec and Orient with respectively 76–91% and 80–94%, but surprisingly the third test with the lower specificity (84–95%) is the one with high cost and fully automated Diasorin technology. Regarding sensitivity, Diasorin is again those that report the lowest yield with 81% followed by the Euroimmune IgG ELISA with the same sensitivity and the Cellex rapid test with 89%. Considering this comparison, the simplicity and low cost of the rapid test are strong competitors of the most complex technologies, which consume more time for the analysis, require specialized personnel and the transport of the samples to the laboratory.

6. Conclusions and future perspectives

The international health emergency due to the worldwide presence of the previously unknown virus SARS-CoV-2, with high spread and mortality in humans, has made essential the rapid development of diagnostic technologies and biosensors for the analysis of this virus.

The first in vitro test for the diagnosis of Covid-19 developed was real-time RT-PCR, the usual commercial gold standard for virus analysis. Today, most of the detection platforms available to stop this pandemic are based on genomic analysis, being incorporated also to the traditional PCR real time LAMP and digital PCR to improve analysis time and sensitivity, respectively. In addition to real time DNA amplification detection, other technologies have been developed and marketed to analyse the amplified Covid-19 genome, such as ELONA and fully automated POCs for DNA detection, but with less success than real time RT-PCR.

Beside genomic analysis, antigen and antibody detection has also found its niche in the market for the diagnosis of Covid-19. The most differentiating factor that positioned immunosensor in the market is the new type of information it offers, the immune response of the infected people, which permit to detect the historical presence of the virus in the organism. For this purpose, different type of platforms has been developed, such as ELISA, Lateral flow immunosensors and fully automated POCs immunoassays. The lateral flow test offers important advantages; minimal or no sample treatment, user friendly, portable and low cost, being able to detect the presence of the virus near the patient, in few minutes, but with less efficiency than other technologies. *ELISA* is not a point of use technology, require specialized personnel and is time consuming, which implies a narrow and limited market. On the other side, fully automated POCs are expensive for being widely distributed in the hospitals. However, its high throughput capacity brings and important advantage when thousands of samples need to be tested every day.

The main IVD players in the Covid-19 diagnosis are the real time RT-PCR and the lateral flow POC immunoassays. The RNA detection with real time RT-PCR/dPCR has demonstrated to be the most reliable and sensitive technique. The amplification of millions of copies of DNA by PCR helps in this regard and even more if digital PCR is used, improving the analysis sensitivity 10 times. Also, the high load of viral RNA for a long period of the diseases, reduce the possibilities of false negatives. However, to run real time RT-PCR and dPCR a structural support is needed, and it is required to deliver the samples to a laboratory conditioned to work with this technology, increasing a lot the cost and the time of analysis. Moreover, the sampling is complex and requires a pretreatment, and specialized handling and transportation. Even so, PCR has not been relegated despite the great advantages of lateral flow immunostrip. These small IVD platforms can be used by anyone without any special training other than reading instructions. The use of the sensor and the data analysis are so simple that they are recommended for home use, like its counterpart pregnancy test based on the same technology. In addition, the short analysis time, just a few minutes, and their low cost, makes them an excellent tool for pandemics and for many other situations. But this technology is limited to sensitivity, not only by the inherent limitation of the technique but also by the lower load of antibodies or viral antigen throughout the disease. The cost of rapid-immunostrip is about 10 €, similar than real time RT-PCR kits; about 16 €. However, in the case of PCR needs also to be counted the cost of the device, ranging approximately from 15.000 to 90.000 € and the cost of the specialized staff, laboratory, and transportation.

So, a combination of real time RT-PCR/dPCR and rapid-immunostrip has demonstrated to be the most effective manner to counterattack this pandemic with the tools currently available. The immunostrip save time on screening patients with symptoms and serve as first-level screening before the confirmatory diagnosis with viral genetic material. It has been the solution adopted by most of the countries fighting against this pandemic. However, this solution requires a double check and a double investment. Because although the immunostrips have great advantages, such as rapid response, low cost, portability, these require the confirmation with the real time RT-PCR, which takes minimum 1 day and extra costs. However, when the evolution of the pandemic causes the number of contaminants to grow exponentially, fully automated immunoassays and PCRs, capable of executing thousands of tests per day, have gain importance, being acquired by most countries.

