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Review of COVID-19 testing and diagnostic methods

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ABSTRACT

More than six billion tests for COVID-19 has been already performed in the world. The testing for SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) virus and corresponding human antibodies is essential not only for diagnostics and treatment of the infection by medical institutions, but also as a pre-requisite for major semi-normal economic and social activities such as international flights, off line work and study in offices, access to malls, sport and social events. Accuracy, sensitivity, specificity, time to results and cost per test are essential parameters of those tests and even minimal improvement in any of them may have noticeable impact on life in the many countries of the world. We described, analyzed and compared methods of COVID-19 detection, while representing their parameters in 22 tables. Also, we compared test performance of some FDA approved test kits with clinical performance of some non-FDA approved methods just described in scientific literature. RT-PCR still remains a golden standard in detection of the virus, but a pressing need for alternative less expensive, more rapid, point of care methods is evident. Those methods that may eventually get developed to satisfy this need are explained, discussed, quantitatively compared. The review has a bioanalytical chemistry prospective, but it may be interesting for a broader circle of readers who are interested in understanding and improvement of COVID-19 testing, helping eventually to leave COVID-19 pandemic in the past.

1. Introduction

Viruses are nanometer-scale pathogens that can only replicate inside a host organism. Viruses infect animals, plants, bacteria, and fungi. The majority of viruses are specific to one species, but some viruses can infect organisms that belong to different species, such as influenza A and C. Some viruses mutate and jump from one species to another, such as coronaviruses. Viruses can not survive without a host. Often viral infections lead to the host cell death. Some viruses do not cause obvious changes in host cells and can stay inactive for years, causing chronic viral infections. The majority of viruses do not cause death of a host organism because it would stop viral spread. However, some viruses result in potentially lethal diseases.

Viruses are grouped based on the nucleic acid they use to encode genetic material: DNA or RNA. The majority of DNA viruses carry double-stranded DNA (dsDNA) and rarely single-stranded DNA (ssDNA). These viruses use host cells' DNA-dependent DNA polymerase for replication. As DNA polymerase has proof-reading abilities, DNA viruses mutate to a lesser extent and are milder. RNA viruses usually carry single-stranded RNA (ssRNA), but some contain double-stranded RNA (dsRNA). ssRNA viruses are subdivided into positive-sense, negative-sense, and ambisense RNA viruses. Positive-sense RNA viruses contain genetic material in the form identical to mRNA that can be translated directly by host cells. Negative-sense RNA viruses contain RNA complementary to mRNA, so that viral RNA serves as a template for host cells' transcription via RNA-dependent RNA polymerase before translation is possible. Ambisense RNA viruses contain RNA that has different parts having positive-sense and negative-sense properties. RNA polymerase does not have proof-reading properties; therefore, RNA viruses are prone to a much higher rate of mutations. RNA viruses are also more pathogenic than DNA viruses, for example, coronaviruses, HIV, hepatitis viruses, Ebola, and others.

Methods of viral detection include detection of viral particles (virions), viral antigen, antibodies to the virus, and viral nucleic acid. We consider two categories of scientific publications: one that reports performance of commercialized and/or government-approved diagnostics methods/kits, and another that describes relatively new not-yet-commercialized and not-yet-approved methods. A detection method is

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accepted as a diagnostic test if it satisfies certain analytical requirements. These requirements are a sufficiently low limit of detection, high selectivity, sensitivity, accuracy and preferably high speed of test run. Limit of detection is the lowest concentration of detection target (analyte) that a test can detect. For qualitative diagnostic tests it is the parameter that defines whether the test can be applied to patients with low viral load. For quantitative diagnostic tests another important parameter is – limit of quantification, which is the lowest analyte concentration that can be correctly measured by the test. Limit of detection is especially important since the purpose of testing is to detect virus at much lower viral load than the one seen at the time of symptom onset which for COVID-19 starts from 10^4 copies/mL [1]. Most of the current PCR-based methods have limit of detection at 100 copies/mL or less, which corresponds to the viral detection at 2–3 days before the onset of symptoms [1]. Limit of detection defines a test's sensitivity – the ability of the test to detect viral infection when a virus is present in samples. Sensitivity is expressed in percentage: 100% - % (false negatives). Specificity refers to the ability of a test to return a negative result in cases when virus is absent in samples. Specificity is also expressed in percentage: 100% - % (false positives). Accuracy reflects overall reliability of a method and is expressed as: 100% - % (false negative) - % (false positive). Sensitivity is the rate of true positives, and specificity is the rate of true negatives, and these rates will not necessarily be the same. Therefore, accuracy is another important analytical parameter that measures the rate of correct results for a certain test, expressed in percentage. Usually to calculate accuracy, sensitivity, and specificity, a test's results are compared to an established "gold standard" test, usually RT-PCR. Some other tests with even more remarkable accuracy, sensitivity, and specificity parameters are being developed, such as mass spectroscopy for detection, which can serve as a reference for other tests. Therefore, development of various detection methods is important. If a test is expensive, requires complicated equipment, and takes a long time to results, but has a very low limit of detection and accuracy, it can be used for scientific research.

Coronaviruses are recurrent in human population, such as MERS-CoV (Middle East Respiratory Syndrome Coronavirus) and SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus) viruses, active SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) and other coronaviruses leading to symptoms of common cold. Coronaviruses are positive-sense ssRNA-containing enveloped viruses, having been detected by multiple methods. SARS-CoV-2 is one of coronaviruses, closely related to bat and pangolin coronaviruses [2]. Coronaviruses are known causes of global pandemics due to high transmissibility and mortality; for instance, SARS-CoV outbreak in 2002–2003 with 9.56% mortality rate [3]. MERS-CoV started an epidemic in Middle East in 2012 with 34.4% mortality rate [4].

Highly transmissible SARS-CoV-2 originated in Wuhan, China at the end of 2019 and caused viral respiratory distress and pneumonia, later named COVID-19. By the end of January 2022 about 350 million people have been tested positively with the virus worldwide, with about 5.6 million deaths [5]. GDP (gross domestic product) dropped worldwide in 2020 with the exception of China and Vietnam that experienced up to 3.8% growth. The level of global economic decline in 2020 due to COVID-19 is unprecedented since WW2 and estimated at about 4.2% by World Bank [6]. The most significant decline in GDP happened in the EU, especially Spain and Italy, and India, which lost more than 10% of GDP compared to 2019. The USA and Russia each underwent less than 5% decline in GDP. GDP is estimated to return to pre-coronavirus value by the end of 2021 and experience growth from 2022 [7]. SARS-CoV-2 has had a profound effect on global economy, resulting in stock market volatility, with some national stock markets not having been able to recover yet, e.g. Coronavirus pandemic has caused loss of jobs and increase in worldwide unemployment rates (related to the lack of new job opportunities) due to lockdowns. Travel and hospitality businesses are the most damaged part of industry, with hundred billions of dollars lost in 2020 and 2021 due to governmental restrictions. This sector is not estimated to recover until 2025 [8]. Restrictions have been introduced in order to prevent the spread of SARS-CoV-2, and while it is a necessary step, it cannot be (and is not) endorsed as the only step to control SARS-CoV-2.

The COVID-19 testing is essential not only for diagnostics and containment of the virus but for resumption of flights, reopening of international travels and resumption of other normal economic activities. The total number of COVID tests taken in the world up to March 11, 2022 is about 6 billion while the largest number of tests have been performed in USA (960 M) India (780 M) UK (490 M)and Spain (470 M) [4]. As for COVID-19 testing, most countries perform 2–3 tests per 1000 people every day. The majority of countries have performed 400–1000 tests per 1000 people by now. This puts an additional constraint on economy during coronavirus crisis. RT-PCR tests that constitute the predominant part of these numbers cost on average $50–100 per test, in addition to the costs of electricity, equipment maintenance, and salaries for staff. For this reason, there is a considerable difference in the number of tests between high- and low-income countries. High income countries such as Denmark, Austria, UAE, United Kingdom, and the USA have performed 2000–15 000 tests per 1000 people, while many low income countries such as Sudan, Haiti, DRC, Yemen, Afghanistan and Nigeria performed less than 20 tests per 1000 people [9]. These statistics clearly demonstrate the need of development of new inexpensive detection methods that can be used in developing countries. COVID-19 is the most tested disease in human history, and the need for testing will be preserved in a foreseeable future even after the time when efficient COVID-19 vaccines would be availed to the most people on our planet. Vaccines became available in many countries in early 2021 and majority of adult population got vaccinated in a few countries by end of summer 2021. Now 65% of world population or 5 billion people received a vaccine [10]. Nowadays Omicron spreads faster and considered to be more contagious than original COVID-19 variants [11]. This new trend drove the demand for COVID testing in many countries, particularly developed countries even higher. For instance, 1.2–1.7 million tests per day were performed in UK as 7-day average in January 2022, while it was only 0.5–0.6 M tests performed per day in UK in January [12].

Detection of SARS-COV-2 is equally important for realistically assessing the epidemiological situation nationally and globally and for controlled return towards pre-coronavirus life as is social distancing. Today clinical detection of SARS-CoV-2 is represented mainly by RT-PCR, and serological detection – by antibody-targeted ELISA. RT-PCR is a sensitive and specific method for checking infection status of a person, but it is lengthy, requires complex equipment and a highly skilled operator. In order to safely return to normal life, a test needs to be rapid, portable, and have enough analytical sensitivity to maintain a 90% or higher true positive rate. In this way, a test can be applied to employees who want to return to offline work, in the airports, cinemas, sport complexes. A test that can be run outside laboratory settings is called a point-of-care test. Ideally, it would be a relatively inexpensive test that can be run with many samples and return results in up to 30 min with naked-eye detection. This also becomes important if the zoonotic origin of SARS-CoV-2 is taken into account: in case of transmission of another coronavirus it will be beneficial to have established robust diagnostic tests that are known to detect a virus from this family. For this reason, the most recent detection methods were assessed in this review, some of which have a potential to become novel point-of-care tests.

Some key features of SARS-CoV-2 relevant to its detection include: its genome consists of six open reading frames (ORFs): replicase (ORF1a/ORF1b), spike (S), envelope (E), membrane (M) and nucleocapsid (N) (genes targeted in RNA-based detection). In addition, ORFs encoding accessory proteins are present within viral genome. The virions have spike protein on the surface that binds receptors of the cells for cell infection, and nucleocapsid protein – these two proteins are targeted by antigen detection methods. SARS-CoV-2 shares 79% genome sequence identity with SARS-CoV, and SARS-CoV-2 spike protein's receptor-binding domain has 73% amino acid similarity with SARS-CoV [2].

This can potentially cause cross-reactivity with SARS-CoV during RNA and antigen detection. However, because nowadays occurrence of SARS-CoV is relatively insignificant, such cross-reactivity should not affect test results.

There are a lot of novel methods for SARS-CoV-2 detection in the fields of antigen, antibody to the virus, RNA, and viral particles (virions) detection. Some conventional methods were improved and commercialized, such as antibody and antigen testing kits for point-of-care use [13], developed methods of SARS-CoV-2 RNA detection, such as RT-LAMP (Reverse Transcription Loop-Mediated Isothermal Amplification) and Cas-Crispr, and testing for virus in saliva in addition to blood, serum, and nasopharyngeal swabs. Some conventional gold standard methods are also tuned for SARS-CoV-2 detection, such as lateral flow immunoassays, sandwich ELISA, RT-PCR, making them for lab-on-a-chip basis and/or increasing their sensitivity. Some less conventional methods arise such as field-effect transistor sensors, chronoamperometry sensors, and even mass spectrometry for detection of viral antigen.

Previous reviews on detection of SARS-CoV-2 have focused on laboratory scientific methods [14], commercialized technologies [15], or advances in detection [16]. A thorough meta-analysis of SARS-CoV-2 detection through antibody testing has been conducted by Kontou et al. who evaluated performance of ELISA, CLIA, FIA, and LFIA tests [17]. Another review of antibody-based detection consolidated early published studies and preprints [18]. Hereafter we present a broad scope review about COVID-19 testing and diagnostics methods and applications. In the present review we attempted to calculate average performance parameters (LOD, accuracy, time to results, etc.) for major detection methods from available literature and we compared those average analytical parameters to the parameters of commercialized methods.

This review describes both: detection of COVID-19 virus and detection of antibodies to the virus as reported in scientific peer reviewed literature, but also it quantitatively compares those methods with validated commercial methods of virus/antibodies detection already approved by FDA or other regulatory agencies. Clinical methods of COVID-19 diagnostics, such as X-ray, CT, and lung ultrasound are also included in the review.

1.1. COVID-19 specimen collection and sample handling

Antigens and virions are detected in nasopharyngeal swab or saliva specimens [19]. Before discussing detection methods, it is necessary to discuss pre-analytical issues in nasopharyngeal swab collection and storage. Pre-analytical handling of nasopharyngeal swabs is critical for obtaining reliable results. According to Pondaven-Letourmy et al., false negative rate of RT-PCR is around 30%, although RT-PCR itself is a highly sensitive detection method. One of the reasons for high rate of false negatives could be an improper nasopharyngeal sampling [20–23]. SARS-CoV-2 uses binding of the spike protein to a cellular receptor ACE2 for cell infection, which is expressed at a higher rate in distal part of a nose in comparison to their expression in a proximal part of a nose [24]. Assuming that the technique for nasopharyngeal swab collection is standardized; storage conditions are coming into consideration. Basso et al. checked how different storage conditions affect sensitivity of RT-PCR test. The lowest values of threshold cycles (lowest detection limit) were observed when nasopharyngeal swabs were stored in the refrigerator at +4 °C in the solution of extraction buffer to preserve RNA. The researchers also concluded that it is very reliable to store swabs in a viral transport media at room temperature for up to 2 days before RT-PCR test. For longer time periods refrigeration is required [25]. As for antigen testing, it is recommended to store nasopharyngeal swabs in sterile, dry, sealed plastic tube. If swabs are stored in a viral transport media, its volume should not exceed 1 mL and it should not contain guanidinium. Swabs can be stored for up to 8 h at room temperature and 1 day refrigerated at +2–8 °C [26].

2. Detection of SARS-CoV-2 viruses

Major methods of detection of SARS-CoV-2 virus is based on detection of viral RNA. Those methods include RT-PCR, as so far, the most common method of viral RNA detection. RT-PCR is represented by publications about non-commercially approved methods, and by reports on commercialized government-approved assays. Other methods include Droplet Digital PCR (ddPCR), multiplex PCR, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), Loop-mediated Isothermal Amplification (LAMP), Recombinase polymerase amplification (RPA), Recombinase Aided Amplification (RAA), and Pulse Controlled Amplification (PCA).

2.1. Detection of RNA by RT-PCR: principles and applications

The golden standard of COVID-19 virus detection is considered a reverse-transcription Polymerase Chain Reaction (RT-PCR). PCR is one of the common techniques used to detect viral nucleic acid. PCR is used to amplify the number of copies of DNA samples. Its creator Kary B. Mullis was awarded the Nobel Prize in Chemistry in 1993 [27], while he died in 2019 from complications of pneumonia [28].

Original PCR method can detect DNA [29], but if RNA is needed to be detected, RNA is reverse transcribed into the complementary DNA (cDNA) by reverse transcriptase. The PCR method used nowadays is mostly Real-Time PCR or, in other words, Quantitative PCR (qPCR). Using this method, one is able to amplify and detect the concentration changes in amplicon concentration in real time, while conventional PCR measures that at the end of the process. Common methods used in qPCR for the detection of PCR products are DNA binding fluorescent dyes and using fluorescent signals produced by DNA probes. Photodetectors are used in the qPCR to collect data by only allowing passage of the wave of desired wavelengths [30].

Fig. 1 shows the process of qPCR. First, the sample is taken from a person and RNA is extracted. This can be done by automated equipment or kits prepared for RNA extraction. Moreover, Arizti-Sanz et al. [31] and Ramachandran et al. [32] developed their own techniques for RNA extraction, such as HUDSON (Heating Unextracted Diagnostic Samples to Obliterate Nucleases), and ITP (Isotachophoresis). Usually, RNA extraction requires 5–30 min and is followed by the amplification and reverse transcription of viral RNA to obtain cDNA.

Then, reverse transcription is performed to form complementary DNA. Using qPCR apparatus cDNA is amplified and its amount is analyzed.

While reviewing analytical methods, it is important to mention the prices of the machines. As of January 30, 2019, the price of a simple PCR machine was 4912 USD (Bio-Rad T100 thermal cycler), while rtPCR costs from $15000 (RotorGene models) to over $90000 (QuantStudio 12k). The price of the apparatus usually differs because of the performance quality of the instrument. Therefore, careful considerations should be taken to choose the appropriate instrument [33].

The PCR method has several advantages. This method requires a small sample amount for analysis and has high sensitivity and accuracy values. In comparison with other diagnostic methods (cultivation of bacteria, etc.) this method is relatively fast, as it takes only several hours. Newly developed methods based on PCR can even identify microorganisms in several minutes [34–36]. However, PCR still has some drawbacks. For example, the method is condition-sensitive and sometimes can produce false-positive results. As it was mentioned before, PCR machines are expensive and apart from the cost of the apparatus, the transportation also contributes to the cost. Workers at the laboratory should have special training on how to use the instrument. Detection of each type of microorganisms' genetic material requires the usage of special primers, which should be purposely manufactured. Given that, scientists are working on a cost-effective and accurate method of the detection of the virus.

RT-PCR methods used for COVID-19 diagnostics are summarized in

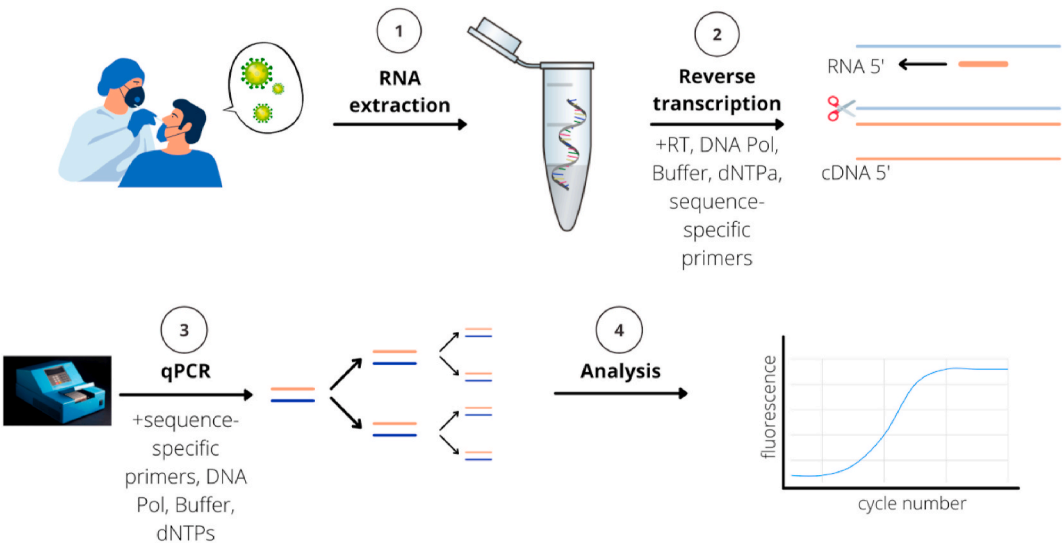

Fig. 1. Illustrated qPCR process from sample collection to result readout (Created in canva.com//Google Spreadsheet//"File:Baby Blue - a prototype polymerase chain reaction (PCR), c 1986. (9663810586).jpg" by Science Museum London/Science and Society Picture Library is licensed under CC BY-SA 2.0s). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Analysis of RT-PCR methods from scientific literature.

