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

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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
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 blind-
folded” 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 high-
light 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 per-
fomed 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 (<1%), 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 con-
tains 4 different type of proteins; spike surface glycoprotein (S), enve-
lope 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 specific proteins of the virus and analysing its specific genomic infor-
mation. The viral dose detected by RNA is very high (10^7–10^8
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) anti-
odies 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 anti-
bodies 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.

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. 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).](chart1)

![Fig. 2. Graph on temporal dynamics on the Infection disease [8,10] versus analyte dose [7,11,12].](chart2)
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 amplified 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 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 who are infected.

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 by day 10 [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, it 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%), fibrobronchoscopic brush biopsy (46%), pharyngeal swabs (32%), feces (20%), 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, 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 double 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, Species 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 Anitooa 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

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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.
| Company (Country) | Kit Name | Regulation (Validation Date) | Collection | Gene | LOD | Analysis Time (min.) |
|------------------|----------|------------------------------|------------|------|-----|---------------------|
| Pathomics Health (China) | Real-Time Fluorescent RT-PCR 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 |
| Bio-Maxima (Poland) | 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 | 4 copies/reaction | <180 |
| 3DMed (China) | Andis® SARS-CoV-2 RT-qPCR | EUA US FDA | Respiratory | ORF1ab, N, E | ≥10 RNA copies | 62 |
| Biomaxima (Poland) | ARS-CoV-2 Real Time PCR LAB | CE Mark for IVD | Respiratory | ORF1ab, N | 9.35 × 10³ copies/mL | 63-90 |
| Co-Diagnostics (USA) | Logix Smart™ 2019-nCoV qPCR test | EUA US FDA (Apr 3, 2020) | Respiratory | RdRp | 10 copies/reaction | 150 |
| ELITech Group (Republic of Korea) | GeneFinder™ COVID-19 Plus RealAmp Kit | EUA US FDA (April 18, 2020) | Respiratory | RdRp, E and N | 200 copies/mL | 30 |
| Luminex Corp. (US) | Roche cobas SARS-CoV-2 Test | HSA (March 19, 2020) | Pharyngeal swab | RdRp, E | 200 copies/mL | 4800 |
| PrimerDesign (United Kingdom) | Genesig 2019-nCoV | EUA US FDA (Mar 20, 2020) | Nasopharyngeal swab | ORF1ab, E | <100 copies | 210 |
| Sanaure Biotech (China) | Sanaure Biotech 2019-nCoV | EUA US FDA | Nasopharyngeal swab | ORF1ab, N | 200 copies/mL | 40 |
| SD Biosensor (Republic of Korea) | Std M nCoV Real Time Kit | EUA US FDA (April 23, 2020) | Nasopharyngeal swabs throat swab | RdRp, E | 5 × 10³ copies/mL | NR |
| LifeRiver Bio-Tech (United States) | LifeRiver Real Time Multiplex RT-PCR | Commercially available | Bronchoalveolar lavage, Sputum | ORF1ab, E | 1 copy/μL | <90 |
| Gencurix (Korea) | GenePro COVID-19 Detection Test + v2 | Received CE Mark for IVD | Sputum, Nasopharyngeal swab | ORF1ab, E | 3.310² copies/mL | 90 |
| AssayGenie (UK) | COVID-19 2019-CE IVD qPCR assay | CE Mark | Nasal swabs, Nasopharyngeal swabs, sputum, bronchial washes | ORF1ab and N | 200 copies/mL | NR |
| Beijing Applied Biological (China) | Multiple Real-Time PCR Kit | EUA US FDA | Nasopharyngeal swabs | ORF1ab, N | 3.310² copies/mL | NR |
| BioFire Defense (UK) | BIOFIRE® COVID-19 test | EUA US FDA | Nasopharyngeal swabs | ORF1ab, RdRp | 1000 copies/mL | 45 |
| Getein Biotech (China) | Novel Coronavirus Real Time PCR Kit | Received CE Mark for IVD | NasopharyngealOropharyngeals bronchoalveolar lavage | ORF1ab, | 200 copies/mL | NR |
| Kogene (Korea) | PowerChex™ Real Time PCR | EUA Korean CDC (Feb 4, 2020) | Nasopharyngeal swabs and throat swab | RdRp, E | 3.310² copies/mL | 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 Analetics (Italy) | REALQUALITY RQ-2019-nCoV Fluorescent RT-PCR Kit, Target: ORF1ab | EUA US FDA (Mar 27, 2020) | Respiratory specimens | RdRp, E | 1-10 copies | NR |
| BGI Genomics (Denmark & China) | PerkinElmer® New Coronavirus NA | EUA US FDA (Mar 24, 2020) | Respiratory specimens | ORF1ab | 100 copies/mL | 180 |
| BioMérieux (France) | SARS-COV-2 R-GENE® & BIOFIRE® FILMARRAY® | EUA US FDA (Mar 24, 2020) | Respiratory specimens | ORF1ab, ORF8 | 100 copies/mL | NR |
| CTK Biotech (US) | Aridita COVID-19 Real-Time PCR test | Australia’s ARTG (April 24, 2020) | Nasal, Sputum | ORF1ab, N | 500 copies/mL | NR |
| Certas Biotech SL (Spain) | VIASURE® Real-Time PCR Detection Kits | Australia’s ARTG (March 21, 2020) | Respiratory specimens | ORF1ab, N | >10 copies | 120 |
| Genomics (Spain) | qCOVID-19 | CE Mark (Mar 6, 2020) | NR | NR | 100% | <120 |
| Liming Bio (China) | StrongStep®Novel Coronavirus Multiplex rPCR | CE Mark | Respiratory specimens | ORF1ab, E, N | NR | NR |
| KH Medical (Korea) | RADI COVID-19/COVID-19 Triple Detection (CE IVD) | EUA US FDA (April 21, 2020) | Respiratory specimens | RdRp, E | 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 | 1copie/25 μL | 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) | Nasopharyngealocularpharyngeal and nasal swab | Nsp2, N | 80 |
| Luminoex 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 |
| Genomics (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 conditions and procedures 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 clinial 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 analysis, enhancing the cost and time for the test. Variables very relevant in the 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 sample the analyse 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 press a button the result should come out, with reliable results, obtained at short time and in a cost-effective way. It may sound chimeric, but fortunately all these needs are already answer with the point of care (POC) diagnostic 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 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 automated 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 NOW®TM 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 read-out. 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 (MB-Dio 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.
Antibody to counterattack the action of the virus. In this serologic test, the most usual antibodies utilized for this purpose are the ones against viral antigens. This type of immune tests is commonly referred as Antigen tests.