Throughout the COVID-19 pandemic in a few months we have had the opportunity to see how IVD technologies have evolved and improved

from traditional virus analysis techniques to more sensitive, automated, faster and with higher performance technologies for SARS-COV2 detection. But it is still critical and necessary to have accurate, portable and rapid diagnostic tests to combat Covid-19, combining the high throughput analysis of both analytes; DNA and antibodies at shorter time in the same device that required minimum sample treatment with an easy-to-use point-of-care platform at an affordable price. Science must be united in knowledge and collaborate between the different scientific expert in technologies based on the detection and diagnosis to fill the still opened gaps. We live in a global world and the problems affecting society know no borders. Also, governments need to become aware of the importance of research and they should invest more than just a little percentage of the budget in research and development or only do so when there is an emergency.

Solving the Covid-19 problem is a global challenge. Millions of people still need to be diagnosed, and a low-cost, reliable rapid test is needed. This is a global problem in which science and research must pay continuous attention to develop and improve point-of-care tests for infectious diseases.

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.

Acknowledgments

This work was supported by Networking Biomedical Research Center (CIBER), Spain. CIBER is an initiative funded by the VI National R&D&i Plan 2008–2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions, and the Instituto de Salud Carlos III (RD16/0006/0012), with the support of the European Regional Development Fund and Spanish Ministry of Science and Innovation through the Severo Ochoa Centers of Excellence Program. This work was funded by the CERCA Program, the Commission for Universities and Research of the Department of Industry and Knowledge (2017 SGR 1079) and the Orfeu program for massive Covid-19 screening of the Generalitat de Catalunya. MM would like to acknowledge her confinement partners; Frank and Cesc for their patience.