Method	Sensitivity	Specificity	Accuracy	Sample size, samples	Time (min)	LOD
RT-PCR [37]	100%	100%	95.45%	44	3-5/batch	1 cp/rxn
RT-PCR [40]	32.5–32.9 per 1000 copies	N/A	N/A	1000	N/A	N/A
RT-PCR [41]	100%	100%	100%	9	N/A	N/A
RT-PCR [38]	29.2%	100%	92%	2923	N/A	5 cp/rxn
RT-PCR [42]	100%	100%	100%	184	N/A	N/A
RT-PCR [43]	91.7%	N/A	91.7%	12	days	N/A
qRT-PCR [35]	100%	100%	100%	51	70	5 cp/rxn
Automated Direct rt-qPCR [39]	100%	100%	100%	2127	57	20 cp/rxn
qRT-PCR [44]	100%	100%	100%	26	120	15 cp/rxn
RT-PCR [45]	N/A	N/A	100%	297	N/A	3.6–39 cp/rxn
Average ± st.dev Range	83.7 ± 30.1% (29.2%; 100%)	100 ± 0% (-; 100%)	97.7 ± 3.6% (91.7%; 100%)	667 ± 1040 (9; 2923)	62.75 ± 47.7 (3–5 min; days)	9.2 ± 7.9 (1; 10)

Note: Average ± standard deviation; Range (x%, y%); Accuracy= (TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

Table 1. The major performance parameters summarized in this table include sensitivity, specificity, and accuracy. The sensitivity is defined as the ability of a particular test to detect a virus when the virus is present in a sample, and is expressed in % (100% or all negative – % of false negative results). Specificity refers to the ability of a particular test to show a negative result when the virus is absent from a sample, and is expressed in % (100% or all positive – % of false positive results). Accuracy denotes the percentage of times at which the performed test results are correct. Mathematically it is expressed as Accuracy = (TP + TN)/(TP + FP + FN + TN), where TP are true positive, TN are true negative, FP are false positive and FN are false negative results. Among detection methods that used RT-PCR to detect SARS-CoV-2 virus, Wang et al. (2020) was the fastest [37]. In this method nasopharyngeal or oropharyngeal swabs were used as samples. First, RT-PCR was performed to amplify viral DNA, in particular, the conserved regions in the genome of the virus. The uniqueness of this method is the usage of upgraded *Pyrococcus furiosus* Argonaute (PfAgo), which were added (along with guide DNAs and molecular beacons) to the PCR products. After the incubation fluorescence signal was detected. Even though the cost of the RT-PCR apparatus is high, the time used was shortened from more than an hour to 3–5 min per batch. Apart from being fast, this method showed high value of specificity and sensitivity (100%) and accuracy (95.45%). The LOD of this method was equal to 1 copy per reaction, which was the lowest among similar methods. Wang et al. (2020) also reported that this method was effective among COVID-19 mutations due to single nucleotide specificity [37]. The authors acknowledge, however, that the performance of the method is probe-sensitive. Therefore, wide application of this method requires thorough prior validation.

Almost all methods reported high sensitivity values, but the method by Lu et al. (2020) showed low sensitivity values (29.22%) [38]. Lu et al. (2020) based their assay design on previous diagnostic assays that had been developed for detection of MERS-CoV and SARS-CoV and targeted the N gene. Also, this study began when a limited amount of genetic material was available and samples from different sources were used. Each of these factors could have affected the final sensitivity value. The main conclusion of this study is that RT-PCR assays are most efficient when samples are taken from the upper respiratory tract.

The highest (worst) LOD was observed in the work of Ji et al. (2020) - 20 copies per reaction [39]. The developed assay was automated and direct, so the process of detection is simple. The method had high sensitivity, specificity and accuracy values (100%) which were tested on 2127 samples. A big sample size guarantees the method's reliability. The whole process takes 90 min and the positive result can be detected in 57

min. These values are faster than other suggested methods based on RT-PCR. Moreover, the apparatus can detect 16 samples simultaneously, and other viruses can be detected as well, so it has a very broad spectrum of usage.

Concluding, the methods mentioned above once again show that RT-PCR is a gold standard for the detection of viruses. Almost all analyzed methods showed 100% specificity, good sensitivity and accuracy values, as well as low limit of detection. However, these methods are relatively slow and they were tested on small sample sizes. Another interesting thing to note is that the number of papers using RT-PCR is relatively small, which could mean that this method has achieved its best, so now science should take another approach and come up with other methods to detect the microorganisms.

2.2. Overview of the results of the commercialized RT-PCR methods

The most important commercial method for diagnostics of COVID-19 is a conventional RT-PCR, which was discussed in detail in the corresponding section. This is a gold standard in SARS-CoV-2 detection, to which other methods are often compared in terms of specificity and sensitivity. In this section modified RT-PCR and other methods that are represented with point-of-care tests and portable kits will be discussed. All point-of-care tests discussed in this section are listed in the table below.

Table 2 summarizes performance of government-approved RT-PCR kits. RT-PCR kits provide the most sensitive detection, with the best methods having detection limit of several copies per reaction. These tests can take up to 3 h to get results. Results are usually detected by a fluorescence analyzer. The sensitivity of these tests tends to be lower than in RT-PCR published in the literature but higher than for antigen and antibody detection. One disadvantage for some of the RT-PCR tests based on kits is that they are not fully automated. For example, "RealStar® SARS-CoV-2 RT-PCR Kit" by Altona comes with software that must be run and controlled by the user, who also needs to prepare purification reagents and control centrifugation [46]. In other kits RNA extraction must be performed manually by the user. This means that even medical staff may need additional training in using these devices. Another caveat is that sometimes RT-PCR kits require materials for detection, but do not provide them. For example, "Real-Time Fluorescent RT-PCR Kit" by BGI in collaboration with Pathomics Health requires RT-PCR system with software, nucleic acid extraction kit, vortex mixer and other materials that are not provided [47].

Among FDA-registered RT-PCR kits the best sensitivity results are by "Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay" (France, 2.65 copies/reaction), "PerkinElmer® New Coronavirus Nucleic Acid Detection Kit" (UK, 3 copies/reaction), "Allplex™ 2019-nCoV Assay" (USA, 1.25 copies/reaction), "1copy™ COVID-19 qPCR Multi Kit" (South Korea, 4 copies/reaction), and "TaqPath™ COVID-19 Combo Kit" (USA, 10 copies/reaction). These kits also have high clinical accuracy, up to 100%. The worst sensitivity results among FDA-registered RT-PCR kits are by "PowerChek™ 2019-nCoV Real-time PCR Kit" (South Korea, 560 copies/reaction), "Logix Smart™ Coronavirus Disease 2019 (COVID-19) Kit" (China, 600 copies/reaction) and "NxTAG CoV Extended Panel Assay" (Canada, 1000 copies/reaction). All 3 of those least sensitive FDA-approved test kits are reported to have an absolute accuracy of 100%, however they report using specimens spiked with viral RNA to the concentration above detection limit. It means that it is unknown if the limit of detection of these kits is enough for detection of RNA in clinical specimens of patients infected with SARS-CoV-2.

Among non-FDA registered tests, the best sensitivity results are shown by "Aridia COVID-19 Real-Time PCR test" (USA, <10 copies/reaction) and "Zena Max – AMD SARS-COV-2" (UK, 10 copies/reaction).

FDA-registered PCR tests demonstrate better sensitivity than non-FDA registered tests. It can be concluded that nearly all registered commercialized kits are suitable for detection of virus in an average clinical specimen with viral infection. If virus has small number of replicates, the most sensitive FDA-registered kits will detect it, as they have much lower detection limits. Average clinical accuracy of non-FDA registered tests is higher than that of FDA-registered tests, but this data is only for 5 non-FDA registered tests with reported clinical accuracy.

Geometric mean of limit of detection of FDA-registered RT-PCR kits is lower than that of non-FDA registered tests, meaning that FDA-registered tests have higher analytical sensitivity. This means that more tests need to be approved by FDA for emergency use authorization. It is also necessary to keep in mind that FDA-registered tests use on average much more samples for validation of results, and all except 3 non-FDA registered tests did not report the number samples used. This contributes to the clinical performance results, which can change upon using a bigger sample pool. Comparison between tests can be seen in the Table 3.

2.3. Other nucleic acid-based methods (ddPCR, multiplex PCR)

PCR kits are widely used in the detection of viral genome and considered to be the golden standard. However, as mentioned earlier, PCR methods have limitations (time of detection, condition-sensitive, cost, etc.) Biosensors are believed to be better at detection. Biosensors showed high sensitivity, selectivity and accuracy. Moreover, biosensors are cost-effective and have faster time of detection. Biosensors for the detection of other respiratory viruses have been developed. For example, Veerapandian, et al. developed dual immunosensor for the detection of influenza A virus [77] or optical biosensor for the detection of SARS-CoV by Huang et al. [78]. At the time of writing this review, two methods for the detection of SARS-CoV-2 have already been proposed.

Biosensors created by several groups of scientists in general were faster in the detection of viral genome than qPCR methods. Alafeef et al. were able to create a biosensor with the detection time less than 5 min [79]. Moreover, this biosensor is low-cost and easy-to-implement. It has a quantitative paper-based electrochemical sensor chip which can digitally detect the SARS-CoV-2 genetic material. It uses gold nanoparticles, capped with highly specific antisense oligonucleotides (ssDNA) targeting viral nucleocapsid phosphoprotein (N-gene). The sensing probes are immobilized on a paper-based electrochemical platform to yield a nucleic-acid-testing device with a readout that can be recorded with a simple hand-held reader. Its detection limit is 6.9 copies per μL, which can be low in comparison with PCR methods [79]. However, this method does not require further amplification, which helps to save time. Another advantage of this method is an ability to detect mutated virus genomes, which is useful given the rise in cases with varied and more contagious types of the virus. The biosensor was tested on clinical samples, when tests swabs were collected from the nasal region or saliva. The sample size was equal to 48, among which 22 are positive samples and 26 are negative. It had 100% sensitivity, 100% specificity and 100% accuracy [79]. However, the sample size is relatively small. Therefore, to achieve higher confidence in those impressive results, a bigger sample size should be tested. Qiu et al. created photothermal biosensors with LOD equal to 0.22 pM. They used two-dimensional gold nanoislands (AuNI chips) with complementary DNA receptors to achieve nucleic acid hybridization, which allowed the sensitive detection of the viral genome. Time required for detection is yet to be reported. Clinical sample size (82) was also small, so this method is yet to be researched. However, low limit of detection shows that this method can be reliable and practical [80].

Droplet Digital PCR (ddPCR) was first introduced by Saiki et al. (1988) [81]. The principle used commonly now was introduced by Diehl et al. (2006) [82]. ddPCR is a relatively new technique and became commercially available in 2011 by BioRad (Hindson et al., 2011) [83]. ddPCR uses Taq polymerase in a standard PCR reaction for the amplification of a target DNA fragment from a complex sample using pre-validated primer or primer or probe assays. During the ddPCR the reaction is partitioned into thousands of reactions prior to amplification. Also, the data in ddPCR is collected at the reaction end point. These

Table 2
Performance of commercialized government-approved RT-PCR kits.

Name	Sample	LOD	Clinical accuracy	Time, min	Ref
BGI Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2	Nasopharyngeal swabs, 750 μL	75 copies/reaction 384 samples	Sens = 88.1% Spec = 99.6% Acc = 87.7%	180	[46,48] US FDA
Altona RealStar® SARS-CoV-2 RT-PCR Kit	Nasopharyngeal swabs, 500 μL	19 copies/reaction 69 samples	Sens = 95% Spec = 100% Acc = 95%	>30	[47] US FDA
3DMed ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit	Nasopharyngeal swabs, 200 μL	40 copies/reaction 136 samples	Sens = 97.2% Spec = 93.1% Acc = 90.3%	30	[49] US FDA
Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay	Oropharyngeal swabs, 700 μL	2.65 copies/reaction 100 samples	Sens = 94.7% Spec = 100% Acc = 94.7%	70	[50] US FDA
GeneFinder™ COVID-19 Plus RealAmp Kit	Nasopharyngeal swabs, 140–250 μL	70 copies/reaction 120 samples	Sens = 100% Spec = 100% Acc = 100%	150	[51] US FDA
Logix Smart™ Coronavirus Disease 2019 (COVID-19) Kit	Nasopharyngeal swabs, 140 μL	600 copies/reaction 180 samples	Sens = 100% Spec = 100% Acc = 100%	50	[52] US FDA
Sansure Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit	Nasopharyngeal swabs, 200 μL	40 copies/reaction 246 samples	Sens = 94.3% Spec = 98.9% Acc = 93.2%	30	[53] US FDA
SD Biosensor STANDARD M nCoV Real-Time Detection kit	Nasopharyngeal swabs, 600 μL	150 copies/reaction 60 samples	Sens = 100% Spec = 100% Acc = 100%	30	[54] US FDA
Beijing Applied Biological Multiple Real-Time PCR Kit for Detection of 2019-nCoV	Nasopharyngeal swabs, 200 μL	40 copies/reaction 757 samples	Sens = 99.1% Spec = 94.9% Acc = 94%	100	[55] US FDA
BioFire® COVID-19 Test	Nasopharyngeal swabs, 300 μL	99 copies/reaction 536 samples	Sens = 97.1% Spec = 100% Acc = 97.1%	45	[56] US FDA
PerkinElmer® New Coronavirus Nucleic Acid Detection Kit	Nasopharyngeal swabs, 70 μL	3 copies/reaction 384 samples	Sens = 100% Spec = 100% Acc = 100%	110	[57] US FDA
BioMerieux ARGENE® SARS-COV-2 R-GENE®	Nasopharyngeal swabs, 200 μL	76 copies/reaction 186 samples	Sens = 100% Spec = 100% Acc = 100%	45	[58] US FDA
Allplex™ 2019-nCoV Assay	Nasopharyngeal swabs, 190–300 μL	1.25 copies/reaction 300 samples	Sens = 100% Spec = 93.1% Acc = 93.1%	70	[59] US FDA
QIAstat-Dx Respiratory SARS-CoV-2	Nasopharyngeal swabs, 300 μL	150 copies/reaction 3801 samples	Sens = 97.2% Spec =	60	[60] US FDA

(*continued on next page*)

Table 2 (*continued*)

Name	Sample	LOD	Clinical accuracy	Time, min	Ref
			96.1% Acc = 93.3%		
NeuMoDx™ SARS-CoV-2 Assay	Nasopharyngeal swabs (500 μL) or saliva (700 μL)	75 copies/reaction (nasopharyngeal swabs) or 35 copies/reaction (saliva) 131 samples	Sens = 100% Spec = 100% Acc = 100%	80	[61] US FDA
NxTAG CoV Extended Panel Assay	Nasopharyngeal swabs, 200 μL	1000 copies/reaction 60 samples	Sens = 100% Spec = 100% Acc = 100%	165	[62] US FDA
TaqPath™ COVID-19 Combo Kit	Nasopharyngeal swabs, 200 μL	10 copies/reaction 120 samples	Sens = 100% Spec = 100% Acc = 100%	40	[63] US FDA
1copy™ COVID-19 qPCR Multi Kit	Nasopharyngeal swabs, 140 μL	4 copies/reaction 120 samples	Sens = 95% Spec = 100% Acc = 95%	110	[64] US FDA
cobas® SARS-CoV-2 Test	Nasopharyngeal swabs, 600 μL	51 copies/reaction 312 samples	Sens = 100% Spec = 95.5% Acc = 95.5%	180	[65] US FDA
GenePro SARS-CoV-2 Test	Nasopharyngeal swabs, 140–200 μL	77 copies/reaction 100 samples	Sens = 100% Spec = 100% Acc = 100%	90	[66] US FDA
PowerChek™ 2019-nCoV Real-time PCR Kit	Nasopharyngeal swabs, 140 μL	560 copies/reaction 140 samples	Sens = 100% Spec = 100% Acc = 100%	90	[67] US FDA
FTD™ SARS-CoV-2	Nasopharyngeal swabs, 200 μL	108 copies/reaction 80 samples	Sens = 100% Spec = 100% Acc = 100%	>60	[68] US FDA
Biomaxima SARS-CoV-2 Real Time PCR LAB-KIT™	Nasopharyngeal swabs	≥10 copies/reaction	Sens = 99% Spec = 99% Acc = 98%	62	[69] URPL, CE-IVD
Liferiver Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit	Nasopharyngeal swabs, bronchoalveolar lavage fluid and deep cough sputum	1×10^3 copies/mL	N/A	N/A	[70] CE-IVD
GenePro COVID-19 Detection Test	Nasopharyngeal swabs	1×10^3 copies/mL 100 samples	Sens = 97.9% Spec = 100% Acc = 97.9%	90	[71] CE-IVD
AssayGenie COVID-19 (SARS-CoV-2) Triplex RT-qPCR Detection Kit	Nasopharyngeal swabs	200 copies/mL	Sens = 100% Spec = 100% Acc = 100%	>45	[72] CE
CTK Aridia COVID-19 Real-Time PCR test	Nasopharyngeal swabs	<10 copies/reaction	Sens = 100% Spec = 100% Acc = 100%	90	[73] CE
VIASURE SARS-CoV-2 Real Time PCR Detection Kit	Nasopharyngeal swabs	≥10 copies/reaction	N/A	N/A	[74] CE-IVD
RADI COVID-19 Detection Kit	Nasopharyngeal swabs	660 copies/mL 764 samples	Sens = 98.9% Spec = 100% Acc = 98.9%	80	[75] CE-IVD
Zena Max – AMD SARS-COV-2	Nasopharyngeal swabs	10 copies/reaction 192 samples	Sens = 100%	N/A	[76] CE-IVD

Sensitivity - Sens = 100% - % (false negative); Specificity - Spec = 100% - % (false positive); Accuracy -Acc = 100% - % (false negative) - % (false positive).

Table 3
Comparison of performance of commercialized government-approved RT-PCR kits.

Group	Geometric mean LOD	Average sens	Average spec	Average acc	Average time	Average number of samples
FDA	41.8 copies/reaction	98.1%	98.7%	96.8%	82.5 min	378
Other	44.1 copies/reaction	99.2%	99.8%	98.8%	73.5 min	352

Note: 322 μL was taken as an average sample volume to estimate LOD.

differences from the standard PCR method allow one to independently quantify DNA without standard curves, which gives more precise and reproducible data. ddPCR can be used for very low-target quantitation from variably contaminated samples [84]. The method is also used for clinical purposes: the first one was CE-marked in 2017 and approved by US FDA in 2019 for diagnosing chronic myeloid leukemia [85]. Studies using ddPCR were cited 36191 times and the number of citations was increasing exponentially since 2016. In 2021 alone such papers were cited 5923 times, which means that the method is becoming more popular each year [86]. In comparison, papers using RT-PCR were cited 381972 times, but this method has been developed much earlier than ddPCR. Also, since 2014 the number of citations has plateaued, increasing significantly only in 2020 (presumably due to newly developed SARS-CoV-2 virus) [87]. The performance parameters of other methods of SARS-CoC-2 RNA detection are summarized in Table 4.

For instance, ddPCR was used in the method proposed by Suo et al. which showed good results [90]. The LOD was equal to 2.1 copies/-reaction for ORFlab primers and 1.8 copies/reaction for N primers. These numbers are much lower than that for RT-PCR (1039 and 873.2 copies/reaction respectively). This method showed high accuracy (95%), specificity (100%) and sensitivity (94%) meaning that this method is reliable.

Multiplex PCR is the simultaneous amplification of more than one target sequence in a single reaction tube using more than one primer pair. Two studies used this type of PCR to detect the virus. Ishige et al. (2020) were the most successful [96]. Their method showed 100% specificity, sensitivity and accuracy, but these results were achieved on tests of 24 clinical samples, therefore, are inconclusive. With only 30 min of detection time and LOD of 21 copies per reaction, this method can be used widely for urgent testing. However, further research is needed. Another study by Visseaux et al. using multiplex PCR showed good results as well [34]. While the specificity and accuracy values were less than that of Ishige et al. (2020) [96], the sample size was bigger (69 samples: 40 positive and 29 negative). The method was slower (67 min) than the method proposed by Ishige et al. [96], but it is still faster than traditional RT-PCR methods.

Wang et al. and Cheong et al. received good results using nanopores and by applying nano PCR [36,95]. Regarding Wang et al. study, the method was able to detect the viral genome in 10 min with LOD of 10 copies/reaction [95]. They developed a nanopore targeting sequence which allows the detection of respiratory viruses simultaneously. The method showed relatively low specificity value (61%), so there is an increased risk of false positive results, but this method has a potential, as it can be further extended for diagnostics of other pathogens and viruses. Cheong et al. used the classical principle of RT-PCR, but integrated it into one device [36]. The main advantage of it is that this device does not require bulky instrumentation and specialized laboratories, it is portable and has small detection time (17 min). The LOD of the device is 3.2 copies per μL, with high accuracy (100%), so this device is efficient and reliable.

