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Merging microfluidics with luminescence immunoassays for urgent point-of-care diagnostics of COVID-19

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Abstract
The Coronavirus disease 2019 (COVID-19) outbreak has urged the establishment of a global-wide rapid diagnostic system. Current widely-used tests for COVID-19 include nucleic acid assays, immunoassays, and radiological imaging. Immunoassays play an irreplaceable role in rapidly diagnosing COVID-19 and monitoring the patients for the assessment of their severity, risks of the immune storm, and prediction of treatment outcomes. Despite of the enormous needs for immunoassays, the widespread use of traditional immunoassay platforms is still limited by high cost and low automation, which are currently not suitable for point-of-care tests (POCTs). Microfluidic chips with the features of low consumption, high throughput, and integration, provide the potential to enable immunoassays for POCTs, especially in remote areas. Meanwhile, luminescence detection can be merged with immunoassays on microfluidic platforms for their good performance in quantification, sensitivity, and specificity. This review introduces both homogenous and heterogenous luminescence immunoassays with various microfluidic platforms. We also summarize the strengths and weaknesses of the categorized methods, highlighting their recent typical progress. Additionally, different microfluidic platforms are described for comparison. The latest advances in combining luminescence immunoassays with microfluidic platforms for POCTs of COVID-19 are further explained with antigens, antibodies, and related cytokines. Finally, challenges and future perspectives were discussed.

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1. Introduction
The COVID-19 is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes a global epidemic. At the moment we organizing this review, there have been more than 520 million confirmed cases and 6 million deaths [1]. Besides, viruses carrying RNA as germ plasm are prone to mutate, producing greater infectivity and harm [2]. There have already been over 1000 mutant strains of the SARS-CoV-2 virus around the world, among which Delta and Omicron strains are highly contagious variants raising global concerns [3]. The Omicron variant has been rapidly spreading world-wide, as it is more contagious than any earlier coronavirus strains. Particularly, Omicron even gained potent capabilities for immune evasion [4]. Early detection and isolation are necessary steps to prevent the further spreading of the epidemic [5–7], which requires rapid, sensitive, and accurate detections and quantifications [8]. Now available methods for COVID-19 diagnosis mainly include nucleic acid assays, immunoassays, and radiological imaging [9–11]. Radiological imaging shows visible results of whether there are lung lesions, but is not able to diagnose the type of virus [12–14]. It also requires large imaging equipment and specialized medical staff [15]. Nucleic acid assays, the gold standard for COVID-19 diagnosis, have played an irreplaceable role in epidemic prevention and control [9,16], which can detect patients in the window period and achieve early diagnosis accurately [17]. However, time-consuming amplification, requirements for professional equipment and operation limit their rapid detection [18]. Alternatively, immunoassays, especially test strips, have been used for COVID-19 diagnosis, reducing false negatives partly and providing household convenience [19–22]. For comparison, nucleic acid assays require long-term processes of nucleic acid extraction, sample pretreatment,
and temperature cycling, while immunoassays enable exceptionally rapid antigen detection without the requirements of pretreatments [23]. More importantly, immunoassays are able to accurately evaluate the infectivity of COVID-19, since there is a strong positive correlation between the antigen level and the infectivity [24]. Thus, immunoassays can provide clinicians with enough diagnostic information to determine the current stages of viral infection in patients. Immunoassays, particularly antibody tests, indicate ongoing or past infections, promoting a better understanding of the transmission dynamic [25,26]. The speed and versatility of immunoassays make them invaluable tests for pandemic monitoring, and commercialization efforts to produce them on a massive scale are beginning to ramp up [27]. Additionally, cytokine storm, known as aggressive inflammatory responses characterized by the elevated release of cytokines, has been described as features associated with life-threatening complications in COVID-19 patients [28,29]. Critical evaluations of cytokine levels and research on the underlying mechanism greatly rely on immunoassays. Therefore, it is urgent to establish proven and effective platforms for immunoassays, since the currently available platforms are designed for traditional immunoassays, which are still limited by the high cost and low automation. Thus, how to enable point-of-care (POC) immunoassays for miniaturization of experimental instruments, simplification of operation methods, and instantaneous reporting of results was urgently required [30–32]. Particularly, the microfluidic chips provide more possibilities for POCTs [18,33–35].

Towards this end, microfluidic chips could be a powerful tool to enable POC immunoassays [36–38]. In 1990, Manz et al. firstly proposed the concept of micro total analysis systems, also latterly termed microfluidic chips or lab-on-a-chip [39]. Microfluidic technology offers great potential to revolutionize the way of sampling, sample separation, mixing, chemical reaction, and detection [40,41]. It is of high throughput, low reagent/sample consumption, and less pollution, which is conducive to miniaturization and automation [42–44].

For POC immunoassays, microfluidic platforms for POCTs consist of two basic parts: analyte recognition and signal detection. There are couples of ways for signal detection in the measurements, which include luminescence [45–47], surface-enhanced Raman scattering (SERS) [48–50], surface plasmon resonance (SPR) [51–53], colorimetry [54–60], distance reading [61,62], giant magnetoresistance effect (GMR) [63,64], and electrochemistry [65–68], and etc. Among them, luminescence detection is one of the most widely-used with several advantages [69–71]. Compared with naked-eye detection, luminescence provides more quantitative and sensitive data [72–74]. Compared with SPR, GMR, etc., luminescence is less costly and more readily available [75,76]. Furthermore, relatively simple peripherals of luminescence detection facilitate integration and automation.

Despite microfluidic chips for POCs having been recently reviewed [40,77–82], as far as we know, very few articles have been written from the perspective of luminescence immunoassays. Besides, our review outlooks the future of the post-pandemic era in the coming years, strengthening the importance of monitoring the severity of COVID-19 in patients and treatment of sequelae. We first present immunological signatures of COVID-19 infection, emphasizing the necessity of immunoassays. Then we introduce microfluidic chips based on luminescence immunoassays, including heterogeneous and homogeneous immunoassays (Fig. 1). Given the importance of sensitivity, digital immunoassays based on luminescence are also discussed in this paper. We also describe the characteristics of the integrated microfluidic platforms. More specifically, the microfluidic devices for POC immunoassays of COVID-19 are highlighted, including antigen, antibody, cytokine storm, and combination tests. Finally, the challenges and future perspectives in the development of POCTs were discussed in depth.

2. Immunological signatures of the life cycles of COVID-19 in patients

It has been more than two years since the outbreak of COVID-19, but no effective antiviral drugs or treatments have been found. Early detection, diagnosis, reporting, isolation, and treatment are the most effective prevention and control means [5,83,84]. Compared with the first outbreak in Wuhan, we now have a more mature health care system and a better plan to cope with it. Omicron, the main circulating strain nowadays, is highly contagious and does little harm [85]. The vast majority of those infected are asymptomatic or mild. Diagnosis and treatment protocol for novel coronavirus pneumonia (Trial Version 9) does not require hospitalization for mild cases. However, we have to admit that the epidemic is still going on, and its effects will continue to be felt. As we can see in the post-pandemic era, the epidemic is far from over, and there are still local outbreaks. We need to establish a perfect coping mechanism to reduce the impact.

