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Miniaturized Real-Time PCR systems for SARS-CoV-2 detection at the Point-of-Care

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ABSTRACT

Over the past two years, SARS-CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus 2) infection has spread globally causing multi-organ disease and severely impacting the healthcare systems of all countries. Accordingly, the development of easy-to-access diagnostic devices has become essential to limit the effect of the virus worldwide.

Real-Time PCR is considered the gold standard to identify SARS-CoV-2 infection due to high sensitivity, affordability, and capacity to detect low viral loads at early disease stage. Advances in lab on a chip technology has led to the development of some Point-of-Care (POC) devices using Real-Time PCR and approved by the United States Food and Drug Administration.

We provide an overview on recently developed POC tests for the rapid diagnosis of COVID-19 infection. Practical applications of miniaturized devices based on viral genome amplification as well as favorable features such as reduced sample processing time, ease of use by non-specialized personnel, and the potential of PCR-based POC technologies will be highlighted and reviewed.

1. Introduction

In late 2019, Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus 2) broke out and spread globally within weeks. The etiologic agent of COVID-19 belongs to the Coronaviridae family, a group of three novel coronavirus including Severe Acute Respiratory Syndrome-Corona Virus (SARS-CoV) and Middle East Respiratory Syndrome-Corona Virus (MERS-CoV) that cause acute disease with frequently poor outcome in case of severe illness [1].

Coronaviruses have a single stranded RNA genome of 26–32 kb [2]. On the circular external surface of the SARS-CoV-2, the Spike protein can bind the human cellular receptor Angiotensin Converting Enzyme 2 (ACE2) on the lung cells membrane and small intestine cells promoting the internalization of the virus into the cell [3].

SARS-CoV-2 can be detected in a variety of body fluids such as blood saliva, sputum, etc., and different tissues. Most of the biospecimens are collected from the upper respiratory tract by using nasopharyngeal and/or oropharyngeal swabs. Samples should be harvested in a viral transport medium (VTM), kept at 2–8 °C up to 72 h after collection, and transferred to the laboratory. Improper storage and uncontrolled movements are one of the most common causes of false negative or invalid results [4]. Moreover, if the test is not sufficiently sensitive, a low viral load could mislead clinicians and patients, and thus promoting the spread of the disease extensively.

In this review, we take into account instruments and tools that may improve the aspects of early but reliable diagnosis by evaluating the state of art of Point-Of-Care (POC) devices and their potential, and comparing seven available market RT-PCR platforms (Reverse Transcription-Polymerase Chain Reaction) specifically designed for the detection of SARS-CoV-2. These devices are easy to use and often do not require particular skills to be performed or heavy laboratory equipment. More importantly, they can be used in geographical contexts where resources are limited. Therefore, the application of POC devices can be

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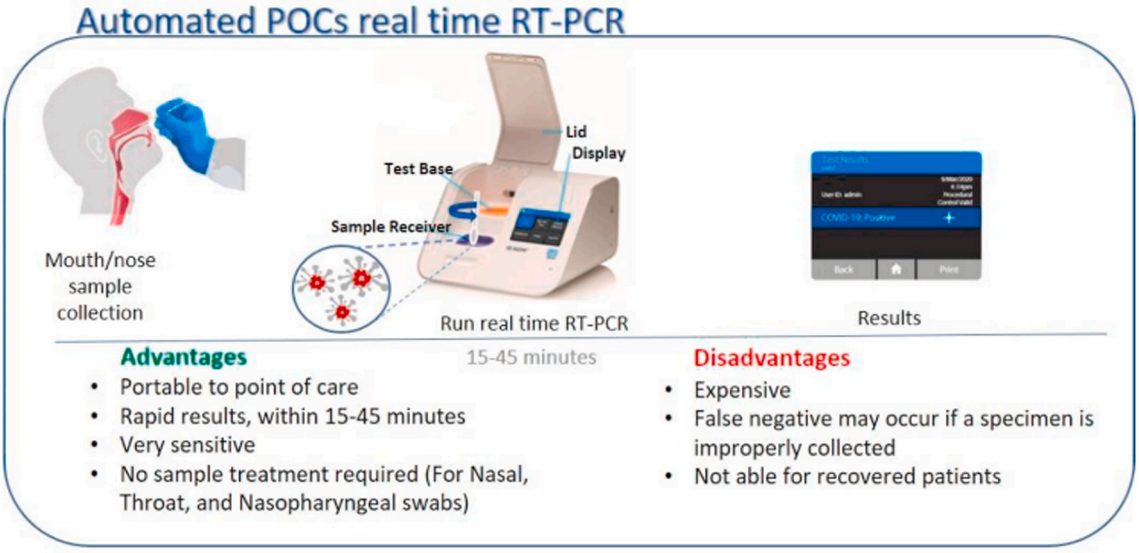

Fig. 1. Advantages and disadvantages of POC RT-PCR devices [22].

done anywhere and delivers results right after a simple procedure. In addition, they exhibit robustness, high specificity, selectivity, short sample handling steps and provide the opportunity to make clinical decisions in a short period of time [5].

In particular, we will describe two kits from Cepheid and one of each of other five companies: Visby Medical, Cuehealth, Abbott, MESA Biotech, and A. Menarini Diagnostics.

2. SARS-CoV-2 and Point-Of-Care (POC) devices

SARS-CoV-2 infection may manifest with either a wide range of symptoms, with different degree of severity, or no symptoms at all, running silent in the individual. Prevalent clinical features are fever (body temperature above or equal to 38 °C), fatigue, vomiting, myalgia, sputum production, headache, hemoptysis, diarrhea [6], cough, sore throat, [7] and dyspnea [8]. The most serious cases are affected by severe acute respiratory syndrome and require oxygen supplies and intervention in the Intensive Care Units. The virus is characterized by a high transmission capacity and velocity which determinates a rapid diffusion of COVID-19 and an exponential trend of infection [9]. If on one hand it is necessary to contain the pandemic and protect the weak and vulnerable population, on the other hand it is hard to monitor and track the virus circulation [10]. Transmission occurs with aerosolized particles that can be transferred by symptomatic or asymptomatic patients through direct contact and/or the oropharyngeal via. The incubation period can last five, eleven or fourteen days, depending on the case [11]. For these reasons, the prevention, control and prompt diagnosis are complex but crucial in the battle against the spread of COVID-19 [12]. A key point is self-diagnosis which can be done only if the biological fluids are easily collected and the test is simple and straightforward [13].