Moitra et al. integrated nanoparticles to develop colorimetric assay [92]. This method also allows the detection of the virus without advanced instrumentation. The assay is based on gold nanoparticles capped with thiol-modified antisense oligonucleotides, which are specific for N-gene of SARS-CoV-2. The mechanism involves selective agglomeration in the presence of target RNA leading to a change in its surface plasmon resonance. The addition of RNaseH leads to the formation of visible precipitate. The LOD of this method was 0.18 ng/μL and the time of the detection was equal to 10 min.

There were other methods that identified SARS-CoV-2 RNA. The ATR-FTIR (Attenuated Total Reflection – Fourier Transform Infrared) spectroscopy method used showed good results as well. Barauna et al. used contrived saliva samples spiked with inactivated γ-irradiated COVID-19 virus particles, which generated infrared (IR) spectra with a good signal-to-noise ratio [93]. The method allowed the detection of the viral content in 2 min which makes it the fastest among all methods. The accuracy of the method was equal to 90% was calculated with the sample size of 181 participants. Also, this method can be used to test people on site, as it does not require reagents or additional procedures. This method had a relatively low LOD (1582 copies/mL). Yu et al. have developed a new lateral flow strip membrane assay that can detect RdRP, ORF3a, N genes of SARS-CoV-2 simultaneously [91]. The assay was tested on 162 clinical samples and showed 100% sensitivity, 99% specificity and 99.4% accuracy. The values are good, but the sample size

Table 4
Analysis of other methods using SARS-CoV-2 RNA.

Method	Sensitivity	Specificity	Accuracy	Sample size, samples	Time (min)	LOD
Plasmonic Photothermal Biosensors [80]	N/A	N/A	N/A	82	N/A	0.22 pM
DNA nanoscaffold [88]	N/A	N/A	N/A	N/A	10	0.96 pM
Electrochemical Biosensor [89]	100%	100%	100%	48	<5	6.9 cp/μL
ddPCR [90]	94%	100%	95%	63	N/A	1.8–2.1 cp/rxn
Lateral Flow Strip Membrane assay [91]	100%	99%	99.4%	162	30	10 cp/test
Nanoparticles for colorimetric assay [92]	N/A	N/A	N/A	N/A	10	0.18 ng/μL
ATR-FTIR [93]	95%	85%	90%	181	2	1582 copies/mL
RdRP [94]	N/A	N/A	N/A	N/A	30	100 aM
Nanopore Targeted Sequencing [95]	100%	61%	85%	61	10	10 cp/rxn
Multiplex PCR [34]	100%	93%	97%	69	67	1000 cp/mL
Multiplex rRT-PCR [96]	100%	100%	100%	24	30	21 cp/rxn
BD MAX [97]	100%	N/A	100%	205	150	50 cp/PCR
Nano PCR [36]	N/A	N/A	100%	75	17	3.2 cp/μL

Note: Accuracy=(TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

is inconclusive, thus further research of this method would determine more practical values. The method is fast (30 min) and has relatively low LOD (10 copies per test). Both methods have a potential to be used widely alone or together with other methods to detect the virus among the population, especially when rapid testing is required (airports, gate controls or different events).

Concluding, methods based on RT-PCR showed high sensitivity, specificity and accuracy values, but in comparison to other methods they required longer time to detect the virus (hours and days). When nanopores or nanomembranes, other PCR methods were integrated, the time of detection was lowered down to 17 min preserving 100% accuracy [36]. Another method had even lower detection time (10 min), but accuracy was lower as well (85%) [95]. Some suggested methods used portable devices with a good limit of detection values and small time of detection. These devices are good when rapid detection is required, especially in airports or events. Biosensors also were used to detect the viral genome. In general, developed biosensors had good LOD (0.22 pM, 0.96 pM, 6.9 cp/μL) [80,88,89], two of them had good detection time (10 min and less than 5 min) [88,89]. They still can lose to RT-PCR methods due to decreased accuracy values (one method showed 100% value [89], but others still need to be tested on clinical samples), but other characteristics make these methods practical.

Comparing 10 RT-PCR methods mentioned in the scientific literature and 30 commercial methods approved by FDA and other organizations, it can be concluded that the latter is more reliable. The average speed of detection of literature methods was 62.8 min (ranging from 3 to 5 min to days) [35,37–45], while the minimal time taken by commercial methods was 30 min [49,53,54]. However, the average time of detection of the literature methods was calculated without one method, which took days to detect the virus. Considering that, the average should have been higher, but To et al. did not mention the exact number of days, so the calculation was impossible. Only several commercial methods showed LOD less than 5 copies/reaction [50,57,59,64] (maximum 1000 copies/reaction) [62], while methods published in literature mostly had lower LOD (maximum 20 copies/reaction) [39]. They were compatible with each other in terms of specificity, sensitivity and accuracy. Even if the average sample size (667) of literature methods was higher than that of commercial (352 for non-FDA approved and 378 for FDA-approved), the standard deviation was higher (1040 for literature and 359, 783 for commercial). Thus, the results obtained from testing the commercial methods are considered to be more reliable. This does not mean that literature methods are bad, but further research is required. Comparing sensitivity values of literature methods (83.7%) and commercial methods (98.1% for FDA approved and 99.2% for methods approved by other organizations), the latter had higher sensitivity. The standard deviation of commercial methods was also low, so all commercial methods had consistently high sensitivity values, while that of literature methods was relatively big. However, literature methods showed high specificity (all methods - 100%) [35,37–45], but there were values less than 100% among commercial methods [46,48,49,53,55,59,60,65,69]. Accuracy values were compatible within each other, but the lowest accuracy was recorded among FDA-approved methods. Even if the average sample size of literature methods was higher than that of commercial, the standard deviation was higher. Thus, the results obtained from testing the commercial methods are considered to be more reliable.

Comparing commercial methods within each other, the numbers did not differ significantly. For example, the difference in average sensitivity and specificity was 1.1%, the average accuracy values differed by 2% (Table 5). The average sample size of FDA approved methods was higher, but looking at standard deviation it can be said that the numbers were more consistent among non-FDA approved methods. The same pattern was recorded in time to detection and the limit of detection. The median values for sensitivity show that literature and FDA approved commercial methods mostly had 100% values, while non-FDA approved methods were not equal to 100% [69–75,98]. But median values for specificity were 100% [69–75,98]. Accuracy median was highest for literature methods, however commercial methods also showed good results. Generally, median values showed the same trend as average values - there is a significant difference between commercial and literature methods, but commercial methods within each other do not differ much. Commercial methods are more reliable as they were tested on bigger sample sizes and more practical - as they can detect the viral genome better.

2.4. CRISPR

In 2002, the term CRISPR short for clustered regularly interspaced short palindromic repeats was introduced by Jansen et al. [99], to the sequences in prokaryotes identified earlier in 1987 [100]. CRISPR were evolutionarily developed to protect the bacteria and archaea from viruses and plasmids analogous to the RNA interference system present in eukaryotes [101]. The defense against the foreign genome by CRISPR includes incorporation of a foreign genomic sequences into host genome, with a subsequent usage of these sequences to attack invader [102]. CRISPR-Cas systems consist of two classes: Class 1 includes multiple Cas proteins for the interference step, while Class 2 depends on one multidomain protein [103]. Signature genes of Class 1 are Cas3 and Cas10 that cut DNA and RNA respectively. In Class 2, Cas 9 and Cas12 proteins make cuts in target DNA, while Cas13 cleaves RNA [104,105]. Due to the efficient genome recognition and editing of CRISPR complexes, and the simplicity of Class 2 proteins, Cas12, Cas13, Cas9 proteins discussed in this review along with the other CRISPR-Cas Class 2 complexes are used for the detection of nucleic acids [106].

The mechanism for the detection of the genome starts with the recognition and cleavage of the targeted nucleic acids by CRISPR-Cas complexes using the guide of designed gRNA. This causes the activation of the non-specific collateral activities of Cas proteins, which is the cleavage of any ssDNA nearby for Cas12 and Cas 9, and ssRNA for Cas13 proteins. Cleavage of reporter molecules during the collateral activity releases a signal for the presence of targeted nucleic acid, which could be SARS-COV-2 gene [107].

Fig. 2 displays the application of CRISPR for the detection of SARS-COV-2. The analyzed methods in this review mostly require the nasopharyngeal swab, while for some methods saliva can also be used as the sample. After obtaining the sample for analysis, the viral RNA should be extracted from the swab. RT-PCR, RT-LAMP, and RT-RPA (Reverse Transcription Recombinant Polymerase Amplification) were applied for amplification in the methods discussed in this review. Depending on the amplification type, the sample to result time, reactants, equipment, and reaction conditions vary significantly. RT-LAMP at RT-RPA, which are

Table 5
Comparison of RT-PCR in scientific literature and commercial methods.

	Sensitivity	Specificity	Accuracy	Sample size	Time (min)	LOD	Ref.
RT-PCR (literature)	83.7 ± 30.1% (29.2; 100%) - 100%	100 ± 0% (-; 100%) - 100%	97.7 ± 3.6% (91.7; 100%) - 100%	667 ± 1040 (9; 2923) - 117.5	62.7 ± 47.7 (3–5 min; days) - 63.5	9.2 ± 7.9 (1; 10) - 5	[35, 37–45]
PCR commercial (FDA approved)	98.1 ± 3.0% (88.1; 100%) - 100%	98.7 ± 2.4% (93.1; 100%) - 100%	96.8 ± 3.8% (87.7; 100%) - 98.5%	378 ± 784 (60; 3801) - 138	82.5 ± 49 (30; 180) - 70	148 ± 254 (2.65; 1000) - 70	[46–68]
PCR commercial (non-FDA approved)	99.2 ± 1.1% (97.1; 100%) - 99.5%	99.8 ± 0.4% (99; 100%) - 100%	98.8 ± 1.3% (97.1; 100%) - 98.9%	352 ± 360 (100; 764) - 192	73.4 ± 19.6 (45; 90) - 80	120 ± 142 (10; 322) - 37.2	[69–75, 98]

Note: Average ± stdev; Range (x; y); Median.

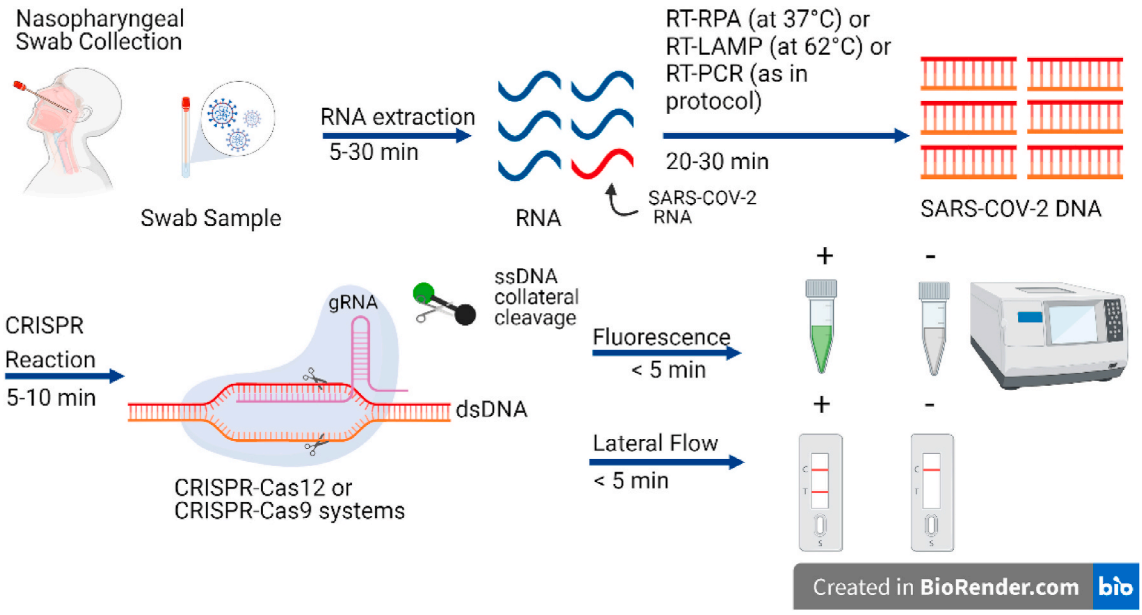

Fig. 2. The illustration of the CRISPR-based amplification assay process for the detection of SARS-COV-2 (created in BioRender.com).

mostly applied for CRISPR-based SARS-COV-2 detection, required the incubation for 20–30 min at 62 °C and at 37 °C correspondingly, while for RT-PCR several temperature changes are needed as it is stated in the protocol. After the double-stranded DNA is obtained as the result of the preceding step, CRISPR reactions may begin. However, if Cas13 protein is selected for the detection, amplified DNA should be transcribed back into RNA and this is possible by application of T7 polymerase. The average CRISPR master mix includes the amplification product, gRNA, CRISPR-Cas protein of choice, a fluorescent probe, buffer, and the nuclease-free water to reach the desired reaction volume. Incubation of products generally occurs at 37° for 5–10 min. The detection and the cleavage of SARS-COV-2 by the CRISPR-Cas system causes the collateral cleavage of the single-stranded DNA, and this results in the fluorescent signal. Next, in less than 5 min results can be obtained by lateral flow or fluorescence detection by naked eye or apparatus depending on the specific method. Generally, around 50 min are needed for SARS-CoV-2 detection with the CRISPR method including the time for setup after RNA extraction.

The lowest LOD among the CRISPR methods presented in the review is 2 RNA copies per sample, obtained by Huang et al. [108]. This method involves the amplification of the target fragments by RT-PCR using 5 μL of isolated RNA followed by DNA amplification protocols or by RT-RPA method on 5 μL of isolated RNA. Then, for the CRISPR reaction, the Cas12a protein and the fluorescent probe were applied, and the signal was observed by SpectraMax i3x Multi-Mode Microplate Reader after 20 min of the incubation at 37 °C in the dark. The primers and gRNA were designed to target the N and ORF1ab regions of the SARS-COV-2. While testing the clinical swab samples, 15 out of 15 positive samples were detected, thus 100% sensitivity was achieved. However, 4 false positives were obtained leading to the specificity of 71.4%. The application of RT-PCR for amplification of RNA extract to increase the sensitivity turned out to be the double sword, which generated the low specificity, because of the false positives.

CRISPR-COVID method developed by Hou et al. is a method with high sensitivity and quick turnaround time [109]. In 30 min, RT-RPA and T7 transcription is performed on the extracted RNA, then additional 10 min are required for the CRISPR-Cas 13a reaction till obtaining the signal. The LOD of CRISPR-COVID is lower than 3 copies per microliter. The clinical sensitivity of the method was tested by using RT-PCR and metagenomic next-generation sequencing (mNGS) methods, and CRISPR-COVID demonstrated 100% accuracy by having the same results as mNGS in all 114 samples, containing SARS-COV-2, SARS-CoV-2-/hCoV+, and healthy genome. Important to note that RT-PCR could not determine the presence of the targeted virus in 5 out of 57 cases detected by mNGS and CRISPR-COVID. This means that the sensitivity of the CRISPR-COVID method is higher in comparison to RT-PCR. Furthermore, according to Hou et al. this method runs at less than $3.5 per test and estimated to cost as low as $0.7 at a production scale. Since CRISPR-COVID is more reliable and cost-effective than RT-PCR, it can be a good alternative to the latter.

Reverse transcription All-In-One Dual CRISPR-Cas12a (RT-AIOD-CRISPR) is the fast assay with a turnout time of 40 min established by Ding et al. [110]. 2.5 μL of RNA extract from the swab is put into the tube containing primers targeting the N gene of SARS-COV-2, avian myeloblastosis virus (AMV) reverse transcriptase, Cas12a-crRNA mix, ssDNA-FQ reporters, buffers, and other components required for both RT-RPA and CRISPR reactions. Generally, the positive result can be obtained after 20 min of incubation at 37 °C and the fluorescence can be detected with the naked eye under an LED light. This assay can detect down to 5 RNA copies per reaction and the clinical results of 28 samples were consistent with the RT-PCR test. The following assay is isothermal and does not require a high temperature, therefore, a low-cost hand warmer can be applied for the incubation. Since RT-AIOD-CRISPR is a quick, reliable, easy, single-step, and low-cost method, it is an excellent method that can be developed for point of care devices. All the necessary reagents can be integrated into the chip, and the RNA extracted from the swab can be added later. After 40 min of incubation with the hand warmer, the programmed smartphone can be used to take photos and return qualitative or semi-quantitative test results. According to the authors, at the current time, the cost of this test is about $6, however, it is expected to decrease significantly at the production scale.

The major part of the methods with viral RNA detection requires the pre-extracted nucleic acid for detection. The extraction of RNA needs expensive instruments and a lot of time. Therefore, the CRISPR method proposed by Ramachandran et al. that directly uses swab samples can be useful to detect SARS-CoV-2 [32]. Isotachophoresis (ITP), which is a two-buffer system consisting of a high-mobility leading electrolyte (LE) and low-mobility trailing electrolyte (TE), is practiced to extract RNA from nasopharyngeal swab and to speed up CRISPR-Cas12 reaction. The purification and the acceleration reactions by ITP are possible because when the electric field is applied, sample ions with effective mobilities are concentrated on the 10 μm zone at the LE-to-TE interface. By using

ITP, RNA can be extracted from the nasopharyngeal swab in 3 min, after pre-incubating the sample for 2 min at 62 °C. After this, for RT-LAMP reaction, 20–30 min incubation is required, and the ITP and CRISPR detection of DNA is performed in less than 5 min. RNA extraction and CRISPR detection are conducted on a chip, which means that this method can be developed into the point-of-care device. The volume of all the reactants required for the CRISPR reaction in this assay is about 100 times less than the average amount being only 0.2 μl, from this it follows that this method is cost-effective and reduces the use of chemicals. Another advantage of ITP based CRISPR method is that time needed from the sample to the result is very rapid, being about 40 min, and still, the method has an average LOD for CRISPR of about 10 copies/μl. The accuracy of the method with the usage of pre-extracted RNA from 40 clinical samples was measured to be 93.8% in comparison with RT-qPCR results. However, the results obtained from the ITP based RNA extraction are less reliable, 3 out of 4 positive samples were detected, thus resulting in the sensitivity of 75% for ITP based nucleic acid extraction and the CRISPR reaction method.

Another CRISPR assay that does not require the extraction of RNA for SARS-CoV-2 detection is SHINE, the method developed by Arizti-Sanz et al. [31]. Only about 50 min are needed to achieve the result after obtaining the swab or saliva of the patient. SHINE, short for Streamlined Highlighting of Infections to Navigate Epidemics, is based on SHERLOCK (Specific High-sensitivity Enzymatic Reporter unlocking) and includes a reverse transcription step (RT) followed by isothermal recombinase polymerase amplification (RPA), T7 transcription and Cas 13 cleavage of single-stranded RNA. SHINE is optimized to be conducted on unextracted nucleic acid, within a single tube to reduces cross-contamination. The results can be visualized by the lateral flow or in-tube fluorescence. The fluorescence visualization was applied for the clinical testing, since it requires less incubation time, and allows the testing of a large number of samples simultaneously, and reduces the risk for cross-contamination. Portable transilluminators (<$500) or small, blue LED lights (~$15) can be used as a required blue light-emitting device, then smartphone applications can analyze the captured photos to provide the result of the testing. 50 clinical nasal swabs tested with SHINE demonstrated 90% sensitivity and 100% specificity in comparison to the conventional RT-qPCR method.

Table 6 contains the information about the specificity, sensitivity, and accuracy of the CRISPR-based methods grouped by their Cas protein of choice. The average sample to result time, the number of clinical samples tested, LOD of the method are also presented in Table 6. For some methods, LOD was displayed in copies per reaction and was converted into copies per μl. The average accuracy for CRISPR-based SARS-CoV-2 detection was calculated to be 96.5%, while the specificity is 98% and the sensitivity is 96%. The specificity is higher than the sensitivity, which means that the possibility of differentiating between different viruses is higher than detecting the SARS-CoV-2. Between different types of Cas proteins, Cas9 showed the best accuracy of 98%, although it is important to note that Cas9 was applied only in one technique presented in this review. In more than half of the cases Cas12 was used for CRISPR reaction and this protein has an accuracy of 96%. The accuracy of Cas13 is considerably high, being 97%, and since Cas 13 was used in 4 methods, it can be assumed that this protein gives the highest accuracy. The sample-to-time result of both Cas 12 and Cas13 is around 50 min, while for Cas 9 about 60 min are required to know the outcome. LOD of Cas 12 is better than of Cas13, being 5.3 and 6.6, respectively. From the data presented in Table 6 it can be assumed that there are no significant differences from the protein of choice, and Cas9, Cas12, Cas13 are all reliable for SARS-CoV-2 detection.