SARS-Cov-2 is a new coronavirus that binds to host cells’ surface via spike proteins and is endocytosed into cells via clathrin-mediated membrane fusion [86–88]. Viruses that are not destroyed by non-specific immunity would be presented to T cells, stimulating the specific immune response. Then specific antibodies, including immunoglobulin G (IgG), immunoglobulin M (IgM), and so on, are produced to remove viruses in the form of immunocomplexes. Generally, IgM can be detected about a week after infection, while IgG comes after IgM at about two weeks [89,90]. When IgG gradually disappeared, IgM content reached its peak [91]. IgM is long-lasting and plays a major role in removing viruses and maintaining long-term immunity [92]. So antibodies in the blood are indirect evidence of COVID-19 infection and reveal the process.

When the immune system is overactivated, excess cytokines are
released, causing positive feedback, and leading to systemic inflammatory responses, eventually forming cytokine storms [28,93]. Studies have shown that cytokine storm is an important point in the transition from mild to severe and critical COVID-19, and is also a cause of death in severe and critical COVID-19 [29,94,95]. And it is well established that cytokine storm is highly correlated with the severity of COVID-19 patients and mortality [96–98]. Hence, real-time monitoring of cytokine levels greatly reduces the risk of COVID-19 patients. Another point is that sequela exist in cured patients, especially in critically ill patients [99–102]. SARS-CoV-2 attacks the human nervous system, causing a proven loss of smell and taste [103–105]. In other words, sequela from infected patients could become a global phenomenon. Hence, prognostic assessment or long-term monitoring is critical.

Although no antibodies are observed in the window period or even early infection, immunoassays still work to distinguish the remaining different stages of the infection [37,106,107]. Additionally, as mentioned above, antibodies can be monitored to ascertain the severity of the disease and recovery progress. Immunoassays are irreplaceable in assessments of severity, risks of cytokine storm, and outcomes of treatments.

3. Luminescence immunoassays on microfluidic chips

Based on whether labels are required to be isolated, immunoassays are divided into two major types: heterogeneous and homogenous immunoassays [108]. The signal can be outputted directly without washing in homogenous immunoassays [109]. In heterogeneous immunoassays, analytes are bound to solid substrates specifically for following cleaning and further readout.

Luminescence is a non-thermal emission of light from substances, including fluorescence, chemiluminescence and so on [110]. It is a process that occurs when photons of electromagnetic radiation are absorbed by molecules, raising them to some excited state, and then, on returning to a lower energy state, the molecules emit radiation or rather luminesce [111]. Luminescence has been engaged with immunoassays for POCTs in many fields.

3.1. Heterogeneous luminescence immunoassays on microfluidic chips

In heterogeneous luminescence immunoassays, identifiers, such as capture antibodies (cAbs) or antigens, are incubated on solid phases [112]. Then, analytes and labels are identified and fixed while the remaining free molecules are washed out. Hence, fixed luminescent labels can be used for quantitative analysis. Currently, heterogeneous luminescence immunoassays are widely-used methods of immunoassays that separate fixed labels to be measured from analysis systems before detection, such as well-known enzyme-linked immunosorbent (ELISA) and chemiluminescence immunoassays [113–115].

3.1.1. Heterogeneous fluorescence immunoassays on microfluidic chips

Usually, heterogeneous fluorescence immunoassays (FIAs) use fluorescently labeled antigens or antibodies to localize, characterize, and quantify analytes by recognizing corresponding antigens or antibodies [79]. Fluorescent molecules as labels for detection show well practicability. The commonly used fluorescent labels are luciferin and rhodamine dyes. Take luciferin as an example, Zhao et al. developed an automated microfluidic system for rapid and quantitative analysis of chloramphenicol (CAP) via competitive immunoassays [Fig. 2A] [116]. Some self-driven microfluidic chips without additional actuators were also described [47,117]. Immunochromatographic assay (ICA) is a typical example with simplicity, and rapidity but uncontrollable flow rates [118–120]. To improve it, Hemmig et al. fabricated a capillary-driven microfluidic chip integrating a self-coalescence and a bead lane module to detect cardiac marker troponin I via one step sandwich FIAs (Fig. 2B) [121].

Besides traditional fluorescent molecules, some nanomaterials have also been widely employed for heterogeneous FIAs due to their excellent fluorescence properties, such as aggregation-induced emission (AIE) luminogens (AIEgens) [122–124], quantum dots (QDs) [125–127], upconversion nanoparticles (UCNPs) [128–130], and time-resolved fluorescent materials [73,131,132]. Unlike conventional fluorescent molecules, the luminescence intensity of AIEgens increases with the concentrations of labels without aggregation-caused quenching. AIEgen-based nanoparticles have been designed to obtain highly efficient luminescence for immunoassays [133–135]. Wu et al. reported a sensitive fluorescence ELISA platform based on AIEgens nanobeads for carcinoembryonic antigen (CEA) quantification [133]. In another work, Tang's group designed a dual-modality readout immunoassay platform based on AIEgens for EV71 virions detection (Fig. 2C) [136]. Two-channel detection has higher fault tolerance for more accurate analysis. Fluorescent microspheres, loading multiple fluorescent molecules, also serve as signal labels for extensive use. For COVID-19 tests combined with ICA, polystyrene nanoparticles loaded with 3.18 × 10^5 dyes (AIE810NP) as luminescent labels detected IgM or IgG in sequential clinical samples earlier than commercial gold nanoparticles (AuNPs) based test strips (Fig. 2D) [137].

QDs are nanoscale, low-dimensional semiconductor materials that have attracted attention with their desirable optical properties, such as broad excitation spectrum, narrow symmetric emission spectrum, precise tunability of emission peak, long fluorescence lifetime, photochemical stability, and negligible photobleaching [138–140]. QDs-ICA employs QDs as readout signals to increase sensitivity, which benefits from both ICA and QDs [46,127,141,142]. As shown in Fig. 3A, Huang et al. used compact and hierarchical magneto–fluorescent assemblies as both target-enrichment substrates and luminescent sensing labels for ICA [143]. Similarly, Zhou et al. developed a quantum dot nanobead-based ICA to detect SARS-CoV-2 total antibodies within 15 min [144]. The platform performed well in the dynamic monitoring of serum antibody levels in the whole course of SARS-CoV-2 infection with the sample added.