The rapid diffusion of the SARS-CoV-2 virus drove the interest towards the development and improvement of POC systems, even though Lab-On-Chip devices have been developed for the prompt diagnosis of other infectious diseases. For example, a device based on the Real-Time PCR method able to detect a single copy of the hemagglutinin gene (HA) of influenza A/H1N1 virus was developed performing a rapid quantitative analysis with high sensitivity and specificity. Results were achieved in 15 min after 30 PCR cycles [14].

Other examples of Real Time-PCR based devices detecting parasites, such as *Plasmodium falciparum* and *Plasmodium vivax*, or bacteria, like multidrug resistant tuberculosis (MDR-TB), *E coli* O157 mono:H7, *Salmonella typhimurium*, and *Vibrio parahaemolyticus citogeni, or* viruses such as Ebola and HIV, are available nowadays [15]. Therefore, this technology has the powerful potential and flexibility to be implemented elsewhere once a tool is optimized for a gene/oligonucleotide/ transcript or a group of genes.

The POC tests are rising as they led to a decentralization of laboratory testing, which now can take place directly "at the patient's bedside" or "on-field" sites. In the last years, these tools helped gathering more information about the diagnostic tests by reducing in the meantime the device's size [16]. Moreover, the improvement of POC allows a remarkable reduction in the time of analysis when compared it to laboratory assays [17–18].

The central idea of the POC diagnostic tests is to be "a simple test near the patient" with the following features: (i) quick result, (ii) accurate, (iii) easy to use, (iv) portable, (v) low-cost, and (vi) able to perform multiple tests simultaneously [19].

In addition, the POC should not require specialized staff while giving back results consistent with validated laboratory outcomes. Devices, reagents and consumables should be safe to use and not expensive [20]. As a result of these major properties, it would be possible to exponentially increase the number of "temporary sites" performing the diagnosis of COVID-19: offices, pharmacies, schools, other institutions and home tests could be set up, granting quick results and monitoring the exposed population.

In the pandemic scenario, indeed, a rapid test result is crucial in screening, diagnosing, and monitoring COVID-19 infection in the population as it would guide the clinicians or public health authorities in making decisions about treatment and/or contact tracing. The advantages of the POC devices can shorten the patient's stay in hospital, reduce morbidity and mortality in some cases [21], and identify and contain clusters of contagions. To be competitive with standard assays, POC devices should feature high sensitivity with a very low limit of detection (LOD) and minimize sample handling and processing. The LOD refers to the smallest quantity of a target molecule that can be measured in $\geq$ 95% sample replicates and identifies the number of copies of viral genomic RNA per milliliter of transport medium.

Fig. 1 summarizes the advantages and disadvantages of POC RT-PCR devices. Portability to the field of use, quick results (within 15–45 min) and a high degree of selectivity can be very effective at the Point-Of-Care. On the other hand, they may be expensive sometimes, unsuitable for recovered patients, and often reporting false negative or positive results, if the specimen was collected in an inadequate way [22].

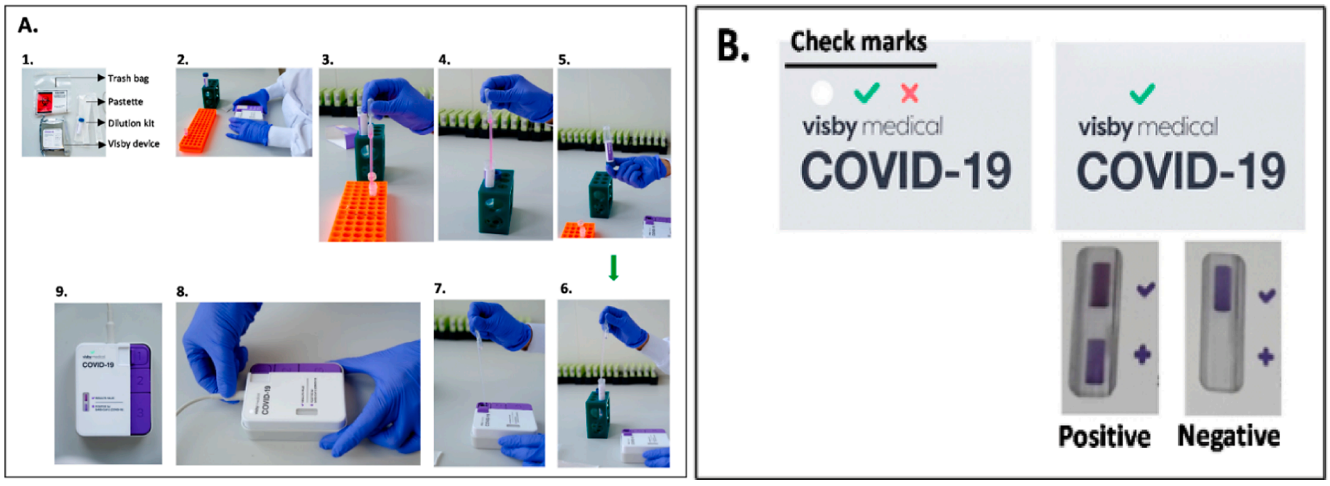

Fig. 2. A. POC device and procedure; **B**. Results of COVID-19 test. Figures reproduced with Copyright permissions from reference [31].

3. RT-PCR as gold standard for COVID-19 diagnosis

RT-PCR remains the gold standard technique for SARS-CoV-2 detection, when it comes to sensitivity, affordability and low-costs for the best rapid COVID-19 diagnostic test, because of the capacity of measuring low viral load [23].

The World Health Organization has approved and standardized assays that are based on RT-PCR method for the identification of COVID-19 worldwide [24]. The SARS-CoV-2 virus was found in various samples, such as respiratory and fecal samples, but tests have been validated on nasopharyngeal and/or oropharyngeal sites [25]. In addition, sampling procedure needs to be standardized and for this reason it is essential to use dacron or polyester flocked swabs for sample collection [26].

The genome of SARS-CoV-2 is characterized by 14 ORFs (open reading frames) encoding 27 proteins. In particular, 4 structural genes code for the Spike protein (S), Envelope Small protein (E), Matrix protein (M), and Nucleocapsid protein (N). Furthermore, genes encoding non-structural proteins, such as RdRP (an enzyme responsible for the viral replication) are also present in the genome [27]. The most detected genes are N and ORF1ab because of their high sensitivity and specificity [28]. The ORF1ab is the largest gene containing overlapping ORFs and it encodes some polyproteins.