Table 7 presents the same features of the methods as Table 6, but the techniques were grouped according to their amplification types, and the average values of these characteristics were quantified. RPA was measured to be the most reliable method with an accuracy of 98%. LOD of RPA is equal to 5.9 cp/μL of RNA that can be considered good in comparison to LOD of LAMP method with 13 cp/μL. However, the lowest LOD of 0.4 cp/μL is obtained while amplifying the RNA with PCR. Overall, RT-PCR is an expensive but highly sensitive method, because of the great sensitivity of RT-PCR, the specificity can be decreased due to contamination. Other types of amplification such as RPA and LAMP, do not require the change of temperature, therefore expensive instruments are not required. Furthermore, the incubation temperature for RPA is about 37 °C, since RPA has the smallest deviation from the ambient temperature, it also has significance as the amplification method for point of care devices.

The average accuracy of all CRISPR methods is about 96.5%, and about 52 min are needed to obtain the results of the test. CRISPR-coupled RT-RPA and RT-LAMP are good alternatives to RT-PCR since these methods are more cost-effective and faster. The majority of the methods relied on RNA extraction by kit, while Ramachandran et al. extracted the RNA by using ITP as described before [32], and heat lysis was performed by Arizti-Sanz et al. to obtain the viral RNA [31]. The average accuracy of these two methods is 95%, while other methods have an accuracy of 97%. The slight differences between these percentages suggest that for CRISPR-based detection, SARS-CoV-2 RNA can be obtained by heat lysis or by the usage of Isotachophoresis (ITP) as well, and still fairly accurate results can be achieved.

2.5. LAMP, RPA, RAA, PCA

Loop-mediated isothermal amplification, also known as LAMP, is one of the popular amplification methods, and, according to Scopus, the number of citations with this keyword is 96532. LAMP was established by Notomi et al., in 2000 and employs a DNA polymerase and 4 (or 6) DNA primers to recognize the 6 (or 8) sequences in the target DNA. The LAMP reaction is initiated by the inner primer, and then the strand displacement DNA synthesis by an outer primer produces single-stranded DNA. After this, the second inner and outer primer generate

Table 6
CRISPR-based SARS-COV-2 detection methods.

Cas	Specificity	Sensitivity	Accuracy	N of samples	N of positive samples	Time (min)	LOD (cp/μL of RNA)	Log addition of LOD	Ref.
Cas 9	100%	97.1%	98.4%	64	35	60	4	4	[111]
Cas 12	95.9 ± 10.8% (71%; 100%) *100%*	96.8 ± 4.5% (88%; 100%) *100%*	95.9 ± 5.5% (86%; 100%) *97.6%*	82; 10; 28; 29; 100; 22; 31	40; 7; 19; 14; 50; 11; 26	51 ± 11 (40; 66) *50*	9.6 ± 9.8 (0.4; 30) *10*	5.3 ± 4.1 (0.4; 30) *10*	[108,110, 112–116]
Cas 13	100%	95.0 ± 4.2% (90%; 100%) *95.0%*	97.2 ± 2.5% (94%; 100%) *97.5%*	50; 114; 64; 154	30; 52; 32; 81	50 ± 14 (40; 70) *45*	7.4 ± 3.4 (3; 10) *8.3*	6.6 ± 1.8 (3; 10) *8.0*	[31,32,109, 117]
Average	97.6 ± 8.3% (71%; 100%) *100%*	96.2 ± 4.1% (88%; 100%) *96.7%*	96.5 ± 4.3% (86%; 100%) *97.8%*			51 ± 11 (40; 70) *50*	8.4 ± 7.7 (0.4; 30) *8.2*	5.2 ± 2.9 (0.4; 30) *8.0*	

Note: Average ± standard deviation; Range (x%; y%); *Median* are shown. LOD conversion from cp/rxn to cp/μL was done by dividing the cp/rxn by the amount of RNA used in μL. Accuracy=(TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

Table 7
CRISPR with different amplification methods.

	Specificity	Sensitivity	Accuracy	N of samples	N of positive samples	Time (min)	LOD (cp/μL)	Ref.
RT-PCR	71.4%	100%	86.2%	29	14	50	0.4	[108]
RT-LAMP	100%	94.3 ± 4.1% (88%; 100%) *94.0%*	96.2 ± 3.9% (89%; 100%) *97.0%*	82; 10; 100; 29; 64	40; 7;50; 26; 32	46 ± 11 (40; 65) *40*	13.0 ± 9.8 (5; 30) *10*	[32,112–114,116]
Best	100%	95%	97.6%	82	40	45	10	[112]
RT-RPA	100%	97.2 ± 3.9% (90%; 100%) *98.6%*	98.4 ± 2.3% (94%; 100%) *99.2%*	64; 28; 22; 50; 114; 154	35; 19; 11; 30; 52; 81	56 ± 11 (40; 70) *55*	5.9 ± 3.5 (2; 10) *5.2*	[31,109–111,115,117]
Best	100%	100%	100%	114	52	40	3	[109]

Note: Average ± standard deviation; Range (x%; y%) and *Median* are shown; LOD conversion from cp/rxn to cp/μL was done by dividing the cp/rxn by the amount of RNA used in μL. Accuracy=(TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

the stem-loop DNA structure by hybridizing the ends of the target. Further by cycling and elongation steps, original stem-loop DNA and one newly synthesized stem-loop DNA with a stem twice as long is produced. In less than an hour, 10^9 of DNA copies are generated through LAMP reaction [118]. Since the nucleic acid in the SARS-CoV-2 virus is RNA, reverse transcription is performed to derive cDNA. Because the isothermal amplification happens at around 62–65 °C, RT-LAMP does not require expensive instruments for temperature changes, and LAMP has the potential to be adapted into portable devices. For SARS-CoV-2 detection with RT-LAMP method, incubation for 30–60 min is required, and by the addition of DNA intercalating object - SYBR Green dye - colorimetric visualization is possible.

Another isothermal amplification technique widely applied for nucleic acid detection is Recombinase polymerase amplification (RPA). The main difference of RPA from other amplification methods is it's use of Recombinase-primer complexes to scan double-stranded DNA, and to facilitate strand exchange at cognate sites. Then, the single-stranded DNA binding proteins stabilize the displaced DNA by binding to it and thus preventing the binding of primers to the displaced DNA strands. Finally, the DNA synthesis at places where the primer is bound to the DNA is initiated by the strand displacing polymerase. The continuous repetition of these steps increases the number or amplification product exponentially to the detectable level. For the RT-RPA process for SARS-CoV-2 detection sample is incubated at around 42 °C and about 15–35 min are required to obtain the result. Due to the lower incubation temperature and short time, RT-RPA is preferable as the point-of-care method to RT-PCR and RT-LAMP.

A brief flowchart of the detection of SARS-CoV-2 from the clinical sample via amplification is presented in Fig. 3. After collecting the sample from the patient, different types of treatments can be applied before nucleic acid amplification. One of the widely used options is the RNA extraction by kit, which might take 5–30 min. Furthermore, heating the sample or magnetic beads can also be used to obtain the RNA. There are also some cases where the clinical specimens were directly subjected to amplification without any pre-treatment. In the majority of the cases the SARS-CoV-2 positive sample that is being amplified can be detected in less than 20 min with a fluorescence reader or a naked eye. If the corresponding color change is not observed after the amplification, then the test result is SARS-CoV-2 negative. In the case of the lateral flow test, the result can be determined in less than 5 min after incubation.

The amplification method with LOD of 80 cp/mL is the SARS-CoV-2 detection by RT-LAMP proposed by Huang et al. [119]. 4 sets of primers each consisting of 6 primers were designed to target N, S, and Orf1ab regions of the viral RNA. After the nucleic acid extraction from the 16 clinical samples using an RNA extraction kit, the RT-LAMP reaction was performed in triplets. In the first and the second tubes, the primers for the N and Orf1ab gene were placed, while human β-actin primers present in the third tube served as a negative control for the experiment. Reactants were incubated for 30 min at 65 °C after which the observation of yellow color with a naked eye was considered as a positive result, while the pink or orange color of the sample similar to the color of negative control implies negative test result. The following RT-LAMP test was 100% in accordance with the result of the RT-PCR test. Thus, this method is accurate, simple, and does not require any expensive instruments for both amplification and visualization of the product. Moreover, RT-LAMP with a sample to the result time less than 70 min is considerably more rapid than the conventional RT-PCR that requires more than 2 h.

Earlier the importance of a portable detecting device was mentioned, and Rodriguez-Manzano et al. already adapted RT-LAMP into the point-

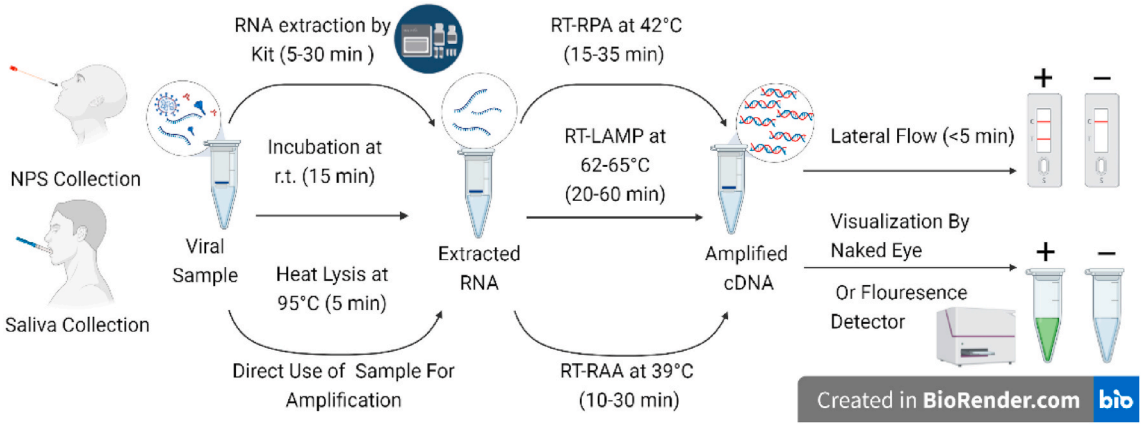

Fig. 3. Schematic Illustration of the detection of SARS-COV-2 by Amplification methods as RT-LAMP, RT-RPA, and RT-RAA (Reverse Transcription Recombinase-Aided Amplification) (Created with BioRender.com).

of-care method [120]. The method relies on detection of changes in pH following nucleotide incorporation during amplification and uses custom lab-on-chip platform with 4368 ion-sensitive field-effect transistors serving as sensors. The reaction on-chip is performed at 63 °C for 30 min. The data is transmitted to the smartphone app via Bluetooth and analyzed in MATLAB. The LOD of this method is 10 copies per reaction, while the sensitivity and specificity for 183 samples are 91% and 100% respectively. The method by Rodriguez-Manzano et al. demonstrates that RT-LAMP can be successfully used in a lab-on-chip platform.

Thi et al. performed the RT-LAMP on different samples as extracted RNA, clinical swab, and heated clinical swab, and the results indicate that the extracted RNA provides the best result after amplification for 1 h with the accuracy for 768 samples being 95% [121]. The test results of 343 hot swabs were in 80% accordance with RT-PCR, while this percentage was decreased to 72% for directly used 235 clinical specimens. Important to note that despite the type of used sample specificity remained high by being 99.7% and 99.5% for extracted RNA and hot swab, the specificity of the not heated sample did not decrease considerably being 94%. The research conducted by Thi et al. suggests that to derive the most reliable result the viral RNA should be extracted from the swab using the kit, or the clinical sample should be at least heated for 5 min at 95 °C to obtain more accurate results than from the directly used swab.

The difference between using saliva and nasopharyngeal swab (NPS) as the sample for SARS-CoV-2 detection with RT-LAMP was studied by Kitajima et al. and the study identified that the LOD for NPS is twice as lower than the LOD for saliva, being 1.0 and 2.3 cp/μL correspondingly [122]. The sensitivity, specificity, and overall accuracy of NPS for 151 samples are equal to 89%, 99%, 93%, while for 88 saliva samples tested by this method the percentages were equal to 83%, 98%, and 93%. These results indicate that using NPS results in slightly higher sensitivity than the usage of saliva samples for SARS-CoV-2 detection.

The impact of targeting different regions of SARS-COV-2 was investigated by El Wahed et al. by applying the primers for RdRp, N, and E gene of the virus; it was found that targeting RdRp provides the most reliable outcome, this claim is supported by LOD and the clinical performance of the different genes. LOD of RdRp was 2 cp/rxn, while for the other two genes it was 15 cp/rxn, when 1 μL of extracted RNA was used for amplification [123]. To identify the clinical accuracy of RT-RPA on RdRp, N, and E genes 36 swab samples were tested, and the following results were obtained: the specificity and sensitivity for RdRp in comparison to RT-PCR was 100% and 94%, while for N gene 94% and 83%, and for E gene 77% and 65%. From the study of El Wahed it can be concluded that targeting the RdRp gene of SARS-CoV-2 will result in better correlation with the RT-PCR, than targeting other genes.

The study conducted by Nawattanapaiboon et al. in Thailand employed RT-LAMP assay targeting RdRp gene on a large number of clinical samples (2120). The study demonstrated high sensitivity and specificity of the test at 95.74% and 99.95%, respectively, values similar to the conventional RT-PCR [124]. In the RT-LAMP reaction that lasted for 60 min at 65 °C, LOD was estimated to be 25 RNA copies per reaction, where the 5 μL of extracted RNA was used for the reaction. Only one false positive and two false negatives were detected from 47 SARS-CoV-2 positives and 2073 negative nasopharyngeal swabs.

Zwirglmaier et al. proposed the novel nucleic acid-based SARS-CoV-2 detection method named Pulse Controlled Amplification (PCA) [125]. The clinical swabs in universal transport medium were treated with 4 vol of AVL buffer and 4 vol of ethanol, or samples were heated at 80 °C for 10 min before performing the RT-PCA. The treated sample and the hybridization buffer were incubated at room temperature for 5 min to allow the hybridization of RNA to the reverse primers. Following this, Pharos Micro was set to 55 °C for 5 min for reverse transcription reaction, then to 67 °C for 60 s for thermalizing step. Then, for the denaturation 250 μsec pulses were applied to the wires suspended into the reaction solution every 3 s for 800 cycles. The clinical accuracy of the following method was 94% for 154 swabs, 74 out of 83 positive swabs were successfully detected by PCA, and no false negatives were observed. Despite the good clinical performance and the LOD of 4.9 cp/μL, PCA requires expensive equipment, therefore it might be difficult to adapt this method to conventional use.

Table 8 presents the information about the clinical performance, time to result, LOD of various virus detection methods that involves amplification, such as previously described LAMP, PCA, RPA, and its alternative RAA. Sample-to-result time was divided into two parts that include time passed after the start of amplification till obtaining the result, and the overall time from swab to result. Some research papers have not included the time needed for RNA extraction with the kit, therefore, these measures were applied. LOD of methods deviated from 0.08 to 62 cp/μL and in order to minimize the effect of the outliers, logarithmic addition was applied. RAA detection provided good results for all criteria with an accuracy of 97%, LOD of 0.7 cp/μL, and overall time of 40 min, however, it is significant to note that only two studies applied this type of amplification. RPA that was used in five experiments showed a high clinical accuracy of 96% and LOD of 7 cp/μL with an average time to result of 45 min. As it was mentioned before, LAMP is widely used for virus detection and 18 different clinical studies discussed in the research were performed by using LAMP, which consequently resulted in the outliers.

As displayed in Fig. 3, the different types of treatments can be performed on the clinical sample before exposing it to amplification. Table 9 presents the analysis of methods depending on their RNA extraction method. RNA extraction with a kit had the best clinical performance with the average accuracy, specificity, and sensitivity of 96.5%, 98%, and 93.5%, respectively. RNA extraction by other treatments had a lower sensitivity of 85%, an accuracy of 93%, and specificity being 99.9%. When the clinical swab or saliva was directly amplified, even though the specificity was not affected and remained 98%, the sensitivity suffered significantly and was equal to 68%, and thus resulting in an accuracy of 84.5%. The specificity is not considerable affected by the existence or absence of any treatment for the sample; however, only after the extraction with the kit SARS-CoV-2 positive samples can be precisely detected. This can be supported by the LOD of the three methods as well, as shown in Table 9. In conclusion, the data in Table 9 supports the findings by Thi et al. that RNA extracted by the kit generates the most accurate results [121].

The performance of different amplification methods discussed previously categorized according to the target region in SARS-CoV-2 RNA is summarized in the Table 10. Data analysis revealed that the best accuracy and LOD are obtained when multiple genes are targeted, 98% and 3.8 cp/μl, correspondingly. Among the single genes, RdRp gives the most reliable results with an accuracy of 96% and LOD of 2.6 cp/μl. In the study of El Wahed et al. RdRp was preferable to other genes as well in terms of clinical performance and LOD [123].

The comparison between the SARS-CoV-2 detection by using RT-LAMP, RT- RPA and RT-RAA, RT-PCR in literature, RT-PCR approved by FDA and other authorization can be viewed in Table 11. The main advantage of using RT-RPA and RT-RAA is short time of the assay which is 20–40 min faster than the other methods. RT-LAMP showed the lowest clinical reliability among all methods of 93.3%. Generally, RT-PCR tests (commercialized and literature) provide more accurate results than the rest. Despite the fact that the specificity of RT-PCR in literature is 100%, and the overall accuracy is 97.7%, the sensitivity of this method is 83.7%, which is the lowest percentage between all detection methods. Even though there is not a significant difference in the specificity and accuracy of the different methods, the stronger deviation in sensitivity can be observed, which can lead to the conclusion that commercialized RT-PCR tests, especially the ones approved by authorizations other than FDA, provide the most sensitive and reliable test results. However, it should be noted that the data about the clinical performance of only five non-FDA registered RT-PCR methods were available.

Table 8
SARS-COV-2 detection by LAMP, RPA, RAA and PCA.

	Specificity	Sensitivity	Accuracy	Time started from reaction, min	Overall time, min	LOD (cp/μL)	Log LOD	Ref.
LAMP	98 ± 4.9% (81%; 100%) *100%*	86 ± 19% (32%; 100%); *91.7%*	93.3 ± 7.6% (72%; 100%) *93.4%*	43 ± 14 (30; 60) *37.5*	61 ± 28 (30; 130) *60*	15.3 ± 22.2 (0.1; 62) *4.9*	4.3 ± 7.1 (0.1; 62) *4.9*	[119–122,124, 126–135]
RPA	98.9 ± 2.5% (94%; 100%) *100%*	92.6 ± 7.4% (83%; 100%) *94.4%*	95.5 ± 4.8% (89%; 100%) *97.2%*	22 ± 8 (15; 35) *20*	45	6.9 ± 5.3 (2; 15) *7.7*	5.4 ± 2.3 (2; 15) *7.7*	[123,136–138]
RAA	98.9 ± 1.5% (98%; 100%) *98.9%*	98.8 ± 1.7% (98%; 100%) *98.8%*	97.3 ± 2.9% (98%; 100%) *97.3%*	28 ± 11 (20; 35) *28*	40	0.7 ± 0.4 (0.4; 1) *0.7*	0.6 ± 1.9 (0.4; 1) *0.6*	[139,140]
PCA	100%	89.2%	94.2%	51	61	4.9		[125]

Note: Average ± standard deviation; Range (x%; y%) are shown; LOD conversion from cp/rxn to cp/μL was done by dividing the cp/rxn by the amount of RNA used in μL. Accuracy=(TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

Table 9
SARS-COV-2 detection by amplification with different pre-treatments to the sample.