- **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 viral antigens. 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 subsequently. However, serologic immunosensor has a very relevant advantage over genomic diagnosis. These platforms can detect the virus even when this virus evolves. 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 antigen detection. 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 response in the first week of infection there is a higher viral load that decreases

![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.](image)

| 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 | RNA swab nasopharyngeal | ORF1b-nsP1,4 & Orf1ab/RdRp | NR | —20 |
| GenMark Diagnostics (US) | ePlex SARS-CoV-2 | EUA from US FDA (Mar 19, 2020) | RNA swab nasopharyngeal | E and N genes | 0.25 copies/μl | 45 |
| Cepheid (US) | Xpert® Xpress SARS-CoV-2 | EUA from US FDA (Mar 03, 2020) | RNA swab nasal nasopharyngeal | RdRp & N genes | 100% | 5-13 |
| Abbott (US) | Abbott ID NOW COVID | EUA from US FDA (Mar 27, 2020) | RNA swab nasopharyngeal | RNA (throat, nasal, sputum, feces) | NR | —25 |
| MicrornaDX (UK) | Rapi Prep COVID-19 | Awaiting CE mark | RNA swab nasal | E and N genes | 12 copies/μl | <45 |
| Mammoth Bioscience (US) | SARS-COV2 DETECTR (LAMP) | EUA from US FDA (August 31, 2020) | RNA swab nasopharyngeal | OrfL and S genes | NR | 0 |
| Biomeme (US) | SARS-CoV-2 Test | EUA from US FDA (August 11, 2020) | RNA swab nasopharyngeal | RdRp & N genes | NR | 0 |
| Fluxery (Germany) | COVID-19 Test at Point-of-Care | CE Mark | RNA swab nasal | E and N genes | 6,75 copies/μl | 40 |
| Sherlock BioSciences (US) | Sherlock CRISPR SARS-CoV-2 | EUA from US FDA (May 06, 2020) | RNA swab nasopharyngeal | ORF1ab, N | 75 copies/μl | 30 |
| Detectchem Inc. (US) | MobileDetect Bio BCC19 Coronavirus | EUA from US FDA (September 1, 2020) | RNA swab nasopharyngeal | E and N genes | 75 copies/μl | 30 |

- **Table 2**

Commercially available DNA base POC for a rapid SARS-COV-2 infection diagnosis in humans.
throughout the disease, reducing the chances of being 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 immunoassays on the market are based on serological detection.

The amount of analyte in the case of the serologic immune test is just 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 immunoassays technologies commercially available for Covid-19 diagnosis; the traditional ELISA platforms, the most cost-effective lateral flow immunoassays and the most evolved microfluidic POC immunoassays.