With the advancement of Lab-On-Chip technologies, miniaturized systems have been developed to remarkably improve the efficiency and affordability of POC tools. Molecular tests consist of five steps: sample lysis, viral RNA purification, reverse transcription of RNA in complementary DNA (cDNA), target region amplification of cDNA, and optical measurement of the amplification product. For a real portability of Real-Time PCR tools and to encourage a transition to the POC market, all these steps should be easily performed in a significant level of miniaturization and integration.

It is particularly important to include a detection system for the amplification. The device generally consists of a photodiode and an emission filter, both located on the same axis as the reaction tube. A LED light and an excitation filter are placed close to the sample. The LED emits blue light through the excitation filter and finally conveys it into the tube containing the sample. The sample will emit green light whose intensity is directly proportional to the amplification product. This green light travels through the emission filter and is conveyed to the photodiode sensor. This generates a voltage proportionally to the intensity of fluorescence. The voltage will be amplified and then read by the microcontroller allowing the quantification of the DNA produced after the PCR cycles [29].

Some machines are able to carry out RNA reverse transcription and DNA amplification in a one-step single reaction. However, false positive and/or false negative results can also occur. False negatives can be the result of a viral load below the detection threshold, sample degradation, presence of inhibitors in the PCR reaction mix, low specificity of primers, probes, and signal fluorescence or issues related to sample collection, storage and transport.

Similarly, false positives may be caused by sample contamination, detection of non-specific coronaviruses, technical issues with kits, probes and type of fluorescence [30].

In the past two years, several RT-PCR devices have been granted authorization from the Food and Drug Administration (FDA) in the United States. Some of them obtained the Emergency Use Authorization (EUA), a particular condition needed to face such an emergency situation with urgent measures. The EUA enables the FDA to strengthen public health protection against chemical, biological, radiological and nuclear threats, including infectious diseases. This facilitates the action of necessary medical countermeasures[a].

The process of development and full approval of the device by the FDA is characterized by 5 steps:

1) D*evice discovery and concept*: the research of new device begins in the laboratory.
2) *Pre-clinical prototype research:* the first *in vivo* tests are carried out and preliminary safety information is gathered.
3) *Pathway to Approval:* the device is tested on people for safety and efficacy.
4) *FDA Review:* FDA teams review the data and decide whether or not to approve the device.
5) *FDA Post-Market Safety Monitoring:* there will be a constant monitoring of device's safety once it is available for public use[b].

4. Market available RT-PCR POC devices for SARS-CoV-2 detection

4.1. Emergency use authorized POC devices

Visby Medical RT-PCR Portable Device is a palm-sized system currently used for SARS-CoV-2 diagnosis. It is based on the amplification of the N gene by RT-PCR. It is an easy to use, innovative and portable instrument that gives results in thirty minutes. It presents sensitivity of

[a] https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization
[b] https://www.fda.gov/patients/learn-about-drug-and-device-approvals/device-development-process

95%, specificity of 100% and LOD of 100 copies/mL, if compared with other tools like Cobas 6800 RT-qPCR assay (Roche).

Visby's device represents an excellent compromise between the molecular detection of COVID-19 and a POC test.

The kit contains Visby device, Pasteur pipettes, dilution kit and a trash bag. The patient blends the diluted sample with the lyophilized reagents by inversion. In particular, 650 μL of diluted nasopharyngeal specimen are collected and placed into the device. Sample extraction, reverse transcription and PCR amplification are then performed. After plugging-in the device, a white light will indicate that sample processing has started. Thirty minutes later, the green light will reveal that reaction was successful, otherwise the light will be red (Fig. 2**A**). In the end, the result is shown in purple in the windows below (Fig. 2**B**) [31].

The cDNA amplification provides a number of DNA fragments that is sufficient to be detectable by a colorimetric system. The amplification product, if present, is specifically hybridized along a flow channel.

The flow channel is designed to perform an enzymatic reaction using the horseradish peroxidase and a substrate that produces purple color when metabolized. The observable color change is a proof of the virus presence in the sample [32].

Visby Medical RT-PCR Portable Device is one of the few devices to have gained the FDA approval[c].

The ***Xpert Xpress SARS-CoV-2/Flu/RSV assay*** is focused on multiplex real time PCR to simultaneously detect (i) SARS-CoV-2, (ii) seasonal influenza virus and (iii) respiratory syncytial virus (RSV). Sensitivity for SARS-CoV-2 virus is 100% with a LOD of 250 copies (cp)/mL. The GeneXpert device targets the Nucleocapsid (N2) and the Envelope (E) genes [36].

This assay was compared with other Xpert assays and the BioFire FilmArray RP2.1 panel, showing an accordance higher than 98%.

The system is composed by a disposable cartridge containing the reagents to carry out the RT-PCR reaction, the instrument that will be connected to the computer, and the software. The cross-contamination in different wells is very unlikely to happen thanks to the specific design of the cartridge. In addition, the cartridge contains two types of control: one to check the sample handling and the other to control the probe. After loading the sample, the cartridge can be inserted into the device and the test can begin without any other manual intervention. Then, the detection of viral RNA by Real-Time will take place.

The patient with a suspected infection collects the sample through the nasopharyngeal swab that is then stored into a tube containing 3 ml of viral VTM or 3 ml of saline solution for transportation [33]. It will be stored at −80 °C until the time of the analysis. Then, 300 μL of resuspended sample in VTM are loaded into the cartridge with a disposable pipette included within the kit. The cartridge cover is then closed and the cartridge inserted into the instrument following the instructions on the GeneXpert display window [34]. Sample processing and RT-PCR for detection of viral RNA will start automatically. Results are ready in less than fifty minutes using one cartridge per nasopharyngeal sample [31–32][c].

It earned an the EUA from the FDA in December 2020.

In October 2021, ***Xpert Xpress CoV-2/Flu/RSV plus*** also received EUA from the FDA. This device has similar features and functions of other mentioned systems but it shows better performance than the Xpert Xpress CoV-2/Flu/RSV [37][c].

The ***Cepheid xpert® xpress SARS-CoV-2 point-of-care test***, like the majority of molecular tests for diagnosis of COVID-19, is based on the amplification of the N and E genes [38].