References

[1] Johns Hopkins University (JHU) and Center for Systems Science and Engineering (CSSE), Coronavirus Resource Center. Available at: https://coronavirus.jhu.edu/map.html (accessed 21st July 2020).
[2] N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, et al., Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study, Lancet 395 (2020) 507–513.
[3] WHO, Director-General's Opening Remarks at the Media Briefing on COVID-19, 2020, 16 March 2020, Available at: www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—16-march-2020, 21st July 2020.
[4] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, et al., A novel coronavirus from patients with pneumonia in China, 2019, N. Engl. J. Med. 382 (8) (2020) 727–733.
[5] A. Wu, Y. Peng, B. Huang, X. Ding, X. Wang, P. Niu, J. Meng, Z. Zhu, Z. Zhang, J. Wang, Commentary genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China, Cell Host Microbe 27 (2020) 325.
[6] B. Robson, COVID-19 Coronavirus spike protein analysis for synthetic vaccines, a peptidomimetic antagonist, and therapeutic drugs, and analysis of a proposed achilles' heel conserved region to minimize probability of escape mutations and drug resistance, Comput. Biol. Med. 121 (2020) 103749.
[7] Y. Pan, D. Zhang, P. Yang, L.L.M. Poon, Q. Wang, Viral load of SARS-CoV-2 in clinical samples, Lancet Infect 3 (2020) 411–412. Dis. 24 de febrero de 2020; 20 (4).
[8] K.K.W. To, O.T.Y. Tsang, W.S. Leung, A.R. Tam, T.C. Wu, D.C. Lung, et al., Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study, Lancet Infect. Dis. 20 (5) (2020) 565–574.
[9] I. Thevarajan, T.H.O. Nguyen, M. Koutsakos, J. Druce, L. Caly, C.E. van de Sandt, Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19, Nat. Med. 26 (2020) 453–455.
[10] X. He, E.H.Y. Lau, P. Wu, et al., Temporal dynamics in viral shedding and transmissibility of COVID-19, Nat. Med. 26 (2020) 672–675.
[11] A.K. Azkur, M. Akdis, D. Azkur, et al., Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19, Allergy 75 (2020) 1564–1581.
[12] L. Bao, W. Deng, B. Huang, et al., The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice, Nature 583 (2020) 830–833.
[13] C.H. Chau, J.D. Strope, W.D. Figg, COVID-19 clinical diagnostics and testing technology, Pharmacotherapy 40 (8) (2020) 860–868.
[14] Bell, S.M. Hodcroft, E., Wagner, C., Müller, N., Hadfield, J., Neher, R., Bedford, T.. GISAID. Available at: www.gisaid.org/epiflu-applications/next-hcov-19-app/(accessed 21st July 2020) 2020..
[15] V.M. Corman, O. Landt, M. Kaiser, et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Euro Surveill. 25 (3) (2020) 2000045.
[16] 2020 WHO. World Health Organization, Laboratory Testing for 2019 Novel Coronavirus (2019-nCoV) in Suspected Human Cases - Interim Guidance 2020, 2020, 17 Jan 2020. Available at: www.who.int/healthtopics/coronavirus/laboratory-diagnostics-for-novel-coronavirus, 21st July 2020.
[17] T. Ji, Z. Liu, G.Q. Wang, X. Guo, S.A. khan, C. Lai, H. Chen, S. Huang, S. Xia, B. Chen, H. Jia, Y. Chen, Q. Zhou, Detection of COVID-19: a review of the current literature and future perspectives, Biosens. Bioelectron. 15 (2020) 112455–112462.
[18] K. Nalla, A.M. Casto, M.L.W. Huang, G.A. Perchetti, R. Sampoleo, L. Shrestha, Y. Wei, H. Zhu, K.R. Jerome, A.L. Greningera, Comparative performance of SARS-CoV-2 detection assays using seven different primer-probe sets and one assay kit, J. Clin. Microbiol. 58 (6) (2020) e00557-00577.