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 steps 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 EUROMURN, 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 impregnated 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.](image-url)
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

| 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 | IgG – 96.6% | 90 |
| Epitope Diagnostics, (United States) | EDI Novel Coronavirus COVID-19 IgG ELISA kit | CE-IVD marked | Human serum | Serologic IgM, IgG | NR | 80 |
| Livzon (China) | Diagnostics kit for IgM/IgG to covid | | Whole blood sample | Serologic IgM, IgG | NR | NR |
| Mount Sinai Laboratory (US) | COVID-19 ELISA IgG Antibody test | | Serum and plasma | Serologic IgG | NR | NR |
| Thermo Fisher Scientific (USA) | OmniPATH COVID-19 Total Antibody ELISA Test | | Serum and plasma | Serologic IgM, IgG | NR | NR |
| ZEUS Scientific, Inc. (US) | ZEUS ELISA SARS-CoV-2 IgG Test System | | Serum and plasma | Serologic IgM, IgG | NR | NR |
| University of Arizona Genetics Core for Clinical Services (US) | COVID-19 ELISA pan-Ig Antibody Test | | Serum and plasma | Serologic IgM, IgG | NR | NR |
| Bio-Rad Laboratories, Inc. | Platelia SARS-CoV-2 Total Ab assay | | Serum and plasma | Serologic IgM, IgG | NR | NR |
| University of Arizona Genetics Core for Clinical Services (US) | COVID-19 ELISA pan-Ig Antibody Test | | Serum and plasma | Serologic IgM, IgG | NR | NR |
| Bio-Rad Laboratories, Inc. | Platelia SARS-CoV-2 Total Ab assay | | Serum and plasma | Serologic IgM, IgG | NR | NR |
| University of Arizona Genetics Core for Clinical Services (US) | COVID-19 ELISA pan-Ig Antibody Test | | Serum and plasma | Serologic IgM, IgG | NR | NR |

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

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 brings 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%