In addition, some enzyme-induced fluorescence substrates are also employed for fluorescent quantitation [145,146]. In particular, Reis's group developed a series of lab-on-a-stick platforms, which combined the simplicity of dipstick tests with the high performance of 10-bore fluoropolymer microcapillary microfluidic devices for heterogeneous FIAs [147–150]. To improve portability, they fabricated a new, simple, and affordable microfluidic platform with the assistance of a smartphone camera for Escherichia coli detection without sample preparation or concentration [151]. Quantitation was achieved by a highly sensitive fluorescence substrate, AttoPhos, cleaved by alkaline phosphatase (ALP) to produce bright green fluorescence when an analyte is enzymatically detected. To reduce manual steps, they also designed a power-free microfluidic device, called gravity-driven microfluidic siphons, for multiplex protein analysis (Fig. 3B) [152].

Compared with aforesaid down conversion fluorescent materials converting short-wavelength light into long-wavelength light, UCNPs capable of converting near-infrared excitation into ultraviolet or visible emissions exhibit significant advantages, such as narrow emission peaks, low toxicity, photobleaching resistance, and stability [154–156]. Notably, the luminescence of UCNPs requires near-infrared excitation without autofluorescence, largely improving the sensitivity [157]. Zhao et al. proposed an electro-driven ICA by electroosmotic flow (EOF) and UCNPs for rapid
As demonstrated in Fig. 3C, signal intensities increased by 64.0% and time was reduced from 15 min to 5 min when EOF was in the same direction as the capillary force-driven flow. Besides, the emission wavelength of UCNPs can be adjusted by changing the type and doping ratio of rare-earth ions for multiplex detection [158–160]. Kazakova et al. described a novel multiplex microarray immunoassay to measure virus-specific IgG and IgM antibodies simultaneously [169]. In this platform, Erbium-UCNPs and thulium-UCNPs enabled 12 different spots for samples from different periods. This platform has potential for vaccine immunity studies with high throughput.

3.1.2. Heterogeneous chemiluminescence immunoassays on microfluidic chips

Chemiluminescence immunoassays (CLIA) determine the content of analytes based on the intensity of the radiated light
produced by chemical reactions, integrating chemiluminescence with immunoassays. Heterogeneous CLIAs are novel immunological analytical methods for detecting trace amounts of protein with low backgrounds. No additional light sources are required in heterogeneous CLIAs, avoiding the interference of scattering light and luminescent impurities. Heterogeneous CLIAs have made great strides and have been extensively used in life sciences [161], clinical diagnosis [162,163], environmental monitoring [164,165], food safety [52,166], pharmaceutical analysis [167,168] and other fields, benefiting from their high sensitivity, wide linear range, no scattered light interference, no radioactive contaminants, and good reproducibility [169].

3.1.2.1. Direct chemiluminescence immunoassays on microfluidic chips. Direct chemiluminescence immunoassays (DCLIAs) require the direct labeling of antigens or antibodies with chemiluminescent agents [170,171]. Immunocomplexes are formed by utilizing the excellent specificity of antigen-antibody interactions. With the introduction of oxidant and pondus hydrogenii correction solution, chemiluminescence agents decompose and emit light for quantification [172]. Nowadays, isoluminol, acridine ester, and their derivatives are commonly used. Lee’s group designed aptamer-antibody on-chip sandwich immunoassays integrated with acridine ester for automated analysis [173–175]. Magnetic beads (MBs) modified with aptamers can not only capture but also separate analytes (Fig. 4A). Pump-driven pneumatic valves can be used for flow closure as well as drainage. Min. et al. reported an automated microfluidic chemiluminescence immunoassay platform for the quantitative detection of ferritin [176]. Besides acridine ester, 6-[N-(4-aminobutyl)]-N,N,N,N-tetraethylammonio-2,3-dihydro-1,4-phenazine (ABEI) is also applied for DCLIAs. However, these chemiluminescence substrates exhibit flash-type light emission, which requires rapid signal acquisition. In addition, it is hard to distinguish simultaneous flashes, hindering further application for multiplex testing. To improve throughput without mutual interference, Cui’s group devised a three-dimensional microfluidic paper-based device to simultaneously detect early acute myocardial infarction (AMI) biomarkers by CoO2−/ABEI functionalized magnetic carbon composites [177]. Three time-resolved chemiluminescence signals were generated in one chemiluminescence detection run by time-delayed transport of H2O2 to different detection zones (Fig. 4B). Another possible approach is turning flash into glow-type through controlled release. For instance, Wu et al. structured a doxorubicin-ABEI chimeric magnetic DNA hydrogel (MDH) as a novel protease-free and long-lasting chemiluminescence system (Fig. 4C) [178]. The MDH effectively delayed the diffusion rate of reactants because of the dense network structure and then transformed flash-type ABEI/H2O2/CoO2− reaction into a glow-type chemiluminescence system, making the chemiluminescence reaction gradually occur.

3.1.2.2. Indirect chemiluminescence immunoassays on microfluidic chips. Indirect chemiluminescence immunoassays (ICLIAs) are usually based on enzyme proteins labeling antigens or antibodies, which catalyze chemiluminescence agents to produce chemiluminescence signals for measurements [114,179]. Currently, the commonly used enzymes are horseradish peroxidase (HRP) and ALP. Liu’s group designed an active droplet-array microfluidic system based on CLIAs to analyze procalcitonin (PCT) automatically (Fig. 5A) [174]. This platform employed ALP as chemiluminescence labels. In comparison, HRP was more commonly applied for ICLIAs. They also designed active droplet-array microfluidics with HRP labels for ICLIAs [180]. Dynamic solid-phase immunoassays with a pseudo-homogeneous format, where MBs coated with cAbs are dispersed in solution, enable fast and sensitive detection due to violent molecular diffusion [181]. Another option is traditional ELISA with cAbs pre-coated on the surface of microfluidic chips [182,183]. For example, Wang’s group fabricated a microfluidic microarray immunoassay platform called BioC, for the determination of 20-target allergens simultaneously (Fig. 5B) [184,185]. Furthermore, Jiang’s group developed a microfluidic platform integrated with on-chip valves and CLIAs for the quantitative detection of multiple biomarkers (Fig. 5C) [186,187]. Then they introduced electrospun microfibers for improvements with larger specific surface areas compared with tin foil for dynamic multiplexed immunoassays [188]. Instead of actuators, self-driven is another choice. Dai et al. designed a flux-adaptable and self-contained microfluidic platform for automated CLIAs (Fig. 6A). [189]. In addition to quantitative analysis sample pretreatment, sample pretreatment is still required in microfluidic systems. Indeed, an ultra-sensitive detection device for proteins extracted from within single cells was developed and validated [190].