[19] J.F. Chan, C.C. Yip, K.K. To, T.H. Tang, S.C. Wong, K.H. Leung, A.Y. Fung, A.C. Ng, Z. Zou, H.W. Tsoi, G.K. Choi, A.R. Tam, V.C. Cheng, K.H. Chan, O.T. Tsang, K. Y. Yuen, Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens, J. Clin. Microbiol. 58 (5) (2020) e00310-e00320.
[20] Fulgent, Coronavirus Testing, 2020. Available at: www.fulgentgenetics.com/covid19, 21st July 2020.
[21] R. Wölfel, V.M. Corman, W. Guggemos, M. Seilmaier, S. Zange, M. Müller, D. Niemeyer, T.C. Jones, P. Vollmar, C. Rothe, M. Hoelscher, T. Bleicker, S. Brünink, J. Schneider, R. Ehmann, K. Zwirglmaier, C. Drosten, C. Wendtner, Virological assessment of hospitalized patients with COVID-2019, Nature 581 (2020) 465–469.
[22] T.E. Miller, W.F. Garcia Beltran, A.Z. Bard, T. Gogakos, M.N. Anahtar, M. G. Astudillo, D. Yang, J. Thierauf, A.S. Fisch, G.K. Mahowald, et al., Clinical sensitivity and interpretation of PCR and serological COVID-19 diagnostics for patients presenting to the hospital, Faseb. J. 34 (2020) 13877–13884.
[23] Y.H. Jin, L. Cai, Z.S. Cheng, H. Cheng, T. Deng, Y.P. FanJin, et al., A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia, Mil Med Res 7 (1) (2020) 4.
[24] Y. Yang, M. Yang, C. Shen, F. Wang, J. Yuan, J. Li, M. Zhang, et al., Laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections, medRxiv (2020), https://doi.org/10.1101/2020.02.11.20021493.
[25] W. Wang, Y. Xu, R. Gao, R. Lu, K. Han, G. Wu, W. Tan, Detection of SARS-CoV-2 in different types of clinical specimens, J. Am. Med. Assoc. 323 (18) (2020) 1843–1844.
[26] CDC, US Department of Health & Human Services (2020) Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19), 2020. Available at: www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html, 21st July 2020) 2020.
[27] WHO, Collecting, Preserving and Shipping Specimens for the Diagnosis of Avian Influenza A(H5N1) Virus Infection, 2006. Available at: www.who.int/ihr/publications/CDS_EPR_ARO_2006_1.pdf, 21st July 2020.
[28] M. Coronavirus Johnson, Test Kits Encounter QC Hiccup in Some States, CDC Reports, 2020. GenomeWeb. 12 Feb 2020 Available at: www.genomeweb.com/pcr/coronavirus-test-kits-encounter-qc-hiccup-some-states-cdc-reports#.XkTV2VIzYcg, 21st July 2020.
[29] FDA. US Food and Drug Administration, Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics, 2020, 29 Feb 2020. Available at: www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-new-policy-help-expedite-availability-diagnostics, 21st July 2020) 2020.
[30] M.J. Kellner, J.J. Ross, J. Schnabl, M.P.S. Dekens, R. Heinen, I. Grishkovskaya, B. Bauer, J. Stadlmann, L. Menéndez-Arias, R. Fritsche-Polanz, M. Traugott, A rapid, highly sensitive and open-access SARS-CoV-2 detection assay for laboratory and home testing, BioRxiv (2020), https://doi.org/10.1101/2020.06.23.166397.
[31] M.A. Lalli, S.J. Langmade, X. Chen, C.C. Fronick, C.S. Sawyer, L.C. Burcea, M. N. Wilkinson, R.S. Fulton, M. Heinz, W.J. Buchser, R.D. Head, R.D. Mitra, J. Milbrandt, Rapid and extraction-free detection of SARS-CoV-2 from saliva with colorimetric LAMP, MedRxiv (2020), https://doi.org/10.1101/2020.05.07.20093542.
[32] R. Lu, J. Wang, M. Li, et al., SARS-CoV-2 detection using digital PCR for COVID-19 diagnosis, treatment monitoring and criteria for discharge, MedRxiv (2020), https://doi.org/10.1101/2020.03.24.20042689.
[33] J. Perkel, Guiding our PCR experiments, Biotechniques 58 (5) (2015) 217–221.
[34] V.M. Corman, T. Bleicker, S. Brünink, M. Zambon, Diagnostic Detection of Wuhan Coronavirus 2019 by Real-Time RT-PCR, World Health Organization, Geneva, 2020, 2020.