| 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)    | Whole Blood       | Serological (IgM, IgG)     | NR          | 15          |
| Rapid Test Methods      | COVID-19 IgM/IgG Lateral-Flow Kit            | CE Mark (March 25, 2020)         | Whole Blood       | Serological (IgM, IgG)     | 95%         | 10          |
| (Ireland)               |                                               |                                  |                   |                            |             |             |
| Era Biology (China)     | Virusee COVID-19 IgG/IgM Lateral Flow        | CE Mark                          | Whole Blood       | Serological (IgM, IgG)     | NR          | 10          |
| AssyGenie (UK)          | Acro Biotech COVID-19 Rapid POC              | CE Mark                          | Serum, plasma or whole blood | Serological (IgM, IgG)     | 100% (IgG), 85% (IgM) | 15          |
| CKT Biotech (USA)       | OnSite COVID-19 IgG/IgM Rapid Test           | Australian’s ARTG (March 19, 2020) | Whole Blood       | Serological (IgM, IgG)     | 96.9%       | 10          |
| Shenzhen 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      | DPP COVID-19 IgM/IgG Test                    | EUA US FDA (Apr 14, 2020)        | Whole Blood       | Serological (IgM, IgG)     | IgM: 50% IgG: 100% | 15          |
| and LumiraDx (USA)      |                                               |                                  |                   |                            |             |             |
| Edinburgh Genetics (UK) | COVID-19 Colloidal Gold Immuonassay Testing Kit | CE-IVD, China-FDA | Serum, plasma or whole blood | Serological (IgM, IgG)     | 99.31%      | 10          |
| Innoviva Biological     | 2019-nCoV Antibody Test (colloidal gold)     | China’s NMPA. Australian’s ARTG | Serum, plasma or whole blood | Serological (IgM, IgG)     | 87.3%       | NR          |
| Technology (China)      |                                               | CE-IVD approved.                 |                   |                            |             |             |
| Everest Links Pte       | VivaDiag™ COVID-19 IgM/IgG Rapid Test        | Australian’s ARTG (Mar 26, 2020) | Serum, plasma or whole blood | Serological (IgM, IgG)     | NR          | NR          |
| (Singapore)             |                                               | CE Mark                          |                   |                            |             |             |
| BioMedomics/Jiangsu     | COVID-19 IgM/IgG Rapid Test                  | China’s NMPA.                    | Serum, plasma or whole blood | Serological (IgM, IgG)     | 88.66%      | NR          |
| Medomics (USA)          | Lateral flow                                 | CE Mark (Mar 8, 2020)            |                   |                            |             |             |
| Mologic (UK)            | Lateral flow immunoassay for SARS-CoV-2      | CE mark.                         | Whole Blood        | Serological (IgM, IgG)     | 99%         | 243         |
| Hangzhou Biotec (China) | 2019-nCoV IgG/IgM Rapid Test Cassette        | CE Mark                          | Serum, plasma or whole blood | Serological (IgM, IgG)     | 87%         | NR          |
| Pharmact AG49 (Germany) | CoV-2 Rapid Test                             | Australia’s ARTG.                | Whole Blood        | Serological (IgM, IgG)     | 100%         | 20          |
| Zhejiang Orient Gene    | COVID-19 IgG/IgM Rapid Test                  | China’s NMPA. CE Mark.           | Serum, plasma or whole blood | Serological (IgM, IgG)     | 97.9%       | NR          |
| Biotech (China)         |                                               | EUA US FDA Australia’s ARTG (April, 1, 2020) |                   |                            |             |             |
| Cellnex (USA)           | qSARS-CoV-2 IgG/IgM Rapid Test               | EUA US FDA (Apr 1, 2020)         | Serum, plasma or whole blood | Serological (IgM, IgG antibodies) | 93.8%       | NR          |
| Mobidiag (Finland)      | Anti-SARS-CoV-2 Rapid Test                   | CE-IVD marked                    | Serum, plasma or whole blood | Serological (IgM, IgG)     | 96.2%       | <15         |
| Qingdao Hightop (China) | SARS-CoV-2 IgM/IgG Antibody Rapid            | Australian’s ARTG (March 31, 2020) | Serum, plasma or whole blood | Serological (IgM, IgG)     | 97%         | 15          |
| SD Biosensor (Korea)    | STANDARD 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 | NR          | 30          |
| SD Biosensor (Korea)    | STANDARD COVID-19 Ag Test                   | CE-IVD marked                    | Nasopharyngeal swab | Antigen | NR          | 60%         |
| Coris BioConcept        | COVID-19 Ag Respi-Strip                      | CE-IVD marked                    | Nasopharyngeal swab | Antigen | NR          | 15          |
| (Belgium)               |                                               |                                  |                   |                            |             |             |
| Shenzhen Bioeasy (China)| 2019-nCoV Fluorescence Ag Rapid Antigen     | CE-IVD marked                    | Nasal swab/sputum  | Antigen | NR          | 10          |
| RapiGEN (Korea)         | BIO-CREDIT COVID-19 Ag Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit | CE-IVD marked | Nasopharyngeal swab | Antigen | 98%         | 5-8         |
| Nirmidas Biotech, Inc.  | SGTi-flex COVID-19 IgG                      | EUA US FDA (September 29, 2020)  | Nasopharyngeal swab | Antigen | NR          | NR          |
| Sugentech, Inc. (Korea) |                                               | EUA US FDA (September 03, 2020)  | Nasopharyngeal swab | Antigen | NR          | NR          |
| Biocan Diagnostics      | Tell Me Fast Novel Coronavirus (COVID-19)    | EUA US FDA (August 25, 2020)     | Nasopharyngeal swab | Antigen | NR          | NR          |
| Inc. (Canada)           | BIOTIME SARS-CoV-2 IgG/IgM Rapid Qualitative Test | EUA US FDA (July 07, 2020)     | Nasopharyngeal swab | Antigen | NR          | NR          |
| Xiamen Biotime          | CareStart COVID-19 IgM/IgG                   | EUA US FDA (July 24, 2020)       | Nasopharyngeal swab | Antigen | NR          | NR          |
| Biotechnology (China)   |                                               |                                  |                   |                            |             |             |
| Access Bio, Inc. (US)   |                                               |                                  |                   |                            |             |             |
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, Edin-burgh Genetics, Mologic, RapiGen among other, which demonstrated similar performance as ELISA and fully automatized 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 sensi-
tivity, and the analysis time.

Although the great advantages of immunostriip, 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 hos-
pitals started to use these rapid test, since high amounts of false nega-
tives 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 labora-
tories 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 to-
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 automatized 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-aminobutyly) -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 H2O2, for getting a chemi-

lmuminescence response.

In April 2020, three companies introduced new equipment for COV
ID diagnosis with a fully automatized 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 equip-
ment, since needs 18 min more to fulfill 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 ARCHICETE 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].

Moonths later, FDA approved three devices with alike magnetic beads separation and chemiluminescence read out, similar to Snibe’s tech-

nology. 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 SARS-CoV-2 from the Dizayne 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 automatized 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 afford-
able benchtop portable device (50 × 50 × 30 cm approximately). Bio-
chek Inc. commercialized the BioCheck SARS-CoV-2 IgM/IgG Antibody Test Kit analysed in the portable Sophonix MS-Fast Automated Chemi-
luminescent 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 automatized 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 automatized immunoassays based on chemiluminescence analysis and the MosaïQ 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 IgG ELISA 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 Euroimmun 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 immunoassay 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 PO 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 pre-treatment, 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 treatment. 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 contaminatns 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-CoV-2 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.

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