The agreement of the assay with the household RT-PCR is 100%. The assay guarantees a rapid detection of the virus providing results in less than fifty minutes, high sensitivity and specificity for the diagnosis of SARS-CoV-2 with a LOD of 8.26 copies/mL.

Single-use cartridges containing the reagents required to carry out the RT-PCR reaction are provided and they are hermetically sealed to minimize the risk of contamination.

The nasopharyngeal specimen on the swab is temporarily stored and transported in 3 ml of VTM or saline solution. In some cases, it is possible to freeze the samples for a short period before the analysis. Like other tests, the sample is mixed by inversion 5 times and then transferred into the cartridge that is uploaded into the GeneXpert platform where the whole process, from RNA extraction to Real Time PCR amplification of retrotranscripted viral RNA in cDNA, is performed without any manual intervention [39].

The GeneXpert cartridge uses a sample handling control, which certifies a correct manipulation of the sample by (i) monitoring the action of inhibitors that may interfere with the PCR reaction and (ii) maintaining the appropriate temperature and conditions along the entire run, and a probe verification control to verify the rehydration of the reagents and the PCR tube filling.

Cue COVID-19 Test is based on the isothermal NAAT technology. It detects the nucleocapsid (N) region of the virus using a Taqman probe specific to this region and bound to a FAM fluorophore showing a LOD of 20 copies of the genome per sample. The specimen from a nasal swab is transferred into disposable cartridge. Successively, the cartridge is inserted into the device and preheated for one minute before the reaction begins. Thus, heating, mixing, amplification, and detection occur in sequence. Inside the instrument, the electron current flow provides a semi-quantitative nanoampere measurement that will be converted into a positive or negative signal. This method has been proven to be specific, sensitive and accurate compared to central laboratory tests.

Nevertheless, the sensitivity is lower in comparison with Cepheid xpert® xpress SARS-CoV-2 point-of-care test [40] and the positive agreement with central laboratory tests is 91,7%.

The Cue health monitoring system is a portable, compact and small device. Moreover, it is possible to connect the device to a digital App (both iOS or Android systems) allowing the user to view the results after almost twenty minutes. Like other POC, it rapidly identifies viral infection and decrees consequent quarantine for the patient in case of positive outcome [41].

In addition, the Cue COVID-19 test is a POC NAATs that received the EUA approval from the FDA and thanks to its features it can be used at home, in schools, pharmacies and other non-healthcare sites [42][d].

ID NOW COVID-19 assay from Abbott is a near-patient testing tool that permits the molecular diagnosis of SARS-CoV-2 in about five minutes starting from 200 μL of saliva swab and amplifying a unique region of the RdRp gene. It showed 84% of sensitivity an 99,8% of specificity [43].

The assay consists of test bases, sample receivers, transfer cartridges, patient swabs, and positive and negative control swabs.

It is an automated device based on the NAAT technology. The sample receiver contains an elution/lysis buffer and a test base including two test tubes. Each tube has a lyophilized pellet inside, a cartridge for transferring the eluted sample to the test base and the ID NOW instrument. Both specimen receiver and test base are inserted into the device. The sample is added to the receiver, then transferred to the test base through the transfer cartridge and eventually the amplification process can begin [44].

Although saliva is easy to collect and store in the transport medium by the individual users, it contains digestive enzymes that could alter the stability of the sample and consequently the analysis, if a rigorous protocol of storage is not observed. Indeed, it has been demonstrated that false negatives can be acquired at very high Ct levels [43].

Results obtained by ID NOW were compared with Xpert® Xpress

[c] https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-molecular-diagnostic-tests-sars-cov-2

[d] https://www.cuehealth.com/about/press/fda-authorizes-cue-health's-covid-19-test-for-at-homeand-over-the/

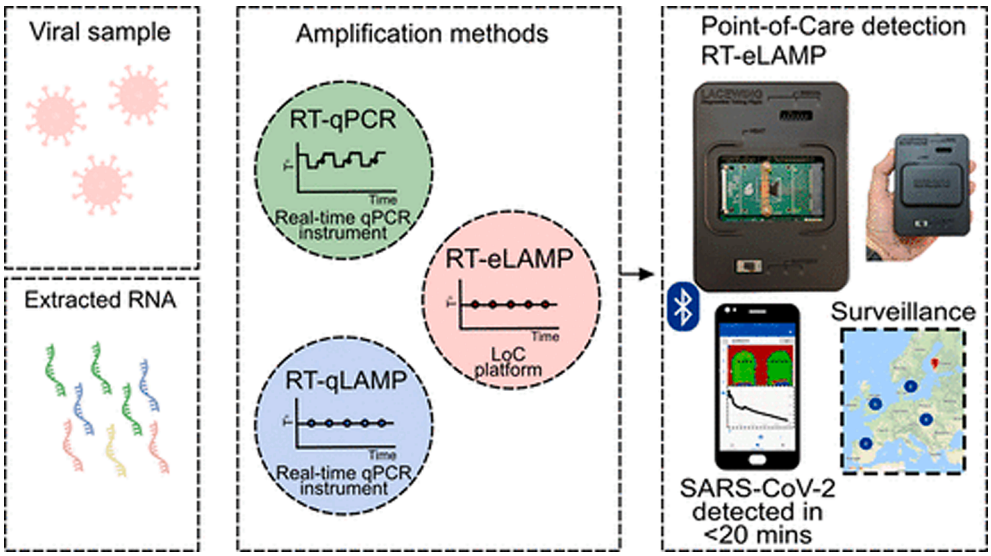

Fig. 3. RT-LAMP miniaturized device connected to smartphone via Bluetooth [51].

SARS-CoV-2 (Cepheid) or Abbott Real Time SARS-CoV-2 (Abbott Molecular) RT-PCR assays[c] granting to ID NOW the EUA approval from the FDA.

Accula SARS-CoV-2 lateral flow is a POC test that amplifies the N gene. The method involves a nasopharyngeal swab collected in VTM or saline solution and a RT-PCR with lateral flow reading. It is a palm size POC device, easy-to-use, with sensitivity and specificity around 95% [45]. Results can be acquired within 30 min from sample collection.

An aliquot of solubilized nasopharyngeal sample in a buffer solution is loaded into a cassette in which the test will be performed. The kit supplies internal positive and negative controls, enzymes, OscAR™ reagents and a result detection strip.

The Accula Dock, the electronic module that guarantees the performance of the *in vitro* test, controls the temperatures, timing and movement of the fluid in the cassette during the reaction. The result is then displayed as blue lines on a detection strip in the cassette when the reaction is over. The control line ensures that the procedure has been successful [46].