Fig. 4. DCLIAs on microfluidic chips. (A) Schematic illustration of the aptamer-antibody DCLIAs on the integrated microfluidic system. (a) Transfer of the MBs to the transportation unit for reaction. (b) Washing with phosphate buffer. (c) Transfer of acridinium ester-labeled antibodies to the transportation unit for reaction. (d) Washing with phosphate buffer once again. (e) Transfer of H2O2 to the transportation unit. (f) Transfer of the complexes to the NaOH chamber for chemiluminescence immunoassays. (Reprinted with permission from Ref. [174]). (B) Schematic illustration of three heart disease biomarkers by magnetic carbon composites and the three-dimensional microfluidic paper-based device. (Reprinted with permission from Ref. [177]). (C) Schematic diagram of novel long-lasting chemiluminescence system. (Reprinted with permission from Ref. [178]).
Antibodies were immobilized on chitosan hydrogel film with an open-network 3D structure, which increased surface coverage and availability (Fig. 6B). It seems that dimensional structure is better for increasing sensitivity because of the high specific surface area \([121, 191-193]\).

### 3.1.2.3. Electrochemiluminescence immunoassays on microfluidic chips

Electrochemiluminescence is an energy-relaxation process by the optical emission of an excited molecule produced by an applied potential at an electrode surface \([195-197]\). Similar to chemiluminescence mentioned above, electrochemiluminescence has a low background as no light sources have been involved \([195, 198, 199]\). Since the electrochemiluminescence immunoassays (ECLIAs) are electrically induced, they are more controllable than chemiluminescence reactions involving uncontrollable reagents. Besides, due to the final balance between redox indicators, namely \([\text{Ru(bpy)}_3]^{3+}\) and \([\text{Ru(bpy)}_3]^{2+}\), the most widely used electrochemiluminescence labels, output signals with circle amplification are of high sensitivity and stability.

Chen et al. developed a paper-based electrochemiluminescence...
method for hepatitis B surface antigen detection combined with screen-printed electrodes (Fig. 7A) [200]. They omitted to apply hydrophobic materials and complex reaction channels to avoid low transportation efficiency, not fully controllable flow direction and inaccurate positioning. However, such a simple design makes it hard to integrate. Valves play an important role in microfluidic chips for precisely controlling fluid movements [184–187]. Sun et al. described a novel rotational paper-based analytical device with integrated rotational valves for ECLIAs [201]. The “On/Off” states of valves were controlled by rotating the auxiliary disc and the washing disc (Fig. 7B). Nonetheless, low emission efficiency and the high cost of electrochemiluminescence are big problems [202]. Hence, new improvements are needed for the further application of ECLIAs. Since the electrochemiluminescence phenomenon of QDs was first observed during the study of silicon semiconductors in 2002 [203], many nanomaterial-based electrochemiluminescence emitters with different sizes, shapes, and compositions have been used for bioanalysis. Li et al. used modified hollow titanium dioxide hollow spheres (THS) and SnS2 QDs to build novel ECLIAs for the ultrasensitive detection of CAP [204]. Additionally, Guo et al. synthesized several ruthenium and iridium complexes for multiplex immunoassays with different electrochemiluminescence emission wavelengths from 491 to 636 nm [205]. These new materials show great potential for electrochemiluminescence, whereas how to improve their properties, even for commercial use, is a big challenge.

3.2. Homogeneous luminescence immunoassays on microfluidic chips

In homogeneous immunoassays, quantitative analysis is achieved without extra steps, such as separation and washing [206,207]. With reagents added and mixed, signals could be read out after reactions. Luminescence resonance energy transfer (LRET) is one of the most common methods for homogeneous immunoassays [208]. It is a non-radiative energy transfer between a donor and an acceptor that occurs at a close enough distance (generally less than 10 nm) [209]. There are many kinds of LRET, mainly including fluorescence resonance energy transfer, bioluminescence resonance energy transfer, and chemiluminescence resonance energy transfer [208]. LRET has been applied widely for biomedical applications, such as clinical diagnosis, environmental monitoring,
and food safety [210–212]. For bioassays, the donors and acceptors get close to each other by antibody-antigen interactions with LRET. The LRET process directly reveals the presence or absence of analytes with only one step. Sandwich immunoassays integrated with these techniques are homogeneous without washing. Compared with the above-mentioned heterogeneous immunoassays, these methods are much simpler. Besides, antibody incubation is not required, making it more suitable for integration and automation. Meanwhile, ratiometric sensors with a ratio value of the fluorescence intensity between receptors and donors have strong anti-interference abilities and more accurate analysis results.

3.2.1. Fluorescence resonance energy transfer immunoassays on microfluidic chips

FRET is a nonradiative process whereby an excited state donor (usually a fluorophore) transfers energy to a proximal ground-state acceptor through long-range dipole-dipole interactions [213]. FRET usually occurs over distances comparable to the dimensions of most biological macromolecules, that is, about 1–10 nm [214]. Notably, the emission spectrum of the donors ought to overlap with the absorption spectrum of the acceptors.

Samson et al. reported responsive FRET signals generated on paper for detecting cyclic AMP (cAMP)-specific phosphodiesterase 4B (PDE4B) inhibitory using inkjet-printing technology with four cartridges [215]. The principle was the competition between the europium (Eu) chelate-labeled cAMP tracer and sample cAMP for binding sites on cAMP-specific monoclonal antibodies labeled with the ULight dye on parchment paper. Compared with conventional fluorophores, time-resolved fluorophores generate signals with higher fluorescence intensity signal-to-noise ratio [216–219]. They use trivalent rare earth ions and their chelates as labels, which can achieve time-resolved imaging with a long fluorescence lifetime [218,220–222]. Time-resolved FIAs integrate time-resolved fluorescence with immunoassays, which exhibit high sensitivity and linearity [131,132,223]. For instance, Rusanen et al. designed a time-resolved FRET immunoassay platform for the detection of both N and S proteins of SARS-CoV-2 simultaneously [224]. A strong association between the sample infectivity and positive antigen test results was observed with high throughput. Nevertheless, sensitivity was lower than those of enzyme-based assays. It might be caused by interference of the sample matrix.

3.2.2. Bioluminescence resonance energy transfer immunoassays on microfluidic chips

Bioluminescent resonance energy transfer (BRET) was developed on the basis of FRET, which was first presented and used for the study of cyanobacterial biological clock proteins by Xu et al., in 1999 [225]. They used a bioluminescent luciferase rather than fluorophore as the donor. Bioluminescence appeared with luciferin substrate added, which served as an energy source for the receptors. Clearly, BRET immunoassays without external light sources are required to feature less background interference than FRET immunoassays.

Merck’s group designed a series of BRET platforms based on smartphones for protein quantification. They presented a new sensor platform called LUMABS, which were single-protein sensors consisting of the blue-light emitting luciferase NanoLuc connected via a semiflexible linker to the green fluorescent acceptor protein mNeonGreen [226]. The presence of analytes disrupted the interaction between the donors and acceptors, resulting in a large decrease in BRET efficiency. Then a fully integrated “sample-in–signal-out” microfluidic paper-based analytical device relying on LUMABS for analyte recognition and colorimetric signal generation was developed [227]. With only 20 μL sample added, the signals could be read out by a digital camera within 20 min, and results were available after simple processing. With improvements of chip design, they presented a thread-based analytical device for POCs (Fig. 8A) [228]. LUMABS and furimazine were positioned on two intertwisted threads in close proximity to each other. Therefore, it achieved higher throughput with simpler paper chips.