[35] BGI, BGI's Real-Time SARS-CoV-2 Test to Detect Novel Coronavirus Receives FDA Emergency Use Authorization, 2020, 27 Mar 2020. Available at: www.bgi.com/us/company/news/bgis-real-time-sars-cov-2-test-to-detect-novel-coronavirus-receives-fda-emergency-use-authorization/, 21st July 2020.
[36] 2020 WHO. Organization WH, Coronavirus (COVID-19) Genesig® Real-Time PCR Assay, 2020. Available at: www.who.int/diagnostics_laboratory/eul_0489_185_00_path_covid19_ce_ivd_ifu_issue_2.0.pdf?ua=1, 21st July 2020.
[37] 2020 FDA. FDA, Logix Smart™ Coronavirus Disease 2019 (COVID-19) Kit, 2020. Available at: http://fda.gov/media/136687/download, 21st July 2020.
[38] SDBiosensors, STANDARD M nCoV Real-Time Detection Kit Information, 2020. Available at, http://sdbiosensor.com/xe/product/7653. Ultra-rapid Real-time RT-PCR Assay for COVID-19, intructions, http://www.anitoa.com/docs/covid-19-rapid-test.pdf. (Accessed 21 July 2020).
[39] Biomaterials Seasun, AQ-TOP™ COVID-19 Rapid Detection Kit PLUS, 2020. https://www.fda.gov/media/142800/download, 1st November 2020.
[40] P.B. van Kasteren, B. van der Veer, S. van den Brink, L. Wijsman, J. de Jonge, A. van den Brandt, R. Molenkamp, C.B.E.M. Reusken, A. Meijer, Comparison of seven commercial RT-PCR diagnostic kits for COVID-19, J. Clin. Virol. 128 (2020) 104412–104422.
[41] Gnomegen, COVID-19 RT-Digital PCR Detection Kit, 2020. https://www.fda.gov/media/136738/download, 1st November 2020.
[42] PreciGenome, FastPlex Triplex SARS-CoV-2 Detection Kit, RT-Digital PCR, 2020. https://www.fda.gov/media/139523/download, 1st November 2020.
[43] Bio-Rad, RT-Droplet PCR, Bio-Rad SARS-CoV-2 ddPCR Kit, 2020. https://www.fda.gov/media/137579/download, 1st November 2020.
[44] CAN, Mass testing of population for coronavirus to be launched, Available at: www.catalannews.com/society-science/item/mass-testing-of-population-for-coronavirus-to-be-launched (accessed 21st July 2020)..
[45] Genomica, COVID-19 Kits, 2020. Available at: http://genomica.com/covid-19/?lang=en, 21st July 2020.
[46] Credo Menarini, Diagnostics Biomedical and a. Menarini Diagnostics Sign an Exclusive Distribution Agreement for Sars-Cov-2 Assay on the Vita PCR Platform, 2020. Available at: www.menarinidiag.es/es-es/home/noticias-y-eventos/detalle-de-noticias/artmid/16444/articleid/91/credo-diagnostics-biomedical-and-a-menarini-diagnostics-sign-an-exclusive-distribution-agreement-for-sars-cov-2-assay-on-the-vitapcrtm-platform, 21st July 2020.
[47] 2020 FDA. US Food and Drug Administration, ID NOW COVID-19 (Abbott Diagnostics), 2020, 27 Mar 2020. Available at: www.fda.gov/media/136522/download, 21st July 2020.
[48] Sherlock BioSciences, SherlockTM CRISPR SARS-CoV-2 Kit, 2020. https://www.fda.gov/media/137746/download, 1st November 2020.
[49] Cepheid, Xpert® Xpress SARS-CoV-2, 2020. Available at: www.cepheid.com/coronavirus, 21st July 2020.
[50] CNBC, FDA Grants 'emergency Use' Coronavirus Test that Can Deliver Results in 45 Minutes, 2020, 21 Mar 2020. Available at: www.cnbc.com/2020/03/21/fda-grants-emergency-use-coronavirus-test-that-can-deliver-results-in-45-minutes.html, 21st July 2020.
[51] R.H.T. Nijhuis, D. Guerendiain, E.C.J. Claas, K.E. Templeton, Comparison of ePlex respiratory pathogen panel with laboratory-developed real-time PCR assays for detection of respiratory pathogens, J. Clin. Microbiol. 55 (6) (2017) 1938–1945.
[52] Mammoth, A CRISPR-Based Detection Platform for SARS-CoV-2, 2020. https://mammoth.bio/covid/, 1st November 2020.
[53] Detectachem, MobileDetect-BIO BCC19 Test Kit for SARS-CoV-2 Detection, 2020. https://www.fda.gov/media/141791/download, 1st November 2020.
[54] L. Guo, L. Ren, S. Yang, M. Xiao, D. Chang, F. Yang, C.S.D. Cruz, Y. Wang, C. Wu, Y. Xiao, L. Zhang, L. Han, et al., Profiling early humoral response to diagnose novel coronavirus disease (COVID-19), Clin. Infect. Dis. 71 (15) (2020) 778–785.
[55] J. Zhao, Q. Yuan, H. Wang, W. Liu, X. Liao, Y. Su, X. Wang, J. Yuan, T. Li, J. Li, S. Qian, C. Hong, et al., Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019, Clin. Infect. Dis. 71 (16) (2020) 2027–2034 (Online ahead of print).
[56] H. Ma, W. Zeng, H. He, D. Zhao, D. Jiang, P. Zhou, L. Cheng, Y. Li, X. Ma, T. Jin, Serum IgA, IgM, and IgG responses in COVID-19,Cell, Cell. Mol. Immunol. 17 (2020) 773–775.