The lateral flow of SARS-CoV-2 Accula showed low sensitivity compared to the EUA SARS-CoV-2 LDT, highlighting the necessity of retesting negative samples using a standardized method [47].

Accula SARS-CoV-2 device has received the EUA approval from the FDA as a POC RT-PCR system for the diagnosis of SARS-CoV-2[c].

5. Other examples of POC devices

The ***Q3, a device by STMicroelectronics***, holds all the features to be classified as a POC device. It is an easy-to-use system that weights 300 g. The disposable cartridges can accommodate a sample volume between 5 and 15 μL. It is one of the main examples of miniaturized devices for the diagnosis of infectious diseases. It is characterized by not disposable and disposable units. The not disposable unit contains the optical system (excitation sources, filters, and optical read-out sensors) in the upper cover while the disposable unit has inside a section that holds single-use cartridge and the control electronics. It has a built-in memory and it can be connected to a PC or smartphone/tablet via USB cable [48].

VitaPCR™ RT-PCR test reaches sensitivity and specificity close to 100% (99,3% and 94,7%, respectively). With this 1.2 kg device, the test is simple and fast, obtaining results in 20 min like in the rapid antigen tests. The assay evaluates three gene targets: N gene of the viral nucleocapsid, a conserved gene sequence among Coronaviruses, and the beta globin gene as a control to assess the DNA quality after the extraction. The amount of sample needed from a nasopharyngeal swab is 30 μL which will be released into a lysis buffer and analyzed with ready-to-use reagents stored at room temperature. Lysis and extraction of nucleic acids are performed in a single solution. This step guarantees sensitivity levels comparable to a laboratory test. The test is based on multi-colored fluorescence detection and the device can be used up to 4 channels maximum.

The instrument is not very large and can be installed away from the laboratory. In addition, the system guarantees protection from the virus as the sample is inactivated by the collection buffer provided by the kit.

It is possible to connect 8 units to a single PC at the same time using the VitaDataLink software[e].

The only drawback of the system is the incapacity to process more than one sample at a time [49].

6. LAMP -based Point-of-Care devices

The LAMP (loop-mediated isothermal amplification) is a gene amplification technique that differs from PCR in real time because the amplification occurs at a single temperature, between 60 °C and 65 °C, and hence it can run without the use of a thermal cycler. Specificity of reaction is high as six primers are used. The amplification product can be analyzed using simple detection methods. Due to its features, the LAMP is the basis of many POC devices [50]. The POC proposed by Rodriguez-Manzano et al. is a handheld device based on the reverse transcriptase and loop-mediated isothermal amplification (RT-LAMP) and semiconductor technology for the identification of COVID-19. It shows a sensitivity of 91% and a specificity of 100% with a LOD of 10 RNA copies per reaction. The main peculiar feature is the ability to connect the device to smartphones via Bluetooth and display both results, within 20 min, and geo-localization (Fig. 3). Using a disposable cartridge, a microfluidic network delivers 5 μL of sample and 5 μL of control reaction mix respectively to two different regions of a connected microchip. The RT-LAMP assay is carried out on an array consisting of more than 4368 sensors called ion-sensitive field effect transistors (ISFETs). The sensors are able to 'feel' the amplification process of DNA molecules through the release of protons each time a nucleotide is incorporated during the LAMP. Moreover, an Ag/AgCl electrode is used as a reference. The bond of the collector on the chip surface is promoted by laser cut double stick adhesive. Materials employed in the manufacture of the chip were

[e] https://www.menarinidiagnostics.it/it-it/Home/Prodotti-di-laboratorio/COVID-19/VitaPCRTM-platform/Caratteristiche

Table 1
The POC devices based on RT-PCR technique and their main features.

System name	Weight and dimension	Reaction time	Reaction volume	EUA Point Of Care approval from FDA	LOD	Sensitivity	Specificity
Visby Medical RT-PCR Portable Device [31]	Small palm size	30 min	650 μL	yes	100 copies/mL	95%	100%
Xpert Xpress SARS-CoV-2/Flu/RSV	Palm size	<50 min [35]	300 μL	yes	<100 viral copies/mL [35]	100%	specificity comparable to the current gold standard techniques can
Cepheid xpert® xpress SARS-CoV-2	Small size cartridge	45–50 min	300 μL	yes	8.26 copies/mL [38]	high sensitivity (% not shows)	99.5%
Cue COVID-19 Test	Small size	20 min	/	yes	20 genome copies/sample	Lower than Cepheid Xpress (61.1%)	91,7% positive percentage agreement with central laboratory tests
ID NOW COVID-19 assay	Small size	5 min	200 μL	yes	125 genome equivalents/mL [43]	84%	99,8%
Accula SARS-CoV-2 lateral-flow	Palm size	30 min	70 μL	yes	/	95%	95%
VitaPCR™ RT-PCR assay [49]	1.2 kg	20 min	30 μL	no	2.73 copies/μl[f]	99,3%	94,7%

chosen because of their low capability of inhibiting the reaction [51]. Another example of highly integrated RT-LAMP based system for self-testing has been recently developed by Tang and coworkers. They realized a compact platform including a microfluidic cartridge for saliva sample collection and processing. Results are then communicated through an application on a mobile phone. The system performs real-time detection of amplification product achieving a LOD of 5 copies/mL of SARS-CoV-2 [52].

7. Point-Of-Care device limitations

Despite the numerous and advantages of POC devices, there are few limitations which can be summarized as follows:

- The incorrect collection and storage of the sample may lead to false negative result.
- When the machine is set up to detect different genetic targets, the instrument is unable to distinguish between the most abundant target genes.
- The incorrect selection of primers or mutations on the target genes may lead to false negative results.
- False negative results may also occur if there are inhibitors in the test sample or if the concentrations of the virus is insufficient.
- The performance of the device may vary according to the circulating viral variants in the population.

8. Conclusion

Over the past two years, there has been an exponential development of POC tests, largely as a result of the COVID-19 pandemic emergency. The highly and quickly diffusion of the virus demands a timely surveillance of new cases to monitor the infection and avoid new outbreaks, promoting self-diagnosis without overburdening national health systems. The RT-PCR remains the gold standard technique for the molecular diagnosis of SARS-CoV-2. The POC must demonstrate high diagnostic reproducibility, sensitivity and specificity compared to standardized devices to this end. Moreover, POC devices will need continuous updates with new sequences of viral variants once the sequences will be known. Miniaturization, reduction of specimen processing time, simplified tests for unskilled personal, home testing and device connection through smartphones or personal computers may lead the progress and expansion of POC worldwide in the next future.