3.2.3. Light initiated chemiluminescence immunoassays on microfluidic chips

Ullman et al. reported luminescent oxygen channeling immunoassays in 1994 for the first time [229]. Molecular oxygen is excited by a photosensitizer and an antenna dye, which diffuses to the second particle and initiates a chemiluminescent reaction. Thus, combined with immunoassays, energy transfer occurs between the two particles in the presence of analytes. On the
contrary, no energy transfer and chemiluminescence appear as molecular oxygen decays. There are some commercial reagents for light-initiated chemiluminescence assay (LICA), such as AlphaScreen [230,231] and AlphaLISA [232,233]. LICA is a chemiluminescence immunoassay but quite different from heterogeneous chemiluminescence immunoassays. Multi-step incubation and washing are required for signal output without exciting light in heterogeneous CLIAs, while light and appropriate distance are requested in LICA.

Stephens et al. presented a batch-fabricated, robust, and mass-producible immunophenotyping microfluidic chip by silicon micromachining processes for specific leukocyte subset isolation immunophenotyping, and personalized immunomodulatory drug screening (Fig. 8D) [234]. The CD21 Jurkat T-cells were isolated by beads, and cytokines were secreted into the immunoassay chamber. Then AlphaLISA with some modification on the chip was conducted for LICA.

### 3.3. Digital immunoassays on microfluidic chips

Some biomarkers have very low concentrations, lower than 10^{-12} M, which exceed the detection limit of conventional immunoassays [235,236]. In consideration of sensitivity, we then introduced digital immunoassays for quantitative analysis of low-abundance protein biomarkers. Digital immunoassays with millions of assays in parallel within femtoliter volume droplets were developed to overcome this concern. The essence of digital immunoassays is counting proteins to achieve absolute quantification with higher sensitivity and lower detection limit compared to conventional immunoassays [236–240]. Based on the way of forming droplets, digital immunoassays can be generally grouped into two categories based on the types of microfluidic chips: microstructured microfluidic chip [240–242] and droplet microfluidic chip [243–245]. Usually, digital immunoassays on microstructured microfluidic chips separate liquids by microchannels or microwells, while digital immunoassays on droplet microfluidic chips are performed by generating droplets with shear force change.

#### 3.3.1. Digital immunoassays on microstructured microfluidic chips

The digital immunoassays on microstructured microfluidic chips seal nL-level liquid in microarrays for step-by-step immunoassays. The whole reaction process is similar to traditional microfluidic immunoassays, but in much more and smaller reaction chambers. MBs are widely used for immobilizing antibodies in digital immunoassays on microfluidic chips [246,247]. In particular, Kurabayashi’s group proposed a pre-equilibrium digital ELISA microarray, which united a spatial-spectral microfluidic encoding scheme and an image data analysis algorithm based on machine learning for single-molecule protein digital counting (Fig. 9A) [241,248]. However, the cost, external magnetic field, high losses, and complex modification process hinder the further applications of MBs. Qian et al. performed bead-free digital immunoassays with polydopamine microspot arrays [249]. Then cAbs were incubated on the polydopamine arrays directly. The method has high sensitivity with a detection limit of 26 fg mL^{-1}. But compared to the bead-based digital assays, the current platform showed limited capture efficiency. In addition, chips with bead-based digital assays are potential for reuse, while chips modified with cAbs directly are one-off.

#### 3.3.2. Digital immunoassays on droplet microfluidic chips

Digital immunoassays on droplet microfluidic chips are performed by generating discrete droplets containing reaction reagents. Droplet formation includes active and passive ways. The active approach applies an external force to microfluidics, including opto-electrowetting [250–252], magnetism [253–255], thermocapillary forces [256,257], surface acoustic waves [258–260], dielectrophoresis (DEP) [261–263], electrowetting-on-dielectric (EWOD) [264–266], etc. However, these methods are suitable for manipulating droplets but not for massive droplet generation to meet digital immunoassays. The passive method generates droplets by adjusting the flow rate and channel structure, mainly including T-channel [267,268], flow focusing [145,269], and coaxial flow focusing [270,271]. Conventional droplet assays can generate a mass of monodisperse droplets but with low throughput. As an improvement, Yelleswarapu et al. designed a microdroplet Megascaler Detector integrated with a parallelized microfluidic droplet generator for faster and multiple detections (Fig. 9B) [240]. Besides, combined with a mobile imaging technique based on cloud computing, this platform achieved throughputs as high as one million droplets per second. Usually, digital immunoassays improve sensitivity by counting single analytes dispersed in tens of thousands to millions of tiny units. However, it greatly decreases the sampling of rare events, leading to excessive Poisson noise. With this in mind, Wat’s group developed droplet digital ELISA to detect proteins in the low attomolar range [272]. The higher sensitivity was achieved by improving the sampling efficiency and counting more target molecules.

As illustrated in Table 1, homogeneous and heterogeneous luminescence immunoassays were compared, and their advantages and disadvantages were discussed. Heterogeneous luminescence immunoassays require solid phases for washing steps, greatly prolonging reaction time and increasing operation complexity, which place a high demand on the design of microfluidic chips and the automation of peripherals. But stepwise reactions reduce nonspecific adsorption to some extent, then sensitivity and specificity are improved. Homogeneous luminescence immunoassays with one step are easier to integrate. Nevertheless, impurities in the tests seriously affect the stability precision and repetition of the results. The hook effect also occurs in an improper proportion of antigens and antibodies, which leads to false-negative errors. Different immunoassays on microfluidic chips achieve effective protein detection but with non-negligible factors (Table 2). Heterogeneous FIAs suffer from a relatively high background in heterogeneous luminescence immunoassays, and new fluorescent molecules with improved optical properties are still a long way from commercial applications. Heterogeneous CLIAs with high sensitivity and wide detection range demand a high level of imaging systems, and the stability of the reagent hinders further application. In contrast, heterogeneous CLIAs with high performance tend to replace heterogeneous FIAs gradually. Homogeneous immunoassays on microfluidic chips with one step require strict reagent and reaction conditions. LRET immunoassays with simple steps are easily disturbed by environmental sample impurities. But we have to realize that these homogeneous immunoassays with unique advantages are likely to become more widely used in the future. Digital immunoassays are of extremely high sensitivity but have a low degree of automation. Hence, more efforts will be focused on how to optimize these methods, integrate them into the POCTs, and further meet clinical needs.