	Specificity	Sensitivity	Accuracy	Time after RNA extraction (min)	Overall Time (min)	LOD (cp/μL)	LOD by Log (cp/μL)	Ref.
Extraction with kit	98 ± 4.9% (81%; 100%) *100%*	93.5 ± 8.2% (71%; 100%) *95.7%*	96.5 ± 4% (89%; 100%) *97.8%*	37 ± 16 (15; 60) *35*	58 ± 12 (40; 70) *60*	7.9 ± 15 (0.1; 62) *3.6*	2.6 ± 5.4 (0.08; 62) *3.4*	[119–124,127–130, 135–137,139,140]
Extraction w/o kit	99.9 ± 0.2% (99%; 100%) *100%*	84.6 ± 19.6% (47%; 100%) *87.9%*	92.7 ± 7.5% (78%; 100%) *93.2%*	39 ± 11 (30; 60) *38*	64 ± 34 (35; 130) *55*	18 ± 22 (3; 50) *9.8*	9.8 ± 3.7 (3; 50) *8.5*	[121,125,131,132, 134,138]
Directly swab	98 ± 3.4% (94%; 100%) *100%*	67.9 ± 31.4% (32%; 87%) *85.0%*	84.5 ± 10.5% (72%; 91%) *90.0%*	40 ± 17 (30; 60) *30*	42 ± 16 (30; 60) *35*	28 ± 36 (3; 54) *28.3*	11.6 ± 8.7 (3; 54) *11.6*	[121,126,133]

Note: Average ± standard deviation; Range (x%; y%); *Median* are shown; LOD conversion from cp/rxn to cp/μL was done by dividing the cp/rxn by the amount of RNA used in μL. Accuracy=(TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

Table 10
SARS-CoV-2 detection by targeting different genes.

	Specificity	Sensitivity	Accuracy	Time started from the reaction, min	Overall time, min	LOD (cp/μL)	Log LOD	Ref.
N gene	97.4 ± 5.2% (81%; 100%) *100%*	85.7 ± 20% (32%; 100%) *91.7%*	92.9 ± 7.9% (72%; 100%) *93.8%*	38 ± 15 (15; 60) *33*	61 ± 31 (30; 130) *60*	18.9 ± 22.6 (0.2; 62) *7.7*	8.5 ± 5.3 (0.2; 62) *10.0*	[120,121,123,125–127, 130–132,134–137,140]
RdRp	99.2 ± 0.9% (98%; 100%) *99.3%*	90.4 ± 6.0% (83%; 96%) *91.5%*	95.9 ± 3.2% (93%; 100%) *95.3%*	36 ± 18 (15; 60) *35*	N/A	2.6 ± 1.7 (1; 5) *2.2*	2.6 ± 1.7 (1; 5) *2.2*	[122–124]
Orf1ab	98.9 ± 1.5% (98%; 100%) *98.9%*	91.3 ± 8.9% (85%; 98%) *91.3%*	93.9 ± 5.5% (90%; 98%) *93.9%*	25 ± 7 (20; 30) *25*	38 ± 4 (35; 40) *38*	1.5 ± 1.5 (0.4; 2.5) *1.5*	1.5 ± 1.5 (0.4; 2.5) *1.0*	[133,139]
Multiple targets	100%	96.7 ± 6.7% (87%; 100%) *100%*	98 ± 3.9% (92%; 100%) *100%*	42 ± 13 (30; 60) *39*	58 ± 13 (45; 70) *60*	3.8 ± 4.3 (0.1; 10) *2.5*	1.5 ± 7.8 (0.1; 10) *2.5*	[119,128,129,138]

Note: Average ± standard deviation; Range (x%, y%); *Median* are shown; LOD conversion from cp/rxn to cp/μL was done by dividing the cp/rxn by the amount of RNA used in μL. Accuracy=(TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

3. Detection of antigens and viral particles

Detection of viral particles and antigen is a viable alternative to RT-PCR. These methods are potentially inexpensive, portable, rapid, and can be used to diagnose patients at the early stage of viral infection. Some methods have already been commercialized and are available as point-of-care tests. They are not required to be performed by a skilled operator and can be run by patients themselves. Structural SARS-CoV-2 proteins such as nucleocapsid protein (nucleoprotein), spike protein, membrane protein, and envelope protein can be detected. Usually, nucleoprotein and spike protein are used as analytes. Detection can be instrumental-based (e.g. mass spectrometry) or chemical reaction-based (e.g. antigen-antibody binding). Fig. 4 provides an overview of detection methods.

In the first method presented on the top of the Fig. 4, the nucleocapsid, spike protein, or any other structural protein of SARS-CoV-2 is detected by the fluorescence generated by the antigen-antibody special interaction. On the right side of the figure, the fluorescence signal vs concentration graph is presented, which is in practice rarely linear and mainly tends to flatten with the rise of concentration. At the bottom, the human clinal sample is digested into peptides and further subjected to mass spectroscopy to find out whether the sample is infected or not by determining the peaks that correspond to SARS-CoV-2 proteins.

Table 11
The comparison of different SARS-COV-2 RNA detection methods.

	RT-LAMP	RT-RPA and RT-RAA	RT-PCR	RT-PCR commerical (FDA approved)	RT-PCR commercial (other authorization)
Time	61 min	43 min	63 min	83 min	74 min
LOD	15.3 cp/μL -mean 4.3 cp/μL - log addition 4.9 cp/μL - median	5.2 cp/μL -mean 2.9 cp/μL - log addition 2.5 cp/μL - median	9.2 cp/rxn	41.8 cp/rxn	44.1 cp/rxn
Specificity	97.9%	98.9%	100%	98.7%	99.8%
Sensitivity	86.2%	94.4%	83.7%	98.1%	99.2%
Accuracy	93.3%	96.5%	97.7%	96.8%	98.8%

Note: Table 11 is a compound table with the data from Table 1, Table 3, Table 8, Table 10. Full clinical data is reported only for 5 non-FDA registered (spec, sens, acc); Average ± standard deviation; Range (x%; y%) are shown; LOD conversion from cp/rxn to cp/μL was done by dividing the cp/rxn by the amount of RNA used in μL. Accuracy=(TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

3.1. Electrochemical detection

Electrochemical detection can be realized using biosensors that measure electrical signals from antigen-antibody binding. Electrochemical biosensors have important advantages over other methods, such as low cost of analysis, quantitative detection, high sensitivity and selectivity, and the potential for portability. Eissa and Zourob developed an electrochemical immunosensor without the need for sample pretreatment. In this device carbon nanofiber serves as an electrode functionalized with diazonium salt as a linker that binds coronavirus nucleocapsid protein. The electrode is covered with cotton fiber used to collect nasopharyngeal swabs. Detection is achieved by competitive assay foolowing exposure of the immunosensor to N protein antibody solution. This device is portable and requires a potentiostat connected to a smartphone for measurements. This immunosensor can be used for clinical diagnostics, as it is sensitive (LOD = 0.8 pg/mL) and analysis time is 20 min [141]. Another electrochemical biosensor that does not require sample pretreatment is a field-effect transistor-based biosensor produced by Seo et al. This device uses carbon (graphene) and detects SARS-CoV-2 spike protein through its binding to anti-spike antibodies. This method is also very fast (analysis in 10 min) and sensitive with a limit of detection of 242 copies/mL in clinical samples [142]. All approaches discussed so far involve anti-SARS-CoV-2 antibodies for specific binding. Some researchers developed an electrochemical biosensor that uses membrane-modified green monkey kidney cells bearing transmembrane anti-spike antibodies for detection of coronavirus spike protein. The biosensor produces very rapid results (in 3 min) with high sensitivity (LOD = 1 fg/mL) [143]. However, this biosensor is difficult to construct because it requires cell engineering and culturing and its performance depends on cell viability. For this reason, this approach has much less potential in clinical diagnostics.

3.2. Immunoassay-based methods

Immunoassay-based methods are old and widespread. These methods are relatively inexpensive, simple, and have a great potential for being point-of-care tests. Some of these methods have been successfully commercialized for rapid point-of-care testing. For example, some methods measure fluorescence signal to quantitatively detect SARS-CoV-2 nucleoprotein in a clinical sample. Two such methods include "STANDARD F COVID-19 Ag" and "Sofia SARS Antigen FIA. Fluorescence immunoassay consists of a single step of a nasopharyngeal swab loading, and results are returned in 30 min. The methods rely on a fluorescence analyzer. Limit of detection of these methods is much higher than for electrochemical methods discussed above - 2.5×10^5 RNA copies/mL [13]. Other portable fluorescence immunoassays were developed to simultaneously detect viral antigen with IgG and IgM antibodies [144].

The other commercialized point-of-care test based on immunoassay is even simpler – a lateral flow immunoassay where a nasopharyngeal swab sample reacts on a test card called "BinaxNOW COVID-19 Ag Card". It is the first FDA-approved rapid antigen test that does not require an instrument. This test provides qualitative results in 15 min, and has a detection limit equal to 4.04×10^4 copies/swab [145].

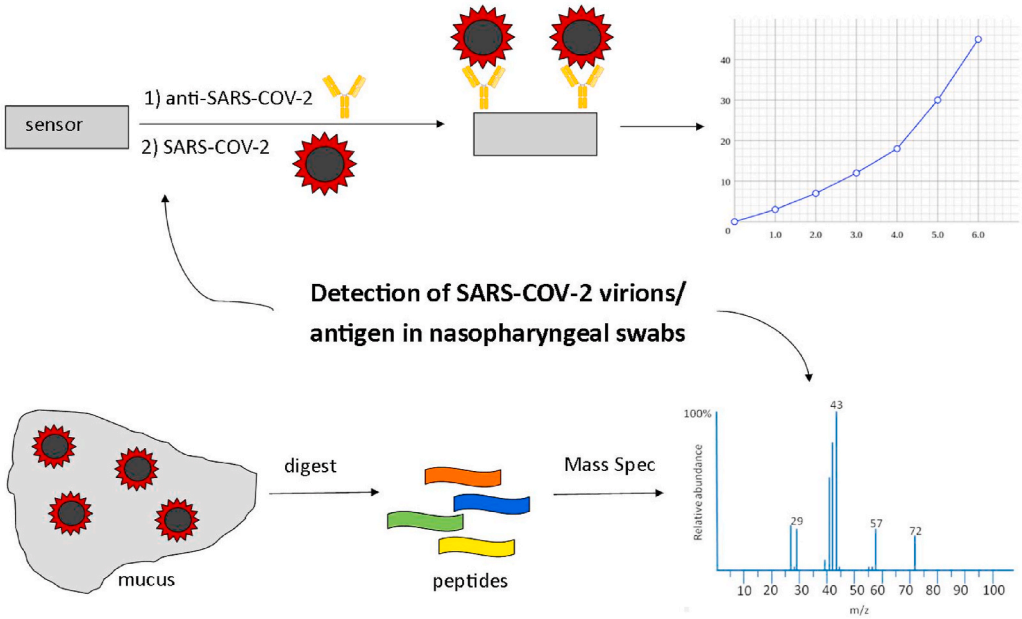

Fig. 4. Detection of viral antigen and particles in nasopharyngeal swabs. Top – fluorescence-based sensor; bottom – mass spectrometry method. (created with MS Publisher).

Overall, sensitivity of rapid antigen testing is much lower than RT-PCR and electrochemical detection. Nevertheless, lower detection limit of 0.65 ng/mL for a portable lateral flow immunoassay was reported [146].

There is another immunoassay-based method described in the literature which utilizes gold nanoparticles functionalized with antibodies and colorimetric readout [147]. This method is depicted on Fig. 5.

3.3. Mass spectrometry

Mass spectrometry for SARS-CoV-2 antigen detection was reported twice. In both studies scientists used targeted mass spectrometry. Cazares et al. reported limit of detection equal to 200 amol, meaning that quantitation and identification of proteins was performed at a single amino acid resolution. However, the analysis takes around 1.5 h to get results. Parallel reaction monitoring was used, and detection proceeded in artificial mucus samples, reflecting how this method can be used in biological matrices [148]. Bezstarosti et al. also received good results using the same method, with a detection limit of 0.9 pg of nucleocapsid protein in 1.5 h [149]. Sensitivity of targeted mass spectrometry is comparable to and can exceed RT-PCR. Parallel reaction monitoring is a very specific method of detection where preselected peptides (digestion products of proteins of interest) are measured, and their fragmentation pattern is identified. Then digested target proteins are analyzed, and results of preselected peptides serve as a control for identification. This way only target proteins from the matrix will be correctly detected. However, mass spectrometry is lengthy, difficult and requires highly trained personnel.

3.4. Detection of viral antigen and particles in saliva

Another interesting approach uses chronoamperometry to detect SARS-CoV-2 virions or spike protein using a first electrochemical reagent-free sensor. In this approach the authors used changes in current caused by binding of viral particles or spike protein to the spike-specific antibody anchored to the electrode through DNA aptamer. The schematic representation of this process can be seen on Fig. 6. Results are obtained in 5 min with a detection limit of 4000 copies/mL – much lower than for reagent-free lateral flow immunoassays. Another important feature of this sensor is its ability to detect coronavirus infection in patients' saliva without pretreatment. The sensor can be put in a person's mouth and give results without even the need for sample collection [150].

Making comparison between scientific laboratory methods with commercial methods is difficult because only a small number of commercial tests report detection limit, and those that do, report $TCID_{50}$/mL or pfu/mL units. To make a comparison, $TCID_{50}$/mL was converted to copies/mL. $TCID_{50}$/mL ≈ 4000 copies/mL using conversion from Liotti et al. [13], but conversion of copies of RNA further into mass is not performed here because antigen was detected in commercial tests, not RNA, so this conversion would be misleading. Thus, commercial tests that reported detection limit were used to estimate their LOD in copies/mL: "Quidel Sofia SARS Antigen FIA" (4.52×10^5 copies/mL), "SD Biosensor STANDARD F COVID-19 Ag Fluorescent immunoassay (FIA)" (2.5×10^5 copies/mL), "BinaxNOW™ COVID-19 Ag CARD" (4.04×10^4 copies), and "SD Biosensor STANDARD Q COVID-19 Ag Test" (1.98×10^6 copies/mL). All of them have LOD worse than LOD of scientific methods that reported LOD in copies/mL: 2.42×10^2 copies/mL [142] and 4×10^3 copies/mL [150]. It can be concluded that scientific methods of detection of antigen have better analytical sensitivity than commercial methods.

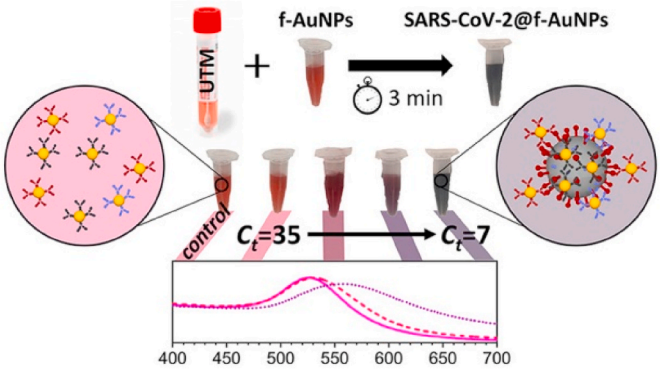

Fig. 5. SARS-CoV-2 viral particles bind to gold nanoparticles functionalized with antibodies targeting three SARS-CoV-2 surface proteins (spike, envelope, and membrane), and color of the solution changes. The binding of the viral antigen to functionalized nanoparticles red-shifts extinction spectrum of the solution. The extent of such shift depends on viral load. Reproduced under Creative Commons License from an open access article by Ventura et al. [147]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

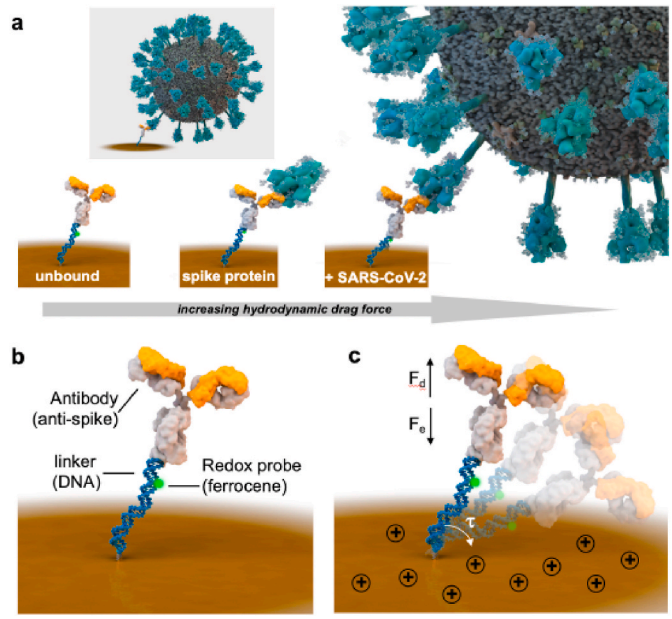

Fig. 6. Reagent-free chronoamperometry sensor. Binding of virions or spike protein to the spike-specific antibody anchored to the electrode through DNA aptamer causes changes in current, which are detected by chronoamperometry. Reproduced with permission granted by American Chemical Society from the article by Yousefi et al. [150].

4. Detection of antibodies to SARS-CoV-2

4.1. Comparison of ELISA, CLIA, and LFIA

SARS-CoV-2 specific IgG, IgM, and IgA antibodies most often become objects of detection using different methods. IgM antibodies appear in the acute phase of infection, and after reaching the maximum, they decrease to diagnostically insignificant levels. IgG antibodies build up more slowly than IgM antibodies, but they remain high in the patient's blood longer. After recovery, IgG antibodies can remain at a low level indefinitely as evidence of a previous illness.

Coronavirus antibody tests are important, as they provide information about whether a person has had a coronavirus infection in the past, i.e. whether he is a potential carrier of the disease and whether he has developed immunity [151–154]. While there is an estimate that antibody testing prevented about 12% of COVID-19 related deaths within the first year of pandemic [155]. Also, the measured level of antibodies provides feedback about vaccination efficiency, which may vary significantly [156]. It may help to predict when second or even third

vaccination is recommended since decrease in antibodies level with time after vaccination is subject to individual variability. This kind of personalized vaccination scheduling may optimize the application of vaccines, which still remains a scarce resource in many countries of the world, and it may decrease some unnecessary side effects of premature vaccination of individuals who still have sufficient antibody titer after natural exposure to virus or after the first/previous vaccination [157]. Since COVID-19 is sometimes asymptomatic, antibodies can be found even in a person who does not feel sick and is sure that he was not sick. Tests for antibodies allow to determine the stage of infection [153,158]. Overall, antibody testing is a complementary method for COVID-19 diagnostics. Besides, those who have antibodies to the coronavirus can donate blood, which will help treat others who are infected [154,159,160]. Plasma transfusion procedure helps people with severe courses of COVID-19. Also, considering the ongoing large-scale vaccination, antibody tests are becoming even more important: those who have recently had COVID-19 can delay vaccinations because they already have natural immunity. And since there are not enough vaccines in many countries, those who have been infected with SARS-CoV-2 are asked to wait three to six months to enable people without natural immunity to be vaccinated first [161]. Moreover, mass testing for the presence of antibodies is of great importance for the state, as it shows a real epidemic picture. The more people are tested, the more accurate the data on morbidity, mortality, severity, and characteristics of the clinical course of a new infection will be. Various nations, including the UK, Germany, and Italy, considered using antibody testing for 'immunity passports and this procedure can be critical to re-opening the economy [162].

Nowadays, methods for detecting COVID-19, taking a short time between collecting a sample from a patient and obtaining test results, are becoming more and more critical. Methods that take no more than 3 h are in demand, so researchers are working in this direction. During antibody testing, patients almost always donate either a blood sample or a saliva sample. However, although a saliva sample is less complicated and more comfortable to obtain, most testing methods work with a patient's blood sample.

Most often, blood for the sample is obtained by venipuncture, but blood obtained by a finger stick is also common. Collected blood samples are centrifuged; different researchers use different centrifuge conditions, for example at 800 g for 5 min [158], at 1000 rpm for 15 min [153], at 1740 g for 10 min [163], at 2200–2500 rpm for 15 min [164]. After that, the supernatant (serum) is removed and sent to storage. In the case of short-term storage, the serum is stored at −20 °C [163,165–169], and in the case of long-term storage the serum is stored at −80 °C [164,165,170,171]. Sometimes researchers do not specify the storage temperature of the serum, but simply write "frozen" [172]. Before analysis, the serum is thawed [163,166] or heat-inactivated at 56 °C [173,174].