[57] F. Amanat, D. Stadlbauer, S. Strohmeier, T.H.O. Nguyen, V. Chromikova, M. McMahon, K. Jiang, et al., A serological assay to detect SARS-CoV-2 seroconversion in humans, Nat. Med. 26 (2020) 1033–1036.
[58] H. Lv, N.C. Wu, O.T.Y. Tsang, M. Yuan, R.A.P.M. Perera, et al., Cross-reactive antibody response between SARS-CoV-2 and SARS-CoV infections, BioRxiv (2020), https://doi.org/10.1101/2020.03.15.993097.
[59] E. Engvall, P. Perlmann, Enzyme-linked immunosorbent assay, Elisa. 3. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes, J. Immunol. 109 (1) (1972) 129–135.
[60] A. Ayouba, G. Thaurignac, D. Morquin, E. Tuaillon, R. Raulino, A. Nkuba, A. Lacroix, N. Vidal, V. Foulongne, V. Le Moing, J. Reynes, E. Delaporte, M. Peeters, Multiplex detection and dynamics of IgG antibodies to SARS-CoV2 and the highly pathogenic human coronaviruses SARS-CoV and MERS-CoV, J. Clin. Virol. 129 (2020) 104521–104532.
[61] P.N. Hedde, T.J. Abram, A.m Nakajima R. Jain, R.R. Assis, T. Pearce, A. Jasinskas, M.N. Toosky, S. Khan, P.L. Felgner, E. Gratton, W. Zha, A modular microarray imaging system for highly specific COVID-19 antibody testing, Lab Chip 20 (18) (2020) 3283–3476.
[62] Chembio Diagnostic System, DPP® COVID-19 IgM/IgG System, 2020, 14 Apr 2020. Available at: https://www.fda.gov/media/136963/download, 21st July 2020.
[63] Coris BioConcept, COVID-19 Ag Respi-Strip, 2020. Available at: www.corisbio.com/Products/Human-Field/Covid-19.php, 21st July 2020.
[64] JMH (Japanese Ministry of Health) Labour and Welfare, Approval of in Vitro Diagnostics for the Novel Coronavirus Infection, Japanese Ministry of Health, Labour and Welfare, 2020. https://www.mhlw.go.jp/content/11124500/000632304.pdf. Accessed 30 May 2020 (accessed 1st November 2020) 2020.
[65] C.H. GeurtsvanKessel, N.M.A. Okba, Z. Igloi, et al., An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment, Nat. Commun. 11 (2020), https://doi.org/10.1038/s41467-020-17317-y, 3436-34.
[66] ECDC, An Overview of the Rapid Test Situation for COVID-19 Diagnosis in the EU/EEA, 2020. Available at: www.ecdc.europa.eu/en/publications-data/overview-rapid-test-situation-covid-19-diagnosis-eueea, 21st July 2020.
[67] Siemens Healthcare Diagnostics, Dimension Vista SARS-CoV-2 Total Antibody Assay (COV2T), 2020. https://www.fda.gov/media/138750/download, 1st November 2020.
[68] Snibe Diagnostic, The World's First 2019-nCoV CLIA Kits Received CE Mark, 2020, 06 Mar 2020. Available at: www.snibe.com/zh_en/en_newsView.aspx?id=576, 21st July 2020.
[69] 2020 FDA. US Food and Drug Administration, VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Reagent Pack (Ortho Clinical Diagnostics) - Letter of Authorization. 14 Apr 2020, 2020. Available at: www.fda.gov/media/136967/download, 21st July 2020.
[70] Abbott, Abbott Launches Covid-19 Antibody Test, 2020. Available at: www.abbott.com/corpnewsroom/product-and-innovation/abbott-launches-covid-19-antibody-test.html, 21st July 2020.
[71] DiaSorin, LIAISON SARS-CoV-2 S1/S2 IgG, 2020. https://www.fda.gov/media/137356/download, 1st November 2020.
[72] Liaison, LIAISON SARS-CoV-2 S1/S2 IgG – FDA Approval, 24 April 2020. Available at: https://www.fda.gov/media/137356/download, 9th October 2020.
[73] Diagnostics Roche, Elecsys® Anti-SARS-CoV-2, 2020. https://diagnostics.roche.com/es/es/products/params/elecsys-anti-sars-cov-2.html, 1st November 2020.
[74] Diazyne, Longitudinal Monitoring of SARS-CoV-2 IgM and IgG Seropositivity to Detect COVID-19, 2020. https://www.diazyme.com/images/products/pdf/Longitudinal-Monitoring-of-SARS-CoV-2-IgM-and-IgG-Seropositivity-to-Detect-COVID-19.pdf, 1st November 2020.
[75] BioCheck, SARS-CoV-2 IgM and IgG Antibody Combo Test Kit, 2020. https://www.fda.gov/media/141251/download, 1st November 2020.
[76] Nanoentek, Qualitative Test for COVID-19 Ag, 2020. http://www.nanoentek.com/theme/nanont2_en/shop/02/product01_view.php?it_id=1602049801#detail_tech1, 1st November 2020.
[77] Becton, B.D. Dickinson, Veritor™ Plus System for Rapid COVID-19 (SARS-CoV-2) Testing, 2020. https://www.bd.com/en-us/offerings/capabilities/microbiology-solutions/point-of-care-testing/bd-veritor-plus-system-for-rapid-covid-19-sars-cov-2-testing, 1st November 2020.
[78] Quotient, The MosaiQ Covid-19 Antibody Microarray, 2020. https://quotientbd.com/covid-19, 1st November 2020.