### 4. Integrated microfluidic chip platforms

Microfluidic chip manufacturing technologies include soft lithography, injection molding, laser engraving, screen printing, and etc. As mentioned above, microfluidic chips integrated with different modules have been employed on sundry occasions, especially in POCTs. For example, Lansionbio Biotechnology Co., Ltd designed a series of active microfluidic platforms with controllable...
reACTION time, flow direction and rate [273]. BluSense Diagnostics company developed centrifugal microfluidic discs-ViroTrack and portable medical testing platforms-BluBox for immunoassays with a drop of blood [274]. Herein, we summarize the types of various integrated microfluidic platforms which would contribute significantly to developing POCTs for the prevention and control of COVID-19. We also compare their characteristics, such as fabrication, principle, and design.

4.1. Lab on a cartridge chip (LOCC)

LOCC is the first micro total analysis system that integrates actuators or self-drive units for flow control. There are no specific requirements for distributing microfluidic channels and chambers, so LOCC with a small size has higher space utilization. It also has low demand for materials. LOCC with active actuators, such as pumps, is relatively less integrated, while LOCC with self-drive units is less stable but more convenient. Hence, it is quite necessary to establish a stable and convenient LOCC system for applications.

Capillary pumps consisting of small parallel microchannels provided main, reliable and adjustable power for self-driven. Gao et al. designed a pump-free LOCC platform based on a comb-like structure channel for driving fluids through the entire channel [275]. Similarly, Wang et al. fabricated a LOCC system integrated with nanorod arrays for flow-through immunoassays [276].
another work, Machado et al. presented an autonomous and cascaded capillary chip aligned with a mobile application for parallel mycotoxin detection [277]. The speed can be adjusted by the size of corresponding capillary pumps. Besides microstructures serving as power for self-driven, gravity and suction are also applied for fluid control [152,278,279]. As shown in Fig. 10A, Xiang et al. present a recyclable gravity-driven LOCC system for competitive immunoassays [278]. In the alternative, finger-driven microfluidic chips have attracted wide attention, eliminating the need for any skilled personnel with simple design and operation [35,280–282]. In particular, Lammertyn’s group presented a new iSIMPLE (infusion Self-powered Imbibing Microfluidic Pump by Liquid Encapsulation) concept, which required liquid encapsulation and a finger-actuated mechanism [283–286]. As an example, they put it into use for therapeutic drug monitoring of adalimumab in patients diagnosed with autoimmune diseases [287]. Alternatively, pumps as external actuators are engaged for fluid control [190,288]. In consideration of unexpected air bubbles, air clogs, and irregular fluidic filling, Bhuiyan et al. designed an artificial intelligence-controlled LOCC system for fluid automation and bubble removal operated by a smartphone [278,289]. The AI image recognition app was implemented to ameliorate several inaccurate microfluidic states, thus resulting in lowering the limit of detection (LOD).

4.2. Lab on a disc (LOAD)

LOAD integrates all parts onto a compact disk (CD)-shaped chip, driven by centrifugal actuators, which was first proposed with the concept of LabCD [290,291]. Usually, microchannels are distributed radially, in the same direction as the centrifugal force. The fluids then move along the network of microchannels away from the center and toward the edge by the synthetic action of centrifugal

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**Table 2**

Comparisons of different luminescence immunoassays.

| Methods                        | Advantages                                | Disadvantages                                |
|--------------------------------|-------------------------------------------|----------------------------------------------|
| Heterogeneous fluorescence immunoassays | Low cost                                  | High commercialization                       |
|                                 | Fluorescent molecules AIEgens              | Aggregation caused quenching, photobleaching |
|                                 | QDs                                       | Synthetic complexity                        |
|                                 | Enzyme catalysis                           | High toxicity, low water solubility          |
| Heterogeneous chemiluminescence immunoassays | High sensitivity, low background, wide linear dynamic ranges | High requirements for imaging systems, enzyme activity effects |
|                                 | DCLIAs                                    | Low stability, short-lived luminescence     |
|                                 | ECLIAs                                    | High cost, weak anti-interference ability   |
|                                 | LRET immunoassays                          |                                             |
|                                 | FRET                                      | High controllability                        |
|                                 | BRET                                      | Low background                             |
|                                 | LICA                                      | High cost, environmental sensitivity, hook effect |
| Digitalimmunoassays             | Low detection limit, absolute quantification | Multi-stage signal amplification           |
|                                 | Droplet                                    | Complex platforms, low level of automation and integration |

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**Fig. 10.** Integrated microfluidic platforms. (A) Schematic illustration of the gravity-driven chip. (Reprinted with permission from Ref. [278]). (B) Photograph and schematic illustration of the fabricated LOAD. (Reprinted with permission from Ref. [296]). (C) Images of Scheme of the chemiluminescent LFIAs for the detection of targets. (Reprinted with permission from Ref. [312]). (D) Schematic diagrams of the µPADs design and the direct ELISA protocol. (Reprinted with permission from Ref. [318]).
forces, capillary forces, Euler forces and Coriolis forces [292–294]. Besides, mixture and washing are much easier by centrifugation and oscillation. Thus, microfluidics could be controlled by regulating speed of centrifugal actuators rather than pumps. It is easy to see that LOAD with simple peripherals has a high degree of parallelization and a huge capacity for total integration [295].

Nowadays, plentiful LOAD systems have been developed for applications in immunoassays, nucleic acid assays, and biochemical analysis. Cho’s group designed a LOAD system integrated with electrospun TiO2 nanofiber and phase change valves for protein detection [Fig. 10B] [296]. By opening the valves one by one, reagents were moved from one chamber to another by centrifugal force, then immunoassays on LOAD were accomplished step by step [297]. In addition, TiO2 nanofibers with high specific surface area and active functional groups capture large amounts of antibodies and improve detection sensitivity. Apart from physical excitation, Delgado et al. present an electrified LOAD (eLOAD) system permitting valve actuation during rotation by wireless control of rotor-based resistive heaters, which was also for additional flow control and sensing capabilities [298,299]. In another work, Xu’s group designed an electromagnet-triggered pillar valve on LOAD for mycotoxin detection [300]. They also designed pinch-valves on LOAD based on magnetic actuation [301]. As an alternative, passive valves are easy to fabricate and simple to actuate with surface modification. For example, Xu’s group fabricated an Euler force-assisted LOAD system for sequential liquid release [302]. They also put forward a centrifugation-assisted lateral flow immunoassay with enhanced sensitivity [303]. Combinations of passive valves and active valves are performed to play to their strengths and mitigate their weaknesses. Henderson et al. combined event-triggered dissolvable film valves with centrifugal-pneumatic siphon structure to control fluid flow [304]. Xu’s group added pillar valves on the centrifugation-assisted lateral flow immunoassay platform mentioned above for better fluid control [305]. Although these have no requirements for additional pumps, they suffer from the unidirectional nature of the centrifugal force. That is the reason for the large size of LOAD as space utilization is relatively low. For more reliable applications, Romero-Soto et al. presented another eLOAD system integrated with electrolys pumps and reversible thermo-pneumatic valves [306]. The new 3D pump design occupied a much smaller space of the disc, which enabled the implementation of more fluidic components to automate complex sequential bioassays.