ELISA (enzyme-linked immunosorbent assay), CLIA (chemiluminescence immunoassay) and LFIA (lateral flow immunoassay) are among the popular serological assays utilized to detect the antibodies of a virus. All these methods begin with the biofluids collection (most often blood) and sample preparation. ELISA, CLIA and LFIA searches in the Scopus database gave 223488, 1830 and 468 citations for each of those methods respectively. Apparently, ELISA is by far the most commonly used method among them. The ELISA method is based on the reaction between specific antigens and antibodies, and the result becomes visible and possible for quantitative analysis due to the enzymatic reaction. CLIA is a laboratory test that was developed in 1983 by Anthony Campbell when he replaced the radioactive iodine used in immunoassays with an acridinium ester that emits its own light [175], cited in Scopus for 1742 times (range 1990–2020). CLIA combines chemiluminescence (electromagnetic radiation caused by a chemical reaction to produce light) with an antigen-antibody immune complex. Both ELISA and CLIA are conducted in microplate wells, but if reaction in ELISA is most often confirmed by the change in color even with the naked eye (microplate reader is needed for quantitative analysis of wells' content), CLIA is verified by the production of light in chemiluminescence analyzer. LFIA, the technical basis of which was invented in 1956 by Plotz and Singer [176], like other methods, includes the reaction between an antigen and its corresponding antibody in biological materials, but it is less popular than above mentioned methods: it is cited in Scopus for only 468 times. However, in 2006 the production of various LFIA formats for a total amount of about $ 2.1 billion was carried by more than 200 companies around the world [176]. LFIA is an immunochemical method of analysis based on the principle of thin layer chromatography, carried out using special test strips, panels or test cassettes. The principle of operation is that when the test strip is immersed in a biological fluid (or other liquid samples), it begins to migrate along the strip according to the principle of thin-layer chromatography. Together with it, labelled specific antibodies applied to the lower part of the test strip move.

Fig. 7 illustrates the differences in working principles of the three most widely used serological assays: The enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA), and lateral flow immunoassay (LFIA). The ELISA and CLIA experimental plates already contain SARS-CoV-2 antigen molecules attached to it. Then, when the serum from the patient's sample is introduced, antibodies against SARS-CoV-2 attach to their antigens. At this point, the main differences between these assays become distinct. In ELISA, secondary antibodies linked with the enzyme bind to the patient antibodies, while in CLIA some enzymes bind directly to the patient's antibodies. The function of these enzymes is to catalyze the reactions of specific substrates (chemiluminescent substrates in CLIA). In ELISA this reaction leads to the measurable color change, while in CLIA detectable amounts of light are produced. These observations help to identify the amount of antibodies present in the patient's organism. The mechanism of LFIA is not much similar to the mechanism of the rest of the serological assays, but it is somewhat similar to one step pregnancy tests, which were introduced by Unilever in 1988 [177]. The sample, containing the SARS-CoV-2 antibodies, is placed on the special strip. Then, the sample flows laterally across the pad, and at the test line COVID-19 antibodies attach to their specific antibodies, and nanoparticle-linked antibodies bind to the virus antibodies. Due to these nanoparticles, the test line becomes visible, indicating the presence of SARS-CoV-2 antibodies in the sample. A control line is needed to verify that the assay actually works: nanoparticle-linked antibodies bind to specific antibodies on the control line, visualizing this line. The negative result is obtained when only the control line is visible; the positive result is obtained when both lines are visible. If the test line is visible while the control line is not, the strip must be defective.

4.2. Validation of CLIA, LFIA, ELISA serological tests by comparison with RT-PCR results

The review paper written by Ejazi, Ghosh, & Ali provides information on the sensitivity and specificity values of serological assays CLIA, LFIA and ELISA [18], which use serum from RT-PCR confirmed patients. First, most of the research based on serological tests was performed in China. According to this review, generally, the sensitivity of IgG antibody detection by CLIA after two weeks of disease is more than 90%. The sensitivity during detection by LFIA in the one-week interval from becoming ill was really low - almost always it did not exceed 30%. For ELISA, sensitivity gets better as the time after infection onset increases; for instance, sensitivity increases from 50 to 81% for IgM and from 81 to 100% for IgG when time increases from 0 to 5 days. In another assay made in China (The rS-based ELISA kit made in Hotgen, Beijing, China) the sensitivity improved from 46% to 91% between 0 and 5 days after the visible start of an infection and 11–15 days after the onset of the infection [178]. A similar trend of a drastic decrease in rates of false-negative results was observed in Germany when the time of the ELISA test shifted from 5-9 days to 10–18 days after onset [179]. So the optimum time for ELISA testing may be within 10–15 days after onset of disease or about 14–21 days after the patient may contract the virus,

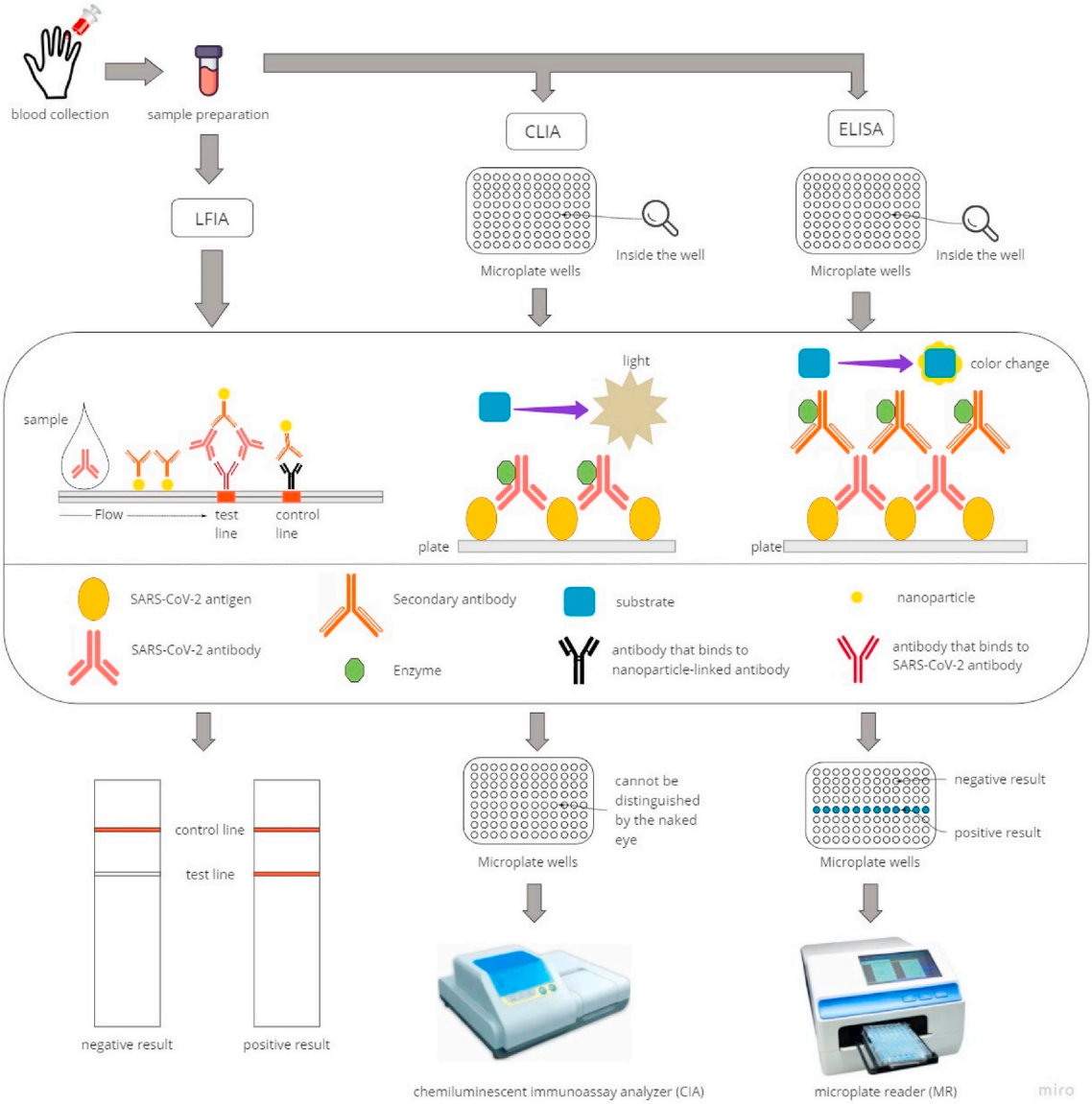

Fig. 7. Schemes of serological assays ELISA, CLIA, and LFIA for detection of SARS-CoV-2 antibodies. CIA is KPS-QQ80 Chemiluminescence Immunoassay Analyzer is from instrumentstrade.com; MR is NS-100 Nano Scan Microplate Reader from hercuvan.com. The figure is created with miro.com.

since the average incubation period is about 4–6 days. Serological tests conducted during this time frame would show the most sensitive results. The tests are not convenient ways to diagnose COVID-19 before the first symptoms appear.

After the incubation time, IgM antibodies that constitute the initial immune response are produced. IgG antibodies are produced next, developing a more specific immune response. However, IgG antibodies' concentration may be several times higher than the concentration of IgM antibodies. The average sensitivity of detection of IgM antibodies by CLIA is lower than that of IgG antibodies (83.6% vs. 94.5%). Based on the analysis of 14 representative ELISA tests, sensitivity for IgG is better than for IgM. For instance, IgG vs IgM was 81% vs 50% after 0 days and 100% vs 81% after 5 days in the relatively representative assay of 178 COVID-19-infected people [180]. As a result, IgG detection is more reliable than detection of other antibodies (IgM and IgA) [181]. Kontou et al. reached the same conclusion in their review [17].

Most of the results of the action of CLIA were able to detect the IgG antibodies separately from the rest of the antibodies, showing that the CLIA method is useful in determining the specific type of antibodies in the analyzed sample; its sensitivity for IgG antibodies constituted 94%. Most of the research on the detection of COVID-19 antibodies by LFIA reported common sensitivity values for all antibodies, not differentiating between IgM, IgG, and IgA. The average sensitivity of separate IgG antibodies (where it was given) was not high - only about 68%. So, LFIA is worse than CLIA in the detection of particular types of antibodies; it can be used only in complex antibodies detection. The specificity values for all methods were almost always higher than sensitivity values and overall high. For CLIA, specificity values were more than 90%, no matter which antibodies were analyzed. The CLIA method, therefore, should be specific for all antibodies even if the sensitivity values for different antibodies, for example, IgM and IgG, differ. From Table 2 in the review paper of Ejazi we calculated the average reported sensitivity of the ELISA method for all antibodies - about 81% - but the average reported false-positives rate that we calculated from the same source was only 3%. The standard error for this specificity was calculated as 1.02 that is lower than the standard error of sensitivity - 3.85. The low standard error for specificity indicates that this specificity value well represents the whole ELISA test. For LFIA, average specificity values are about 92%, which is much higher than average sensitivity values of 63%.

Another trend in ELISA testing is that S-protein based ELISA is

preferable to N-protein based ELISA in terms of sensitivity and earlier term of antibodies detection [178]. ELISA test in the USA achieved 97% sensitivity and 100% specificity when performed with the S1 domain the spike protein of the virus [182]. It is much more likely to get false-negative results than false-positive results with ELISA serological testing and overall false-negative results are far more likely in serological tests for antibodies in comparison to PCR tests for viral RNA.

4.3. Other assays used to detect SARS-CoV-2 antibodies

Single-step, wash-free digital immunoassay has also demonstrated that it is possible to detect human IgG antibodies to COVID-19 in 15 min [160]. This method is an adaptation of the AC + DC assay method. In general, during IgG antibodies incubation with a test sample and gold nanoparticles (2oAb-AuNPs), the recombinant PC biosensor serves as a surface to form a sandwich immunocomplex, which provides antibody detection. This method showed high accuracy: using 4 μL of serum, 26.7 ± 7.7 pg/mL of antibodies were detected. The limit of quantification (LOQ) was 32.0 ± 8.9 pg/mL.

Cady and others' team has described another method for the rapid detection of antibodies against COVID-19 [153]. With multiplexed grating-coupled fluorescent plasmonics (GC-FP) biosensor platform, the entire biosensing procedure took less than 30 min. They used human blood serum and dried blood spot samples as a sample to detect IgM, IgG and IgA antibodies against COVID-19. In terms of sensitivity and specificity, this method showed 100% sensitivity and 100% specificity for serum IgG. The test results almost always coincided with two commercial COVID-19 antibody tests based on enzyme-linked immunosorbent assay (ELISA) and a Luminex-based microsphere immunoassay that shows the viability of multiplexed grating-coupled fluorescent plasmonics (GC-FP) biosensor as a method of detection of antibodies against the coronavirus SARS-CoV-2.

A lateral flow immunoassay is another method to test for antibodies in a blood sample quickly. For example, Cavalera et al. reported that multi-target lateral flow immunoassay allows the evaluation of test results for all immunoglobulin classes (IgG, IgM, IgA) with the naked eye 20 min after the addition of a blood sample [183]. This study reported 100% diagnostic specificity and 94.6% sensitivity. Such high sensitivity values were obtained due to the double-line LFIA which increased the likelihood of detecting SARS-CoV-2 antibodies when they are present in the sample.

Another report improved a lateral flow immunoassay immunosensor to obtain dual lateral flow optical/chemiluminescence immunosensors to detect salivary and serum IgA [184]. As with the previous method, only 80 μL of diluted sample were required, and results that were visible to the naked eye were obtained in a short time of 15 min. No false positives were obtained in this study. Antibodies from saliva samples were also detected in a study conducted by MacMullan et al. [164]. In this study, a commercially available, serum-based enzyme-linked immunosorbent assay (ELISA) was adapted to work with a saliva sample. The specificity of this method was 100%, while the sensitivity only reached 84.2% for a set of 149 clinical samples. This indicates that the immunoassay in the saliva sample requires further research and refinement to improve sensitivity. Considering that collection of saliva samples is much more convenient, faster and safer than the collection of blood, saliva-based sample methods will be in wide demand after improvement of sensitivity values.

The statistical analysis could be applied to ELISA, CLIA, and LFIA assays reported in the literature. In the majority of the reports, the sensitivity and specificity of the immunoassays currently used in the world are compared. It was observed that for all ELISA, CLIA, and LFIA assays, like in Ejazi review, the fewer days have passed since the onset of the disease, the less sensitive the method was to antibodies (both IgG and total antibodies). For example, the lowest sensitivity of Euroimmun IgG ELISA kit was 20% and it was observed when less than 3 days from onset of disease have passed, while the maximum sensitivity for the same kit was 100% and it was observed when 22–30 days from onset of the disease have passed [170]. For CLIA the lowest sensitivity value was 0% for IgG detection by SARS-CoV-2 IgG assay from Abbott Laboratories, USA, when less than 3 days from the onset of symptoms have passed [185] - it was the worst sensitivity value for IgG detection among all CLIA assays. For total antibodies detection, the lowest sensitivity of 40% was shown by Elecsys Anti-SARS-CoV-2 assay from Roche Diagnostic GmbH, Germany, and SARS-CoV-2 Total COV2T assay made by Siemens Healthineers, Germany [186] when 0–7 days passed from disease onset. But if the CLIA tests were conducted when at least more than 14 days have passed from the onset of symptoms, sensitivity values were much higher, even maximum: 100% for the detection of total antibodies by Elecsys immunoassay (Roche Diagnostics, Germany) [185] and SARS-CoV-2 Total COV2T (Siemens Healthineers, Germany) [186]; and 100% for the detection of IgG antibodies by SARS-CoV-2 IgG (Abbott Laboratories, USA) [185]. The same trend can be seen in LFIA assays: the lowest sensitivity of 0% for total antibody detection was observed in Genrui Biotech Inc. (Shenzhen, China) and CTK Biotech Inc. (Poway, CA, USA) when less than 7 days from the disease onset have passed, but when the time period was more than 7 days, the sensitivity values for both assays exceeded 80% [187]. The maximum sensitivity for total antibody was detected when more than 14 days from symptoms onset have passed and constituted 97.6% [186]. Overall, more favorable values of sensitivity (>90) are obtained after at least 2 weeks from disease onset.

Also, it was found in ELISA that average sensitivity and specificity values for IgA antibodies are very similar to that for IgG antibodies: 70% sensitivity for IgA and 72% sensitivity for IgG and 97% for specificity for both antibodies. From the same data, it can be clearly seen that the average sensitivity values are lower than the average specificity values. Situation is similar with the CLIA assays: average sensitivity for IgG is lower than the average specificity for IgG (77% vs 98%). In CLIA and LFIA average sensitivity values for IgG detection are lower than that for total antibody detection, being 77% and 83% for CLIA and 42% and 56% for LFIA respectively, while average specificity values are close to each other: 98% and 99% for CLIA and 99% and 100% for LFIA, respectively. From the same data it can be derived that the average sensitivity values for LFIA are much lower than average sensitivities of ELISA and CLIA. Kontou et al. also reported this conclusion in their review [17]. Another difference between LFIA and other methods is that almost all of its immunoassays detect total antibodies with only a small part of the immunoassays detecting IgG. Another point to mention is that the standard error for specificity of IgG antibodies is much lower than the standard error for sensitivity of IgG antibodies in both ELISA and CLIA: 1.0 vs. 5.7 in ELISA and 0.6 vs. 5.6 in CLIA. Low standard errors for specificity indicate that these specificity values well represent the whole ELISA and CLIA tests. But, since the standard error for specificity in CLIA is slightly lower than that in ELISA, whole CLIA tests are better represented by their specificity values compared to ELISA tests.

According to the Table 12, if we take the average sensitivity and specificity of assays, regardless of antibodies detected, the sensitivity of ELISA is higher than the sensitivity of both CLIA and LFIA, as when less than 7 days and more than 7 days have passed after the onset of the disease. The specificity values at <7 days after the onset of the disease were absent for ELISA and LFIA but were very high for CLIA: 99.9%. Among all assays, the highest specificity at >7 days after the onset of the disease was recorded in CLIA and was equal to 98.4%.

Comparison of the mean values of the sensitivity and specificity of ELISA and CLIA kits taken from research articles with FDA approved and not FDA approved automated immunoassay tests shows the superiority of the sensitivity and specificity of both FDA approved methods (96.6% and 99.5%, respectively) and not FDA approved methods (86% and 99.5%, respectively). The situation is similar with LFIA kits. When comparing LFIA immunoassays from research articles with FDA-approved and not FDA-approved strip immunoassay tests, it is immediately noticeable that LFIA kits are lower in sensitivity and specificity

Table 12
Average sensitivity and specificity values for ELISA, CLIA, and LFIA kits.

	ELISA kits			CLIA kits			LFIA kits		
	Sens.	Spec.	Ref.	Sens.	Spec.	Ref.	Sens.	Spec.	Ref.
<7 days	33.8 ± 20% (20%; 56.3%)	N/A	[170,186]	45.1 ± 34% (0%; 100%)	99.9 ± 0.2% (99.6; 100%)	[185,186]	26.6 ± 18% (0%; 54%)	N/A	[186,187]
>7 days	81.3 ± 20% (15%; 100%)	95.5 ± 7% (69.5%; 100%)	[158,164,167,170,173,185,186,188–190]	83 ± 18% (32.9%; 100%)	98.6 ± 2%; (90.5%; 100%)	[167–169,171,185,186,188,190,191]	71.8 ± 19% (40%; 97.6%)	98 ± 4% (90%; 100%)	[167,183,186,187]

Note: Average ± standard deviation; Range (x%; y%) are shown. Accuracy=(TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

than FDA-approved tests (average sensitivity 97.5%, average specificity 98.1%) and not FDA-approved tests (94.9% and 98.2%, respectively). Most likely, this indicates that the ELISA, CLIA, and LFIA antibody detection kits show lower sensitivity and specificity values than those stated by the manufacturers. Among the FDA-approved automated immunoassay tests for detection of antibodies the greatest clinical performance is shown by "OmniPATH COVID-19 Total Antibody ELISA Test" (USA) with 100% accuracy and "ZEUS ELISA SARS-CoV-2 IgG Test System" (USA) with 99.1% accuracy. Nevertheless, some of the new assays from research papers approached the level of the FDA approved methods. For example, the In-house ELISA showed 100% sensitivity to IgG antibodies after 22–30 days from the onset of the disease [170], and human anti-IgGAM SARS-CoV-2 ELISA showed 94.7% sensitivity and 98.4% specificity for total antibodies detection when more than 2 weeks have passed since the onset of the disease [166]. The sensitivity of these assays is already higher than the average sensitivity of the CE-IVD approved methods (86%), therefore, it is possible that in the future they will be used widely.