In addition, tests must comply with CLIA (Clinical Laboratory Improvement Amendments) parameters [53], an approval from the Center for Medicare and Medicaid Services (CMS) for specific clinical settings to conduct diagnostic tests on human samples. It also assures developing countries that are often lacking of health facilities [54] to better control and map pandemics [55] even in low-resource conditions.

From this point of view, the introduction of POC could help to bridge the social gap during a pandemic. Hopefully, these tests could be set up for other respiratory viruses. The implementation of POC is likely to be achieved by reducing treatment costs and improving robustness [56].

In this review, we analyzed some examples of POC tests for rapid diagnosis of COVID-19, mainly based on nucleic acid amplification of SARS-CoV-2. Some have received approval from FDA, others are still in a preliminary experimental phase. Table 1 summarizes the devices analyzed by some of their characteristics (LOD, specificity, FDA approval). All of them have the potential to overcome the gap between the laboratory and the marketplace and to self-diagnose more accurately and affordably, in comparison with commonly used lateral-flow tests characterized by a high number of false negative results.

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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References

[1] B. Hu, H. Guo, P. Zhou, Z.L. Shi, Characteristics of SARS-CoV-2 and COVID-19, Nat. Rev. Microbiol. 19 (2019) 141–154, https://doi.org/10.1038/s41579-020-00459-7.

[2] H.R. Mollaei, A. Aghaei Afshar, D. Kalantar-Neyestanaki, M. Fazlalipour, B. Aflatoonian, Comparison five primer sets from different genome region of