4.3. Lateral flow immunochromatographic assays (LFIA)

A test strip for LFIA consists of an absorbent pad, nitrocellulose membrane marked with test lines and control lines, conjugate pad embedded with detection antibodies, sample pad, adhesive backing, and base cover, which is the most classical POCTs based on immunoassays [307]. Generally, the whole detection procedure is as follows: a small volume of sample is dropped onto the sample pad, migrates on the conjugated pad, then carries conjugated particles to the test pad; Target antigens in the given sample are recognized and bonded with detection antibodies on reporter surface in the conjugated pad, where complexes interact with CâBs on test line and free reporters bound on control line [308]. In other words, the results can be obtained in one step after the sample added without additional operations. Because of their convenience, LFIA have been commercialized in multiple areas, including COVID-19 antigen test. AuNPs as labels for LFIA with naked eye show relatively good performance [309]. Despite that, they suffer from low sensitivity and semi-quantity. To overcome it, a series of improvements, such as new materials, was introduced as mentioned above. AIEs, QDs, and UCNPw were synthesized as luminescent labels, and sensitivity was improved to varying degrees [76,127,130,137,143,153]. Nanohybrids, as labels, catalysts or for signal amplification, have enhanced the practical application of test strips [310,311]. Chemiluminescence was also contained by freeze-drying oxidants to achieve self-contained chemiluminescent LFIA (Fig. 10C) [312]. In addition, mobile health platforms were established to read and transfer results [73,76,313]. Given throughput, scientists put arrays into use for LFIA [117,314]. Particularly, Jiang’s group embedded skiving stacked sheets of papers into test strips for rapid and multiplexed immunoassays [315]. These platforms showed great potential for practical clinical applications, but the stable materials and the matching equipment need to be developed or improved.

4.4. Microfluidic paper-based analytical devices (µPADs)

Whitesides’s group firstly proposed paper-based microfluidic analytical devices (µPADs) for glucose and protein tests [316]. The working principle of µPADs is to build microchannels on paper for fluid control by physical and chemical methods. Typical physical methods are wax jet printing, ink jet printing, and screen printing, while chemical methods are chemical vapor deposition, photolithography, and plasma etching [317]. With the advantages of low cost, simple processing and conversion, µPADs have attracted widespread attention upon discovery and are widely used in medical diagnosis, environmental monitoring, food safety and other fields.

Fu et al. developed a paper-based microfluidic analytical device (µPAD) based on shape memory-polymer-actuated fluid valves for automated multi-step immunoassays (Fig. 10D) [318]. Reagents were preloaded on the storage zone, while a portable colorimetric reader was developed to control the on-chip valve operations, quantify the colorimetric signal output, display the results, and wirelessly transmit the data to a smart phone for the application of telemedicine. In another work, Chen et al. designed a three-dimensional surface-modified origami-paper-based analytical device (3D-soPAD) combined with a sliding strip as a valve to control the serial steps of sample addition, antigen-antibody interaction, incubation, washing, and detection of the ELISA reaction by simply sliding the tabs to a different position [54]. Besides sliding, rotating also has been employed for valve control. For example, Li et al. integrated hand-powered centrifugation with a rotational µPAD with blood-in-answer-out capability [319]. These platforms exhibited high operability, even for untrained users in environments where access to electricity cannot be assumed. Nevertheless, sensitivity and robustness need to be further improved.

The respective features of different microfluidic chip platforms are listed in Table 3. Although these platforms show great potential for POCTs, problems still exist in practical applications. LOCC, with its small size and various actuators, enables multivariable fluid control but suffers from complex peripherals and relatively low integration. LOAD driven by centrifugal actuators is of high throughput but large size. For µPADs, they display excellent performance at low cost, simple assembling and no extra actuators. However, they are limited as fluid control is single and not suitable for multilayer structures. LFIA have been widely deployed in POCTs for commercial applications due to their low cost, rapidity, and simple and convenient operations, but they have limited capabilities to achieve accurate quantitative and high throughput analysis with relatively low specificity and sensitivity. We ought to admit that all existing platforms are problematic in dealing with the current situation. We need to simplify and optimize the whole process for user-friendly and high performance. Meanwhile, how to reduce cost is still considerable. Therefore, new or modified platforms are claimed to achieve “sample-in/result-out” analysis mode.
5. Luminescence immunoassays for COVID-19 POCTs

The continuous widespread of COVID-19 has damaged not only the health of individuals but also the economy of the global society. So it is urgent to develop global wide POCTs targeting the COVID-19. Thankfully, scientists have made great efforts to develop practicable systems to get us through this mess [30,38,320,321]. At this stage, many immunoassays for the rapid detection of SARS-CoV-2 can be broadly divided into two categories. The first is direct detection of SARS-CoV-2 biomarkers, such as spike (S) glycoprotein, envelope (E) protein, nucleocapsid (N), and membrane (M) protein. The second is the detection of antibodies of the immune system responding to SARS-CoV-2, including IgG and IgM. Antibody levels reveal the staging of the body’s immune response. Besides, cytokines as evaluation indicators reveal the severity of COVID-19. Next, we introduced some relatively mature microfluidic platforms based on these for the POCTs of COVID-19.

5.1. Luminescence immunoassays for COVID-19 antigen tests

SARS-CoV-2 encodes at least 29 proteins in its RNA genome, including four structural proteins: the S, M, E, and N proteins [322,323]. In particular, S protein plays a pivotal role of virus binding to host cell membrane receptors and membrane fusion and is a key therapeutic target for neutralizing antibodies and vaccine design [324–326]. Therefore, quantifying these antigen proteins is crucial for diagnosing, treating, preventing, and controlling COVID-19.

Up to now, scientists have developed a series of COVID-19 antigen detection platforms [327–330]. Among them, test strips are typical representatives. For example, Guo et al. proposed a UCNP-based ICA platform combining the Internet of Medical Things (IoMT) and 5G for proactive detection of S and N protein, with LOD of 1.6 ng mL\(^{-1}\) and 2.2 ng mL\(^{-1}\) for S and N protein [331]. Besides, the results were accessible to edge hardware devices through Bluetooth and transmitted to the fog layer of the network and 5G cloud server for both individuals and hospitals. Apart from capillary for self-driven, external force combined with magnetism also works. Lumiradx Co LTD designed a cheap microfluidic chip device for parallel immunoassays by polyethylene terephthalate screen printing technology (Fig. 11A) [332]. The microfluidic chip automatically controlled the movement of liquid in a single capillary by the different extrusions of the gas chamber to achieve accurate control of each reaction process. Take SARS-CoV-2 nucleocapsid immunoassays as an example [333]. The sample was introduced into the first chamber under negative pressure and nucleocapsid protein was captured by cAbs labeled MBs to form complexes. Analogously, the MB-cAb-NP compound bound to Latex labeled detection antibodies (dAbs) in the second chamber. Then the pressure of the gas chamber was fully released, and all reagents entered the detection chamber. The excess liquid was pressurized through the gas chamber and pumped out to the waste liquid chamber, while the Latex-dAb-NP-cAb-MB remained in the detection chamber via an extra magnetic field for fluorescence intensity quantification.