4.4. Overview of the results of the commercialized antibodies detection methods

Rapid antigen testing is currently represented by immunostrip or fluorescence-based detection in nasopharyngeal swab samples, including two FDA-approved point-of-care tests, which were discussed in a corresponding section: "Sofia SARS Antigen FIA" and "BinaxNOW COVID-19 Ag Card". Other tests have been developed and registered in other regulation centers. These tests are much less sensitive than RT-PCR kits, but provide results in 30 min or less and are much easier to use. They either require adding several drops of extraction buffer to a nasal swab as a single manual step ("BinaxNOW COVID-19 Ag Card") or are fully automated ("Sofia SARS Antigen FIA"). The tests are lateral flow-based and fluorescence-based, respectively.

Antibody testing is achieved by using serum, plasma, or blood of a patient using immunostrip or ELISA-based methods. "Euroimmun Anti-SARS-CoV-2 ELISA" is one of the first FDA-registered automated ELISA kits. These methods can give results in as short as 10 min, but detection limit for the majority of them is not established and antibody testing is meaningful only at least 2 weeks after the onset of symptoms. Therefore, antibody testing cannot be used to diagnose a current infection. Another disadvantage is the need to draw blood from a patient. This procedure needs to be done under sterile conditions, preferably by a health care provider. For this reason, these tests have a limited scope of application compared to rapid antigen testing. The performance of government-approved immunoassay tests is summarized in Table 13.

Among the FDA-approved automated immunoassay tests for detection of antibodies the greatest clinical performance is by "OmniPATH COVID-19 Total Antibody ELISA Test" (USA, 100% accuracy) and "ZEUS ELISA SARS-CoV-2 IgG Test System" (USA, 99.1% accuracy). Among non-FDA registered immunoassays, the highest clinical performance is achieved by EDI™ Novel Coronavirus COVID-19 IgG ELISA Kit (100% accuracy). Clinical performance of FDA-approved tests is higher than that of non-FDA approved tests, and sensitivity is considerably higher. This is in addition to a higher number of samples used to validate the clinical results. Statistical comparison of various types of immunoassay tests is shown in Tables 14–18.

Among FDA-approved strip immunoassay tests for detection of antibodies, the best clinical performance is displayed by "Assure COVID-19 IgG/IgM Rapid Test Device" (100% accuracy for IgM and IgG) and "BIOTIME SARS-CoV-2 IgG/IgM Rapid Qualitative Test" (100% overall accuracy), which give results in 15–20 min. Both tests use lateral flow immunoassay principle.

Among non-FDA approved tests, "BioMedomics COVID-19 IgM-IgG Combined Antibody Rapid Test" (98.8% overall accuracy) and "BELTEST-IT COV-2 Rapid Test" (97.9% IgG accuracy and 97.6% IgG accuracy) show the highest clinical performance. Manufacturers of both tests

Table 13
Performance of automated commercialized government-approved immunoassay tests for detection of antibodies.

Name	Sample	LOD	Accuracy	Time, min	Ref
Euroimmun Anti-SARS-CoV-2 ELISA	Blood (IgG), 10 μL	N/A 519 samples	Sens = 90% Spec = 100% Acc = 90%	120	[192] US FDA
WANTAI SARS-CoV-2 Ab ELISA	Serum, plasma (IgG, IgM), 100 μL	N/A 736 samples	Sens = 98.7% Spec = 98.6% Acc = 97.3%	85	[193] US FDA
Mount Sinai COVID-19 ELISA IgG Antibody test	Serum, plasma (IgG)	OD = 0.15 114 samples	Sens = 92% Spec = 100% Acc = 92%	N/A	[194] US FDA
OmniPATH COVID-19 Total Antibody ELISA Test	Serum (IgG, IgM, IgA), 50 μL	OD = 0.2 299 samples	Sens = 100% Spec = 100% Acc = 100%	80	[195] US FDA
ZEUS ELISA SARS-CoV-2 IgG Test System	Serum, plasma (IgG), 100 μL	OD = 0.198 249 samples	Sens = 100% Spec = 99.1% Acc = 99.1%	85	[196] US FDA
University of Arizona COVID-19 ELISA pan-Ig Antibody Test	Serum (IgG, IgM, IgA)	OD = 0.389 360 samples	Sens = 97.5% Spec = 99.1% Acc = 96.6%	N/A	[197] US FDA
Platelia SARS-CoV-2 Total Ab	Serum, plasma (IgG, IgM, IgA), 15 μL	N/A 650 samples	Sens = 98% Spec = 99.6% Acc = 97.6%	90	[198] US FDA
EDI™ Novel Coronavirus COVID-19 IgM ELISA Kit	Serum (IgM), 10 μL	OD = 0.0669 274 samples	Sens = 73.1% Spec = 100% Acc = 73.1%	80	[199] CE-IVD
EDI™ Novel Coronavirus COVID-19 IgG ELISA Kit	Serum (IgG), 10 μL	5 U/mL 84 samples	Sens = 100% Spec = 100% Acc = 100%	80	[200] CE-IVD
MIKROGEN recomWell SARS-CoV-2 IgG and IgA	Serum, plasma (IgG, IgA), 10 μL	N/A 241 samples	IgG: Sens = 98% Spec = 98.7% Acc = 96.7% IgA: Sens = 73% Spec = 99.3% Acc = 72.3%	120	[201] CE-IVD

Note: OD = optical density.

Table 14
Comparison of performance of automated commercialized government-approved immunoassay tests for detection of antibodies.

Group	Average sensitivity	Average specificity	Average accuracy	Average time	Average number of samples
FDA	96.6%	99.5%	96.1%	100 min	418
Other	86%	99.5%	85.5%	92 min	210

used over 100 samples (106 and 1809, respectively) to validate clinical accuracy, which is a sufficient sample pool for reliable statistical analysis.

FDA-registered tests have higher sensitivity and accuracy than non-FDA registered tests, while tests from other authorization centers use more samples for validation, both requiring nearly the same time to get results. Overall, accuracy of IgG detection is better than that of IgM detection for both FDA and non-FDA registered tests.

It can be seen that testing for antibodies and viral RNA is more developed than testing for viral antigen, although clinical performance of rapid strip antigen tests is comparable to that of rapid strip antibody tests. Commercialized antigen point-of-care tests need to be developed as they provide information on active infection status in 15–30 min, with immunostrip tests being easy to use for general public.

5. Diagnostics of COVID-19 by clinical imaging techniques (X-ray, CT, ultrasound)

It is possible to determine whether a person is infected with SARS-CoV-2 not only using the PCR test, but also by applying clinical imaging techniques, the most common which are X-ray imaging, computed tomography (CT) scan, and ultrasound. A simplified process for the usage of these techniques to detect COVID-19 in a patient is presented in Fig. 8.

5.1. X-ray diagnostics

Medical X-ray imaging is the diagnostic method which uses X-rays and relies on differences in absorption of these rays by different tissues [230]. X-rays were discovered on November 8, 1895 by Wilhelm Konrad Röntgen. When the scientist exposed his hand to the mysterious rays, he saw a clear image of it on the screen, and the bones were visible much more clearly than soft tissues. After this discovery, the first X-ray machines and films were made in 1896 [231].

Fig. 8 demonstrates that initially the patient is irradiated with X-rays, and then the radiologist or artificial intelligence analyzes the image for the presence or absence of COVID-19 in the patient. Currently, a lot of research is aimed at developing and debugging, using deep learning and other machine learning methods, artificial neural networks that can determine from an X-ray image whether a person is infected with SARS-CoV-2 or not. Statistical analysis of these research articles shows how sensitive, specific, and accurate created neural networks are. Neural networks are aimed at dividing X-ray images into 2 classes (covid and non-covid), 3 classes (covid, other infections - most often pneumonia, and healthy patient), and 5 classes (covid, tuberculosis, bacterial pneumonia, viral pneumonia, and healthy). According to Table 19, it can be seen that the distribution by 2 classes exceeds the distribution by 3 classes in sensitivity, specificity, and accuracy. In other words, it is more difficult for artificial intelligence to simultaneously detect not only COVID-19, but also some other infections. In general, the sensitivity of all types of distributions is lower than the specificity and accuracy, while the latter are quite close to each other (98% vs 97% for the 2-class distribution, 87% vs 88% for the 3-class distribution, 96% vs 97% for all distributions). There was no data on sensitivity and specificity for 5-class distribution, but its average accuracy of 96% is only slightly lower than the 97% average accuracy of 2-class distribution, showing that it is

Table 15
Performance of automated commercialized government-approved immunoassay tests for detection of antigen.

Name	Sample	LOD	Accuracy	Time, min	Ref
Quidel Sofia SARS Antigen FIA	Nasal swabs (nucleocapsid protein), 120 μL	1.13×10^2 $TCID_{50}$/mL 209 samples	Sens = 99.4% Spec = 100% Acc = 99.4%	30	[202] US FDA
SD Biosensor STANDARD F COVID-19 Ag Fluorescent immunoassay (FIA)	Nasopharyngeal swabs (nucleocapsid protein)	62.5 $TCID_{50}$/mL (2.5×10^5 RNA copies/mL) 359 samples	Sens = 47.1% Spec = 98.4% Acc = 45.5%	30	[13] CE, KOREA MFDS

possible for neural networks to accurately classify COVID-19 among a few other diseases. The minimum accuracy in this type of distribution was shown by VGG-16 and was 93.9% [232], while the maximum accuracy of 99.9% was achieved by Modified MobileNet [233].

Considering all distributions, the DenseNet201 neural network showed the minimum value of specificity and accuracy (69.3 and 38.2%, respectively) [234], and the minimum sensitivity of 30% was demonstrated by ANOGAN when the specificity parameter was adjusted to 90% [235]. At the same time, the LBP Bag of Tree, HOG K-ELM, and LBP K-ELM neural networks achieved 100% specificity [236], the Respire., Emerg., And Rad-5th neural networks reached 100% sensitivity [237], and the neural network Modified MobileNet achieved a maximum accuracy of 99.9% [233].

5.2. CT diagnostics

Computed Tomography is a widely used diagnostic tool that is based on the reconstruction of the image data acquired by multiple X-ray techniques with a help of a computer [243]. The first commercially available CT scanner was created by Sir Godfrey Hounsfield of EMI Laboratories in 1972, and later in 1979 for this invention, Hounsfield was awarded the Nobel Prize in Physiology and Medicine along with the co-inventor of the CT technology, Dr. Allan Cormack [244].

Chest CT scan can be used to detect different infections, and COVID-19 with the associated pneumonia is not the exception. In order to determine the hallmarks of SARS-CoV-2 infection, Bernheim et al. studied the CT scans of 121 symptomatic patients and found out that bilateral and peripheral ground-glass opacities and consolidative pulmonary opacities are the most common patterns [245]. Apart from these features, vascular enlargement in the lesion and traction bronchiectasis are other characteristics of SARS-CoV-2 CT scans according to Zhao et al. [246]. Furthermore, Wang et al. studied the difference of CT scan for the patients infected with SARS-CoV-2 and influenza, to increase the specificity of the CT scan, and even though both infections had ground-glass opacities with consolidations, these viruses can be differentiated by the fact that 92.3% scans of SARS-CoV-2 infected patients had peripheral and non-specific distributions, while this percentage was as low as 3.3% for people with influenza in the following research. Moreover, SARS-CoV-2 infection presented the balanced lobe localization, shrinking contour, and clear lesion margin in comparison to influenza [247]. The progression of SARS-CoV-2 in 26 patients was studied by Pan et al. and depending on the CT scans of the patients, 4 stages of the infection were defined, in Stage 1 (0–4 days), mainly the ground-glass opacities were detected, and CT infection score of patients continuously raised till peaking in Stage 3 (9–13 days) and consolidation was observed in 91% of the CT scan at Stage 3, finally, the decrease in the infection CT score and in consolidation was noticed in stage 4 (>14 days) [248]. From the study of Pan et al. it can be concluded that the infection peaks after 9–13 days once the symptoms are noticed, and this statement can be supported by the findings of Wang et al. who found out that the peak of the illness is observed after 6–11 days of the onset of the symptoms [248, 249]. In addition, Wang et al. found that 66 of 70 discharged patients from the hospital after obtaining negative RT-PCR results still had residual disease patterns on their final CT scans, which means that CT scan might be more effective than RT-PCR in observing the progression, regression of the infection, and full recovery from SARS-CoV-2 [249]. Another benefit of using CT for SARS-CoV-2 detection is the fast speed of the procedure, as presented in Fig. 8. Only after a few minutes CT chest scan of the patient can be available, and if Deep learning systems classify the result as SARS-CoV-2 positive or negative, the results can be obtained in less than a minute. Radiologists with experience can also quickly detect the infected lung, however, a qualified expert is required to distinguish SARS-CoV-2 from other diseases and the healthy scan.

In order to assess the sensitivity of CT scanning in detecting SARS-CoV-2, a total of 17 methods were studied, where in 5 of the methods radiologists assigned the scans as healthy and infected, while in other 12 research papers artificial intelligence was applied to categorize the scans correspondingly. Deep learning technologies were created by teaching AI and showing the infected and healthy CT scans, and then the technology was tested. Table 20 displays the clinical performance of the CT scan-based methods, where in some studies RT-PCR results were used as a reference, while in other studies patients were diagnosed as SARS-COo-2 positive by physicians according to the symptoms, and exposure history, which made possible the evaluation of RT-PCR accuracy. It should be noted that five sources [250–254] had not used any healthy samples, so the results lack specificity, and the accuracy is equal to the specificity in those methods. As it can be seen from Table 20, CT scan can precisely find the infected patients, with the sensitivity of 94.5%, however, unlike other detection methods discussed in this review, the average specificity is quite low, being around 84%. This could due to the fact that CT-scan is more sensitive than RT-PCR, and some samples assigned to be negative by RT-PCR, are actually SARS-CoV-2 positive. This assumption can be supported by four studies [250–252,255] that assessed the sensitivity of both RT-PCR and CT-scan in detecting the SARS-CoV-2 and used the symptoms of patients as a reference point; the average sensitivity of RT-PCR and CT-scan for these papers were 85% and 96%, respectively.

The lowest specificity among the discussed papers is 25%. The study by Ai et al. (2020) [256] investigated 1014 patients, and employed RT-PCR as a reference material. As a result, RT-PCR had 601, CT scan had 888 positive results. According to the authors, using RT-PCR with low positivity rate as a reference caused the underestimation of specificity. This is because 81% of people with negative RT-PCR but positive CT scan results are considered highly likely cases of SARS-CoV-2 by the symptoms and exposure history of patients [256].

Following analysis of Table 20, it can be concluded that there is not any significant difference between the assessment of scans by radiologists or by AI technology, which means that deep learning technologies can be used to analyze the chest CT scans and provide accurate results, thus reducing the work load of the radiologists.

Table 16
Performance of commercialized government-approved strip immunoassays for detection of antibodies.

Name	Sample	LOD	Accuracy	Time, min	Ref
Assure COVID-19 IgG/IgM Rapid Test Device	Blood, serum, plasma (IgG, IgM), 50 μL	N/A 110 samples	IgG: Sens = 100% Spec = 100% Acc = 100% IgM: Sens = 100% Spec = 100% Acc = 100%	15	[203] US FDA
Cellex qSARS-CoV-2 IgG/IgM Rapid Test	Blood, serum, plasma (IgG, IgM), 10 μL	N/A 378 samples	Sens = 93.8% Spec = 96% Acc = 89.8%	20	[204] US FDA
Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit	Serum, plasma (IgG, IgM), 10 μL	N/A 568 samples	IgG: Sens = 100% Spec = 94.8% Acc = 94.8% IgM: Sens = 97.1% Spec = 94.8% Acc = 91.9%	15	[205] US FDA
SGTi-flex COVID-19 IgG	Blood, serum, plasma (IgG), 10 μL	N/A 419 samples	Sens = 96.7% Spec = 100% Acc = 96.7%	10	[206] US FDA
CareStart™ COVID-19 IgM/IgG	Blood, serum, plasma (IgG, IgM), 10 μL	N/A 246 samples	IgG: Sens = 100% Spec = 98.8% Acc = 98.8% IgM: Sens = 90% Spec = 98.8% Acc = 88.8%	10	[207] US FDA
Tell Me Fast Novel Coronavirus (COVID-19) IgG/IgM Antibody Test	Serum, plasma (IgG, IgM), 10 μL Blood (IgG, IgM), 20 μL	N/A 233 samples	Sens = 100% Spec = 99.4% Acc = 99.4%	15	[208] US FDA
BIOTIME SARS-CoV-2 IgG/IgM Rapid Qualitative Test	Serum, plasma, blood (IgG, IgM), 10–15 μL	N/A 380 samples	Sens = 100% Spec = 100% Acc = 100%	20	[209] US FDA
Innovita 2019-nCoV Ab Test (Colloidal Gold)	Serum, plasma (IgG, IgM), 10 μL Blood (IgG, IgM), 20 μL	N/A 468 samples	Sens = 100% Spec = 98% Acc = 98%	15	[210] US FDA
WANTAI SARS-CoV-2 Ab Rapid Test	Serum, plasma, blood (IgG, IgM), 10 μL	N/A 403 samples	Sens = 94.7% Spec = 98.9% Acc = 93.6%	15	[211] US FDA
Livzon The Diagnostic Kit for IgM/IgG Antibody to Coronavirus	Serum, plasma (IgG, IgM), 10 μL Blood (IgG, IgM), 20 μL	N/A 644 samples	Sens = 90.6% Spec = 99.2% Acc = 89.8%	15	[212] CE
AIDIAN 2019-nCoV IgG/IgM Rapid Test Cassette	Blood (IgG, IgM), 20 μL	N/A 16 samples	IgG: Sens = 100% Spec = 98% Acc = 98% IgM: Sens = 85% Spec = 96% Acc = 81%	10	[213] CE-IVD
Wondfo SARS-CoV-2 Antibody Test	Serum, plasma, blood (IgG, IgM), 10 μL	N/A 596 samples	Sens = 86.4% Spec = 99.6% Acc = 86%	25	[214] CE, CFDA
CTK OnSite™ COVID-19 IgG/IgM Rapid Test	Serum, plasma, blood (IgG, IgM), 30 μL	N/A 126 samples	IgG: Sens = 98.8% Spec = 100% Acc = 98.8% IgM: Sens = 88.2% Spec = 100% Acc = 88.2%	15	[215] CE-IVD
Virusee COVID-19 IgG/IgM Lateral Flow	Serum, plasma, blood (IgG, IgM), 10 μL	N/A	Sens = 97.3% Spec = 99.2% Acc = 96.5%	10	[216] CE, CFDA
UNscience COVID-19 IgG/IgM Rapid Test Kit	Serum, plasma, blood (IgG, IgM), 20 μL	N/A 421 samples	Sens = 98.8% Spec = 98% Acc = 94.8%	10	[217] CE
BioMedomics COVID-19 IgM-IgG Combined Antibody Rapid Test	Serum, plasma (IgG, IgM), 10 μL Blood (IgG, IgM), 15 μL	N/A 106 samples	Sens = 100% Spec = 98.8% Acc = 98.8%	10	[218] CE-IVD
BELTEST-IT COV-2 Rapid Test	Serum, blood (IgG, IgM), 50 μL	N/A 1809 samples	IgG: Sens = 98.2% Spec = 99.7% Acc = 97.9% IgM: Sens = 98.1% Spec =	20	[219] CE

(continued on next page)

Table 16 (*continued*)

Name	Sample	LOD	Accuracy	Time, min	Ref
			99.5% Acc = 97.6%		
Hightop SARS-CoV-2 IgM/IgG Antibody Rapid Test	Serum, plasma (IgG, IgM), 10 μL Blood (IgG, IgM), 20 μL	N/A 550 samples	Sens = 94.2% Spec = 93.9% Acc = 88.1%	20	[220] CE
SD Biosensor STANDARD Q COVID-19 IgM/IgG Duo Test	Serum, plasma, blood (IgG, IgM), 10 μL	0.02 mg/mL 504 samples	Sens = 99.1% Spec = 95.1% Acc = 94.2%	15	[221] CE-IVD

Table 17
Comparison of performance of commercialized government-approved strip immunoassays for detection of antibodies.