COVID-19 for detection of virus infection by conventional RT-PCR, Iranian J. Microbiol. 12 (2020) 185–193, https://doi.org/10.18502/ijm.v12i3.3234.
[3] W. Zhen, R. Manji, E. Smith, G.J. Berry, Comparison of four molecular in vitro diagnostic assays for the detection of sars-cov-2 in nasopharyngeal specimens, J. Clin. Microbiol. 58 (2020) 1–8, https://doi.org/10.1128/JCM.00743-20.
[4] K. Munne, V. Bhanothu, V. Bhor, V. Patel, Detection of SARS-CoV-2 infection by RT-PCR test : factors influencing interpretation of results, VirusDisease 32 (2021) 187–189, https://doi.org/10.1007/s13337-021-00692-5.
[5] S. Kumar, M. Nehra, S. Khurana, N. Dilbaghi, V. Kumar, A. Kaushik, K.H. Kim, Aspects of point-of-care diagnostics for personalized health wellness, Int. J. Nanomed. 16 (2021) 383–402, https://doi.org/10.2147/IJN.S267212.
[6] O. Belmehdi, M. Hakkour, N. El Omari, A. Balahbib, F.E. Guaouguaou, T. Benali, A. El Baaboua, M. Lahmoud, N. Elmenyiy, A. Bouyahya, Molecular structure, pathophysiology, and diagnosis of COVID-19, Biointerface Res. Appl. Chem. 11 (2021) 10215–10237, https://doi.org/10.33263/BRIAC113.1021510237.
[7] B.E. Young, S.W.X. Ong, S. Kalimuddin, J.G. Low, S.Y. Tan, J. Loh, O.T. Ng, K. Marimuthu, L.W. Ang, T.M. Mak, S.K. Lau, D.E. Anderson, K.S. Chan, T.Y. Tan, T.Y. Ng, L. Cui, Z. Said, L. Kurupatham, M.I.C. Chen, M. Chan, S. Vasoo, L.F. Wang, B.H. Tan, R.T.P. Lin, V.J.M. Lee, Y.S. Leo, D.C. Lye, Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore, JAMA – J. Am. Medical Assoc. 323 (2020) 1488–1494, https://doi.org/10.1001/jama.2020.3204.
[8] M. Rezaei, S. Razavi Bazaz, S. Zhand, N. Sayyadi, D. Jin, M.P. Stewart, M. Ebrahimi Warkiani, Point of care diagnostics in the age of COVID-19, Diagnostics 11 (2020) 9, https://doi.org/10.3390/diagnostics11010009.
[9] R. Li, S. Pei, B. Chen, Y. Song, T. Zhang, W. Yang, J. Shaman, Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2), Science 368 (2020) 489–493, https://doi.org/10.1126/science.abb3221.
[10] M. Döhla, C. Boesecke, B. Schulte, C. Diegmann, E. Sib, E. Richter, M. Eschbach-Bludau, S. Aldabbagh, B. Marx, A.-M. Eis-Hübinger, Rapid point-of-care testing for SARS-CoV-2 in a community screening setting shows low sensitivity, Public Health 182 (2020) 170–172, https://doi.org/10.1016/j.puhe.2020.04.009.
[11] V. Koteswara Rao, Point of care diagnostic devices for rapid detection of novel Coronavirus (SARS-nCoV19) pandemic: a review, Front. Nanotechnol. 2 (2021) 1–9, https://doi.org/10.3389/fnano.2020.593619.
[12] J.R. Choi, Development of Point-of-Care Biosensors for COVID-19, Front. Chem. 8 (2020) 517, https://doi.org/10.3389/fchem.2020.00517.
[13] V. Garzarelli, F. Ferrara, E. Primiceri, M. Serena, MethodsX Biofluids manipulation methods for liquid biopsy in minimally-invasive assays, MethodsX J. 9 (2022), https://doi.org/10.1016/j.mex.2022.101759.
[14] H. Song, J. Kim, H. Ryu, D. Lee, S. Kim, D. Kim, B. Suh, D.Y. Choi, K. In, S. Kim, H. Park, Polymeric LabChip Real-Time PCR as a point-of-care- potential diagnostic tool for rapid detection of influenza A/H1N1 virus in human clinical specimens, 7 (2012) 1–8. https://doi.org/10.1371/journal.pone.0053325.
[15] C. Wang, M. Liu, Z. Wang, S. Li, Y. Deng, N. He, Point-of-care diagnostics for infectious diseases: from methods to devices, Nano Today 37 (2021), 101092, https://doi.org/10.1016/j.nantod.2021.101092.
[16] P.B. Luppa, C. Müller, A. Schlichtiger, H. Schlebusch, Point-of-care testing (POCT): current techniques and future perspectives, TrAC - Trends Anal. Chem. 30 (2011) 887–898, https://doi.org/10.1016/j.trac.2011.01.019.
[17] S. Zoupanou, M.S. Chiriacò, I. Tarantini, F. Ferrara, Innovative 3D microfluidic tools for on-chip fluids and particles manipulation: From design to experimental validation, Micromachines. 12 (2021) 104, https://doi.org/10.3390/mi12020104.
[18] F. Ferrara, S. Zoupanou, E. Primiceri, Z. Ali, M.S. Chiriacò, Beyond liquid biopsy: Toward non-invasive assays for distanced cancer diagnostics in pandemics, Biosens. Bioelectron. 196 (2021), 113698, https://doi.org/10.1016/j.bios.2021.113698.
[19] E. Primiceri, M.S. Chiriacò, F.M. Notarangelo, A. Crocamo, D. Ardissino, M. Cereda, A.P. Bramanti, M.A. Bianchessi, G. Giannelli, G. Maruccio, Key enabling technologies for point-of-care diagnostics, Sensors (Switzerland) 18 (3607) (2018), https://doi.org/10.3390/s18113607.
[20] A. St John, C.P. Price, Existing and emerging technologies for point-of-care testing, Clin. Biochemist Rev. 35 (2014) 155.
[21] C.E.S. Ferreira, J.C.C. Guerra, N. Slhessarenko, M. Scartezini, C.N. França, M. P. Colombini, F. Berlitz, A.M.O. Machado, G.A. Campana, A.C.L. Faulhaber, C. A. Galoro, C.M. Dias, W. Shcolnik, M.D.V. Martino, K.R. César, N.M. Sumita, M. E. Mendes, M.H.W. Faulhaber, J.R.R. Pinho, I.V. Barbosa, M.C. Batista, C. Khawali, V.M. Pariz, A. Andriolo, Point-of-Care testing: general aspects, Clin. Lab. 64 (2018) 1–9, https://doi.org/10.7754/Clin.Lab.2017.170730.
[22] M.M. Briza Pérez-López, Commercialized diagnostic technologies to combat SARS-CoV2: Advantages and disadvantages, Talanta. (2020), https://doi.org/10.1016/j.talanta.2020.121898.
[23] Q. Song, X. Sun, Z. Dai, Y. Gao, X. Gong, B. Zhou, J. Wu, W. Wen, Point-of-care testing detection methods for COVID-19, Lab on a Chip (2021).
[24] W.F. Sule, D.O. Oluwayelu, Real-time RT-PCR for COVID-19 diagnosis: challenges and prospects, Pan African Med. J. 35 (2020) 121, https://doi.org/10.11604/pamj.supp.2020.35.24258.
[25] A. Basu, T. Zinger, K. Inglima, K.M. Woo, O. Atie, L. Yurasits, B. See, M.E. Aguero-Rosenfeld, Performance of abbott id now covid-19 rapid nucleic acid amplification test using nasopharyngeal swabs transported in viral transport media and dry nasal swabs in a New York city academic institution, J. Clin. Microbiol. 58 (2020) 1–7, https://doi.org/10.1128/JCM.01136-20.
[26] A. Tahamtan, A. Ardebili, Real-time RT-PCR in COVID-19 detection: issues affecting the results, Expert Review of Molecular Diagnostics. 20 (2020) 453–454, https://doi.org/10.1080/14737159.2020.1757437.
[27] H. Tombuloglu, H. Sabit, H. Al-Khallaf, J.H. Kabanja, M. Alsaeed, N. Al-Saleh, E. Al-Suhaimi, Multiplex real-time RT-PCR method for the diagnosis of SARS-CoV-2 by targeting viral N, RdRP and human RP genes, Scientific Reports. 12 (2022) 1–10, https://doi.org/10.1038/s41598-022-06977-z.
[28] X. Zhang, M. Li, B. Zhang, T. Chen, D. Lv, P. Xia, Z. Sun, X. Shentu, H. Chen, L. Li, W. Qian, The N gene of SARS-CoV-2 was the main positive component in repositive samples from a cohort of COVID-19 patients in Wuhan, China, Clin. Chim. Acta 511 (2020) 291–297, https://doi.org/10.1016/j.cca.2020.10.019.
[29] G. Mulberry, K.A. White, M. Vaidya, K. Sugaya, N. Kim, 3D printing and milling a real-time PCR device for infectious disease diagnostics (2017) 1–18. https://doi.org/10.1371/journal.pone.0179133.
[30] D.S. Mouliou, K.I. Gourgoulianis, False-positive and false-negative COVID-19 cases: respiratory prevention and management strategies, vaccination, and further perspectives, Expert Rev. Respir. Med. 8 (2021) 1–10, https://doi.org/10.1080/17476348.2021.1917389.
[31] A. Renzoni, F. Perez, M.T. Ngo Nsoga, S. Yerly, E. Boehm, A. Gayet-Ageron, L. Kaiser, M. Schibler, Analytical evaluation of visby medical rt-pcr portable device for rapid detection of sars-cov-2, Diagnostics 11 (2021) 1–9, https://doi.org/10.3390/diagnostics11050813.
[32] Visby Medical COVID-19 Test, (n.d.).
[33] GeneXpert, Xpert ® Xpress SARS-CoV-2/Flu/RSV Instructions for Use, (2021).
[34] E.C.M. Leung, V.C.Y. Chow, M.K.P. Lee, K.P.S. Tang, D.K.C. Li, R.W.M. Lai, Evaluation of the Xpert Xpress SARS-CoV-2/Flu/RSV assay for simultaneous detection of SARS-CoV-2, influenza A and B viruses, and respiratory syncytial virus in nasopharyngeal specimens, J. Clin. Microbiol. 59 (2021) 1–9, https://doi.org/10.1128/JCM.02965-20.
[35] G. Johnson, A. Zubrzycki, M. Henry, C. Ranadheera, C. Corbett, A.F.A. Meyers, P. A. Sandstrom, M.G. Becker, Clinical evaluation of the GeneXpert ® Xpert ® Xpress SARS-CoV-2 / Flu / RSV combination test, J. Clin. Virol. Plus 1 (2021), 100014, https://doi.org/10.1016/j.jcvp.2021.100014.
[36] R.W. Lai, Evaluation of the Xpert Xpress SARS-CoV-2/Flu/RSV Assay for Simultaneous Detection of SARS-CoV-2 , Influenza A and B Viruses , and Respiratory Syncytial Virus in Nasopharyngeal Specimens (2019).
[37] X.F.L.U. Rsv-, Xpert ® Xpress CoV-2/Flu/RSV plus, (2021) 1–42.
[38] F. Wolters, J. Van De Bovenkamp, B. Van Den Bosch, S. Van Den Brink, M. Broeders, N.H. Chung, B. Favié, G. Goderski, J. Kuijpers, I. Overdevest, J. Rahamat-Langedoen, L. Wijsman, W.J. Melchers, A. Meijer, Multi-center evaluation of cepheid xpert® xpress SARS-CoV-2 point-of-care test during the SARS-CoV-2 pandemic, (2020). https://doi.org/10.1016/j.jcv.2020.104426.
[39] GeneXpert, Xpert ® Xpress SARS-CoV-2, (2020).
[40] T. Phan, Z. Cravener, M. McCullough, A. Mays, J. Gribschaw, A. Wells, Clinical evaluation of the Cue's COVID-19 diagnostic test to detect, J. Med. Virol. (2022) 3517–3519, https://doi.org/10.1002/jmv.27805.
[41] L.J. Donato, V.A. Trivedi, A.M. Stransky, A. Misra, B.S. Pritt, M.J. Binnicker, B. S. Karon, Evaluation of the Cue Health point-of-care COVID-19 (SARS-CoV-2 nucleic acid amplification) test at a community drive through collection center, Diagn. Microbiol. Infect. Dis. 100 (2021), 115307, https://doi.org/10.1016/j.diagmicrobio.2020.115307.
[42] E. Valera, A. Jankelow, J. Lim, V. Kindratenko, A. Ganguli, K. White, J. Kumar, R. Bashir, COVID-19 point-of-care diagnostics: present and future, ACS Nano 15 (2021) 53, https://doi.org/10.1021/acsnano.1c02981.
[43] Y.P. Tu, J. Iqbal, T. O'leary, Sensitivity of id now and rt–pcr for detection of sars-cov-2 in an ambulatory population, ELife. 10 (2021) 1–23, https://doi.org/10.7554/ELIFE.65726.
[44] Angela Drysdale Abbott Diagnostics Scarborough, Scarborough, ME 04074 Device : EUA Number : Company : Indication : ID NOW COVID-19 Abbott Diagnostics Scarborough, Inc. Qualitative detection of nucleic acid from the SARS-CoV-2 virus in direct anterior (2021) 1–9.
[45] S.H. Yoon, S. Yang, H. Cho, S. Eun, C.M. Koo, M.K. Kim, Point-of-care testing for the detection of sars-cov-2: A systematic review and meta-analysis, Eur. Rev. Med. Pharmacol. Sci. 25 (2021) 503–517, https://doi.org/10.26355/eurrev_202101_24422.
[46] A.S.- Ifu, SARS-CoV-2 Test, 7 (2021) 1–23.
[47] C.A. Hogan, N. Garamani, A.S. Lee, J.K. Tung, M.K. Sahoo, C.H. Huang, B. Stevens, J. Zehnder, B.A. Pinsky, Comparison of the accula sars-cov-2 test with a laboratory-developed assay for detection of sars-cov-2 RNA in clinical nasopharyngeal specimens, J. Clin. Microbiol. 58 (2020) 7–11, https://doi.org/10.1128/JCM.01072-20.
[48] M. Cereda, A. Cocci, D. Cucchi, L. Raia, D. Pirola, L. Bruno, P. Ferrari, V. Pavanati, G. Calisti, F. Ferrara, A.P. Bramanti, M.A. Bianchessi, Q3: a compact device for quick, high precision qPCR, Sensors (Switzerland). 18 (2018), https://doi.org/10.3390/s18082583.
[49] P. Fournier, C. Zandotti, L. Ninove, E. Prudent, Contribution of VitaPCR SARS-CoV-2 to the emergency diagnosis of COVID-19, J. Clin. Virol. J. (2020), https://doi.org/10.1016/j.jcv.2020.104682.
[50] E.R. Adams, G.J. Schoone, A.F. Ageed, S. El Safi, H.D.F.H. Schallig, Development of a reverse transcriptase Loop-Mediated Isothermal Amplification (LAMP) assay for the sensitive detection of leishmania parasites in clinical samples, 82 (2010) 591–596. https://doi.org/10.4269/ajtmh.2010.09-0369.
[51] J. Rodriguez-Manzano, K. Malpartida-Cardenas, N. Moser, I. Pennisi, M. Cavuto, L. Miglietta, A. Moniri, R. Penn, G. Satta, P. Randell, F. Davies, F. Bolt, W. Barclay, A. Holmes, P. Georgiou, Handheld point-of-care system for rapid detection of SARS-CoV-2 extracted RNA in under 20 min, Cite This: ACS Cent. Sci. 7 (2021) 307–317, https://doi.org/10.1021/acscentsci.0c01288.