Group	Average sens	Average spec	Average acc	Average time	Average number of samples
FDA	97.5%	98.1%	95.6%	14.5 min	356
Other	94.9%	98.2%	93.1%	15 min	530

Table 18
Performance of strip immunoassays for detection of antigen.

Name	Sample	LOD	Accuracy	Time, min	Ref
BinaxNOW™ COVID-19 Ag CARD	Nasopharyngeal swabs (nucleocapsid protein), 50 μL	4.04×10^4 copies/swab 460 samples	Sens = 84.6% Spec = 98.5% Acc = 83.1%	15	[145, 222] US FDA
SD Biosensor STANDARD Q COVID-19 Ag Test	Nasopharyngeal swabs (antigens), 350 μL	$3.12 \times 10^{2.2}$ $TCID_{50}$/mL 1659 samples	Sens = 84.9% Spec = 98.9% Acc = 83.8%	15	[223] CE-IVD
ACRO Biotech Inc. COVID-19 Antigen Rapid Test	Nasopharyngeal swabs (antigens), 100 μL	N/A 145 samples	Sens = 97.7% Spec = 99% Acc = 96.7%	15	[224] CE
COVID-19 Ag Respi-Strip	Nasopharyngeal swabs (nucleocapsid protein), 100 μL	5×10^3 pfu/mL 189 samples	Sens = 91.2% Spec = 99.4% Acc = 90.6%	30	[225] CE-IVD

Despite the high sensitivity of CT scanning in detecting SARS-COV-2, it is significant to note the patients are exposed to ionizing radiation, and the amount of radiation received from chest CT scan is 400 times more than that from chest X-ray, and the similar amount of background radiation is received in more than two years [267]. Therefore, Tofighi et al. (2020) proposed to apply Low-Dose and Ultra Low-Dose CT scans that cause 5–8 times and 20–25 times less radiation and still retain the significant accuracy [268]. The expensiveness of CT scan machines can be a possible obstacle for the popularization of the method, since the machine cost ranges between $80,000-$300,000 [269], while the price for taking the chest CT scan is around $675 – $8600 in the US [270].

5.3. Ultrasound diagnostics

Lung ultrasound is also considered a potential method for detecting COVID-19 in patients. Due to the fact that it does not carry ionizing radiation [271–278], this method is beneficial for those patients who should not be exposed to radiation, such as pregnant women. As shown n Fig. 8, a special device sends an ultrasound signal to the organ being examined. The signal is reflected from the organ and picked up by the sensor. The information received is processed by a computer, and the image of a cross-section of an organ appears on the screen. Sound waves were first discovered by the Italian L. Spallanzani in 1794, who proved that a bat with plugged ears ceases to navigate in space. Experiments aimed at using ultrasound waves as a tool for diagnosing neoplasms began in the 40s of the 20th century. In 1947, the Austrian physician Karl Dussik and his brother physicist Friedrich introduced the hypersonography method, which was able to detect a brain tumor by measuring the intensity, with which an ultrasound wave passed through the patient's skull [279].

Currently, studies are underway on the effectiveness of the use of lung ultrasound examination for the detection of COVID-19. These examinations fall into two categories: examinations conducted in emergency departments using point-of-care lung ultrasound (POCUS) and conventional lung ultrasound (LUS) examinations. According to Table 21, although POCUS is worse than conventional LUS in specificity (58% versus 84%), it is better than it in sensitivity (95% versus 64%). Also, POCUS outperforms conventional LUS in the AUC (area under the curve) parameter: 0.94 and 0.83, respectively. When all lung ultrasound examinations are considered, the greatest sensitivity of 100% was demonstrated by Walsh (2020), who worked with POCUS [275]. However, in this study, CT was used as the gold standard and not all patients took an RT-PCR test for comparison, so the results may be slightly inaccurate. Bosso (2020) achieved the highest specificity of 89% when working with conventional LUS [271].

Overall, lung ultrasound is a relatively non-invasive and harmless method, but its specificity and sensitivity still need to be improved before it may approach the test accuracy of CT, and so far, this technique may only be preferable in scanning of pregnant women and other radiation vulnerable patients.

A receiver operating characteristic (ROC) curve, also known as an error curve, is used in addition to sensitivity and specificity to analyze and compare the methods of medical diagnostics that involve artificial intelligence used to detect COVID-19. This curve shows the relationship between true positive rate and false positive rate demonstrated by some method. The area bounded by the ROC curve and the false positive rate axis is a quantitative interpretation of the ROC curve and known as the area under the curve (AUC) value. The higher the AUC indicator, the better the method in terms of classification (the maximum AUC value is 1), while the value of 0.5 demonstrates the unsuitability of the selected classification method (corresponds to random fortune-telling). A value less than 0.5 indicates that the classifier acts exactly the opposite: if positive are called negative and vice versa, the classifier will perform better.

The brief summary of X-ray, CT-scan and lung ultrasound-based detection of SARS-CoV-2 can be found in Table 22. According to Table 22, despite the fact that CT scan sensitivity is superior to X-ray sensitivity, being 94.5% and 80.4% correspondingly, the specificity and accuracy of X-ray detection is slightly higher than that of the counterpart. At the same time, lung ultrasound performs worse than the other two methods in both parameters, showing the specificity of 68.4% and the sensitivity of 79.7%. Regarding AUC, X-rays and CT scans show a fairly high value of 0.95, while its value for lung ultrasound is only 0.87. Overall, lung ultrasound appears to be the most unreliable method for detecting COVID-19; its only advantages are that the patient is not exposed to ionizing radiation during the examination and that the cost of the ultrasound machine is relatively low (starts from $10,000). Regarding cost, the CT scan machine is almost twice as expensive as the

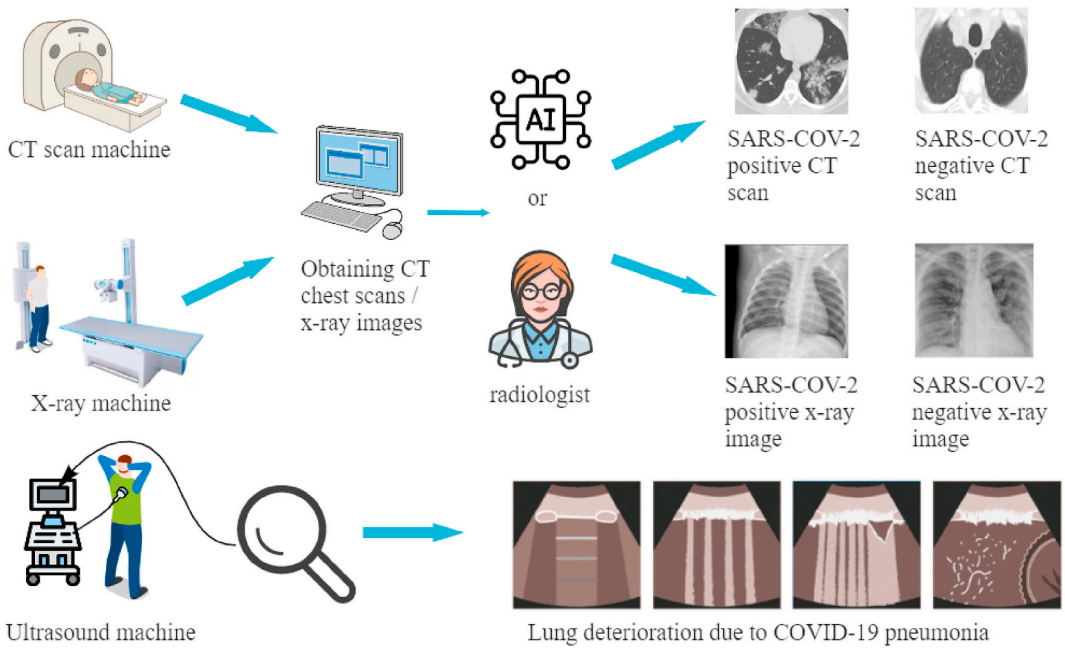

Fig. 8. SARS-CoV-2 Detection Process by X-ray and CT scan. Positive and Negative X-ray and CT scans are retrieved with permission from an open-access article by Saha et al. (2021) [226]. Lung ultrasound images are retrieved with permission from an open-access article by Smith et al. (2020) [227]. X-ray machine icon is obtained from Alibaba (2021) [228]. Lung ultrasound machine icon is obtained from Flaticon (2021) [229]. CT scan machine icon from CleanPNG (2021), and Computer icon from PinClipart (2021). Note: Figure was made with miro.com.

Table 19
Statistical results of detection of COVID-19 from X-ray images.

	Specificity	Sensitivity	Accuracy	N of samples	N of ill patients	Ref
2-class distribution (COVID and non-COVID)	98 ± 3.4% (88.7%; 100%) *99.35%*	89 ± 10% (56.8%; 100%) *92.4%*	97 ± 2.0% (91.4%; 99.7%) *98.2%*	727 ± 379 (162; 1332) *625*	118 ± 52 (62; 276) *125*	[236–239]
3-class distribution (COVID, other infection/s, healthy)	87 ± 9.5% (69.3%; 98.6%) *88%*	67 ± 24% (30%; 98.6%) *59.3%*	88 ± 17% (38.2%; 99.7%) *96%*	988 ± 651 (336; 2276) *632*	272 ± 189 (112; 573) *160.5*	[233–235, 239–242]
5-class distribution (COVID, tuberculosis, bacterial pneumonia, viral pneumonia, healthy)	N/A	N/A	96 ± 1.6% (93.9%; 99.9%) *95.9%*	804 ± 125 (680; 1000) *680*	119 ± 58 (51; 200) *51*	[232,233]
All distributions: Average	96 ± 5.3% (69.3%; 100%) *98%*	83 ± 19% (30%; 100%) *91.2%*	97 ± 3.5% (38.2%; 99.9%) *96.7%*	896 ± 500 (162; 2276) *632*	186 ± 153 (51; 573) *125*	[232–242]

Note: Average ± standard deviation; Range (x%; y%); *Median* are shown. Accuracy=(TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

Table 20
Accuracy, Sensitivity, and Specificity of CT scan-based SARS-CoV-2 Detection.

	Specificity	Sensitivity	Accuracy	N of samples/scans	N of ill patients/scans	Ref
Assessed by Radiologists	25% (only from Ref. [256])	95.9 ± 4.4%; (89%; 100%) *97.0%*	90.1 ± 13.1%; (68%; 100%) *95.8%*	43; 51; 167; 1014; 295	43; 51; 167; 601; 295	[250–253,256]
Assessed by AI	90.8 ± 11.1%; (66%; 99.6%) *94.9%*	94.0 ± 5.1% (82%; 100%) *94.7%*	92.5 ± 7.3%; (77%; 99.4%) *94.5%*	434; 189; 400; 73; 510; 496; 203; 167; 668; 495; 105; 374	127; 83; 200; 34; 275; 250; 98; 95; 445; 244; 105; N/A	[254,255, 257–266]
Average Range *Median*	84.2 ± 23.3%; (25%; 99.6%) *94.7%*	94.5 ± 4.8%; (82%; 100%) *95.8%*	91.8 ± 9.0%; (68%; 100%) *94.5%*	43; 51; 167; 1014; 434; 189; 400; 295; 73; 510; 496; 203; 167; 668; 495; 105; 374	43; 51; 167; 601; 127; 83; 200; 295; 34; 275; 250; 98; 95; 445; 244; 105; N/A	[250–266]

Note: Average ± standard deviation; Range (x%; y%); *Median* are shown. Accuracy = (TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

Table 21
Statistical results of detection of COVID-19 from lung ultrasound images.

	Specificity	Sensitivity	Accuracy	AUC	N of samples	N of ill patients	Ref
POCUS	58 ± 33% (21.3%; 88%); *64.9%*	95 ± 4.3% (92%; 100%); *93.3%*	33.3 (only one ref)	0.94 (only one ref)	184 ± 174 (77; 384); *90*	102 ± 160 (5; 287); *15*	[273, 275, 280]
Conventional LUS	84 ± 7.1% (79%; 89%); *84%*	64 ± 11% (52%; 73%); *68%*	N/A	0.83 ± 0.12 (0.75; 0.92); *0.83*	66 ± 15 (53; 83); *63*	42 ± 35 (19; 82); *26*	[271, 272, 274]
All LUS	68 ± 28% (21.3%; 89%); *79%*	80 ± 18% (52%; 100%); *82.5%*	33.3 (only one ref)	0.87 ± 0.11 (0.75; 0.94); *0.92*	125 ± 128 (53; 384); *79.5*	72 ± 109 (5; 287); *22.5*	[271–275, 280]

Note: Average ± standard deviation; Range (x%; y%); *Median* are shown. POCUS - point-of-care lung ultrasound, LUS - lung ultrasound. Accuracy=(TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

Table 22
Comparison of the Clinical Performance in Detecting SARS-CoV-2, Cost and Radiation Dose of CT scan, X-ray, and lung ultrasound.

	CT scan	Ref.	X-ray scan	Ref.	Lung ultrasound image	Ref.
Specificity	84.2 ± 23.3% (25%; 99.6%) *94.7%*	[255–257,259–263, 265,266]	94.1 ± 7.9% (69.3%; 100%) *98%*	[234–238,242]	68.4 ± 28.1% (21.3%; 89%) *79%*	[271–273, 275, 280]
Sensitivity	94.5 ± 4.8% (82%; 100%) *95.8%*	[250–266]	80.4 ± 19.7% (30%; 100%) *91.2%*	[234–237]	79.7 ± 18.4% (52%; 100%) *82.5%*	[271–275, 280]
Accuracy	91.8 ± 9.0% (68%; 100%) *94.5%*	[250–266]	93.2 ± 12.0% (38.2%; 99.9%) *96.7%*	[232–234,236, 238–242]	33.3% (only one ref)	[273]
AUC	0.95 ± 0.04 (0.89; 0.996) *0.953*	[254,257,260, 262–266]	0.95 ± 0.06 (0.77; 0.9997) *0.9799*	[235,237,238,241, 242]	0.87 ± 0.11 (0.75; 0.94) *0.92*	[271, 272, 275]
Cost	Machine: $80,000-$300,000	[269]	X-ray room: $40,000-$175,000	[281]	Machine: $10,000-$200,000	[282]
Radiation dose	Average dose: 7–8 mSv Low-dose: 1–1.5 mSv Ultra low-dose: 0.3 mSv	[268]	Approximate effective radiation dose for chest x-ray: 0.1 mSv	[283]	No ionizing radiation	[271–278]

Note: Average ± standard deviation; Range (x%; y%); *Median* are shown. AUC - Area under the curve. Accuracy=(TP + TN)/(TP + TN + FP + FN); Sensitivity = (TP)/(TP + FN); Specificity = (TN)/(TN + FP). TP – true positive, TN – true negative, FP – false positive, FN – false negative.

X-ray room, and the average radiation dose of chest CT scanning is 70–80 times higher. From Table 22 it can be concluded that X-ray detection, which is a more accurate, cost-effective and less harmful method, can be a more promising detection method than CT scan and lung ultrasound.

6. Conclusion

COVID-19 pandemic is still continuing, while new variants such as delta-variant [284] and omicron are spreading among the population. In this review paper, different COVID-19 detection methods, both widely used since the beginning of the pandemic and new ones, adapted in research laboratories, were summarized and their benefits and limitations were discussed.

In this review, Figs. 1–8 illustrate the processes and visual results of detection of COVID-19 with different methods such as qPCR, CRISPR, RT-LAMP, RT-RPA, RT-RAA, mass spectrometry, fluorescence-based sensor, reagent-free chronoamperometry sensor, ELISA, CLIA, LFIA, X-ray, CT, ultrasound.

RT-PCR is the main method for detecting viral nucleic acids of COVID-19 throughout the world and according to Table 1, which reviewed publications about not yet commercialized RT-PCR research methods, it has an average accuracy about 97.7%

Commercial kits were reviewed as well, and, in general, their performance was better than that of the scientific methods that are not yet implemented widely. Table 5 displays the comparison between scientific and commercial RT-PCR, and following this table, average sensitivity of 84% of RT-PCR from scientific literature is comparably less than the average sensitivity of FDA-approved commercial PCR methods that has the value of 98%. Also, as the commercial kits were tested on a bigger number of clinical samples, they have confirmed their worth and therefore are more reliable. Comparison between commercial kits was different depending on the method used (RT-PCR, immunoassay tests, strip immunoassay tests, etc.), but the values did not differ significantly. Performances of commercialized government-approved kits are shown in Tables 11–18

Although RT-PCR is considered to be the gold standard for detection of SARS-CoV-2, many different other methods have already been developed, and Table 2 compares the performance of these methods. For example, biosensors showed good results, and even though in comparison to RT-PCR they had lower accuracy, they are still practical and can be used for rapid detection of COVID-19. Also, several portable devices showed promising results in detection of viral genome. The advantage of portable devices is that they can be used to quickly detect COVID-19 both in the hospital and outside the hospital, for example, at the patient's home by an ambulance team.

Another alternative for RT-PCR methods are CRISPR, RT-RPA and RT-LAMP, as they are cost effective and require less time for detection that can be seen from Tables 6–9 and Table 11, which compare SARS-CoV-2 RNA detection methods including CRISPR, RT-LAMP, RT-RPA, RT-RAA, PCA RT-PCR. However, it is important to mention that RNA should preferably be extracted by kits, as other methods may negatively affect accuracy values.

Detection of viral particles and antigens could be another alternative

to RT-PCR. The main advantage of these methods is rapid and early detection, which can also be done by patients themselves. This is highly convenient for patients because the need to visit the hospital disappears, and patients save money and time on the road to the hospital and in the hospital itself.

Testing the level of antibody to SARS-CoV-2 is another detection method of patients infected with COVID-19. Analyzing performance of ELISA, CLIA and LFIA from comparative Table 10, it can be concluded that among these methods CLIA showed the best results in terms of sensitivity and specificity. However, it is important to note that antibody testing is not 100% helpful compared to previously mentioned methods like RT-PCR when early detection of the virus is required. The antibody testing methods are useful for other practices, like producing accurate statistics on the spread of the virus, showing number of infected and recovered people, determination of vaccination schedules, determination of whether the vaccine is working (whether antibodies appear in the body of a vaccinated person), etc.

X-ray, ultrasound and CT scans are also used to detect the virus. When using these methods, the presence of a disease in a patient (some percentage of lung damage) is most often determined by a doctor, for example, a radiologist. However, at present, much attention is paid to the development of artificial intelligence to accomplish this task. Humans use thousands of images to train neural networks to detect COVID-19 in patients from images obtained via clinical imaging techniques. The development of artificial intelligence in this direction has already yielded positive results. As Table 20 shows, the sensitivity and accuracy of artificial intelligence in identifying COVID-19 patients from CT images is comparable to that of a radiologist (average sensitivity 94–96% and average accuracy 90–93%).

If we want to compare these clinical imaging techniques, according to Table 22, which demonstrates different characteristics of CT scan, X-ray scan and lung ultrasound image, among three methods X-ray and CT scans showed similarly high clinical performance (average accuracy 92–93%) relative to lung ultrasound imaging, for which accuracy of 33.3% was known from only one research article [273]. But, of course, these two methods, due to the patient's exposure to radiation (average radiation dose for CT scan is 70–80 times higher than approximate effective radiation dose for chest X-ray scan), are inferior in safety to ultrasound that produces no ionizing radiation. In order to avoid the harmful effects of these methods, it is recommended for use only for initial inspection: to identify the level of lung damage and determine the next course of action, i.e. only examine those who tested positive for COVID-19 in RT-PCR or other reliable tests.

In conclusion, testing for SARS-CoV-2 of people with symptoms similar to those of the COVID-19 and regular testing of people working and visiting public places is still crucial during the pandemic. Early diagnosis of the disease with testing will help identify carriers of the virus and limit their interaction with other people through quarantine. Future research in this area should focus on developing portable, harmless, fast and accurate methods of detection of COVID-19 markers, which can be used by patients even at home, eliminating the need to visit hospitals at the times when it is unsafe to go to public places. This is also important for rapid testing of a big number of people, as we want and do our best to return to pre-pandemic life.

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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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