[52] Z. Tang, J. Cui, A. Kshirsagar, T. Liu, M. Yon, S.V. Kuchipudi, W. Guan, SLIDE: saliva-based SARS-CoV-2 self-testing with RT-LAMP in a mobile device, ACS Sensors (2022), https://doi.org/10.1021/acssensors.2c01023.
[53] E. Valera, A. Jankelow, J. Lim, V. Kindratenko, A. Ganguli, K. White, J. Kumar, R. Bashir, COVID-19 point-of-care diagnostics: present and future, ACS Nano (2021).
[54] P.B. Luppa, C. Mu, A. Schlichtiger, H. Schlebusch, Point-of-care testing (POCT): current techniques and future perspectives (2011) 30. https://doi.org/10.1016/j.trac.2011.01.019.
[55] N. Gupta, S. Augustine, T. Narayan, A. O'riordan, A. Das, D. Kumar, J.H.T. Luong, B.D. Malhotra, Point-of-Care PCR Assays for COVID-19 Detection, 2021. https://doi.org/10.3390/bios11050141.
[56] D. Ong, K. Huang, V. Schweitzer, C. Skevaki, P.P. Nelson, B.A. Rath, P.C. Fragkou, E. Antalis, S. Tsiodras, Current and future point-of-care tests for emerging and new respiratory viruses and future perspectives, Front. Cellular Infection Microbiol. | Www Frontiersin. Org. 1 (2020) 181, https://doi.org/10.3389/fcimb.2020.00181.