5.2. Luminescence immunoassays for COVID-19 antibody tests

COVID-19 antibody tests can monitor antibody levels produced by the body’s humoral immune system, mainly for IgM and IgG antibodies [334–336]. For those vaccinated against COVID-19, antibody tests can reflect the immune state of the population and assess the effectiveness of the vaccine [337,338]. For those not, antibody tests can determine whether previously infected by SARS-CoV-2 and different stages of viral infection [339–341]. However, existing standards and traditional platforms are not enough. There is an urgent need to develop novel and practical POCTs for monitoring antibody levels.

The above-mentioned test strips were also used for antibody quantification [311,342]. Apart from capillary for self-driven, gravity works as well. Innovatively, Chilkiot’s group developed DA-D4 POCTs for COVID-19 serology based on gravity and capillary (Fig. 11B) [343]. The sample was first introduced and filled with a reaction chamber. With wash buffer added, it slowly flowed through the time channel, and at the same time, immune complexes formed. The D4 assay was also designed by them before to interrogate multiple analytes from a drop of blood [344]. For antibodies targeting S1 and RBD, the sensitivity of DA-D4 assay reached 100% two weeks after symptom onset, while for antibodies targeting N is 96.3%. But between 6 and 14 days after symptom onset, the sensitivity was 78.9%, 89.5%, 78.9%, respectively. Maybe it’s because of the low concentration of antibodies once after symptom onset. Centrifugal microfluidic chips are extensively used because of their high integration density, simple equipment and convenient operations [60,345,346]. For instance, VACURE Co LTD fabricated a LOAD immunoassay platform for detecting biomarkers, including SARS-CoV-2 IgM and IgG [347]. Reagents were freeze-dried and could be stored at room temperature for one year. Moreover, time-resolved fluorescence microspheres with large stokes shift and long decay time improved specificity. The whole test could be done within 18 min for nine parallel and independent tests.

5.3. Luminescence immunoassays for cytokine storm tests

Cytokine storm refers to the phenomenon of rapid and massive production of cytokines such as IL-6 in body fluids caused by microbial infection, which might cause acute respiratory distress syndrome, multiple organ failure, and even death [93]. There is clear evidence that COVID-19 patients, especially those with severe pneumonia, have high levels of inflammatory cytokines [348]. In other words, early detection and close monitoring of cytokine levels enable rapid identification of high-risk COVID-19 patients, which is crucial to assess the severity and improve survival rates [349,350]. Besides, cytokine storm enables predict COVID-19 severity and survival as hyper-inflammatory response induced by SARS-CoV-2 is a major cause of disease severity and death [351]. Hence, it is more than necessary to achieve highly accurate, sensitive, stable and fast immunoassays for cytokine storm tests.

Beckman Coulter, Inc. developed an ACCESS system based on ALP for CLIAs, including IL-6 [352]. Paramagnetic particles with a diameter 2–4 μm and sonic cleaning ensured the adequacy of the whole test. Meanwhile, the tectorial membrane reduces
volatilization of the stored liquid and can be directly mechanically punctured to reduce contamination. The shipboard microfluidic chips have low processing requirements but low integration degrees, which do not fully exploit the advantages of microfluidic chips. The above-mentioned pre-equilibrium digital ELISA system proposed by Kurabayashi’s group has been applied to monitor COVID-19 cytokine storm clinically [353]. The digital assay worked as a promising candidate for continuous cytokine profiling with a combination of speed and sensitivity. As discussed, cytokines are highly correlated with the degree of COVID-19 inflammation, but still do not meet the diagnostic criteria for COVID-19. Thus, scientists need to grapple with the problem of measuring cytokine levels in COVID-19 patients.

5.4. Luminescence immunoassays for COVID-19 combination tests

As immunoassay platforms for protein quantification are of general applicability, numerous devices are suitable for antigen and antibody tests. One example is the programmable bio-nanochip (p-BNC) platform for biomarkers testing designed by McDevitt’s group [354–358]. Inspired by the taste buds of the tongue, they developed an “electronic tongue” [359,360]. Agarose gel microspheres modified with antibodies are able to capture analytes and antibodies for quantification. The platform also used QDs as readout signals [361,362]. Phosphate buffer saline washing buffer is stored in two blisters, which are embedded in the microfluidic chips [356]. The movement of the fluid is controlled and stabilized by the actuator’s piercing and pressing mechanism. Fluorescence signals obtained by CCD are then quantified by an algorithm and displayed through an interactive interface. The platform was also used for determining disease severity in patients with COVID-19 (Fig. 12A) [363]. The COVID-19 Severity Score was established to predict mortality and risk factor via combining multiplex biomarker measurements and a statistical learning algorithm. As a representative of LOAD system, Superchip Technology Co LTD developed a POC microfluidic platform integrated a homemade fluorescence analyzer for the detection of IgG/IgM/Antigen of SARS-CoV-2 within 15 min (Fig. 12B) [364]. The microfluidic chip consisted of a top layer with a sample loading chamber, a middle layer with a fluid channel, and a bottom layer with a waster reservoir [365]. Under the capillary effect, sample flow first flowed toward the
Table 4: Comparisons of different luminescence immunoassays on microfluidic chips.

| Detection Methods | Analytes | LOD | Time | Driving force | Fabrication | Immobilizing matrix | Year | Refs. |
|-------------------|----------|-----|------|--------------|-------------|---------------------|------|-------|
| **Heterogeneous fluorescence immunoassays** | CAP | 0.05 μg L⁻¹ | 20min | Pump | Soft lithography | Microspheres | 2021 | [116] |
| | cTnI | 4 ng mL⁻¹ | 25min | Capillary force | Soft lithography | PMMA beads | 2020 | [121] |
| | EV71 virions | 1.4 copies μL⁻¹ | 10min | Magnetism | – | | | |
| | COVID-19 IgM & IgG | 0.236 μg mL⁻¹ | – | Capillary force | Paper | Nitrocellulose membrane | 2021 | [137] |
| | PCT | 0.031 ng mL⁻¹ | 18min | Magnetism | Capillary force | Paper | 2021 | [143] |
| | COVID-19 total antibodies | – | 15min | Magnetism | Capillary force | Paper | 2021 | [144] |
| | *Escherichia coli* | 240 CFU mL⁻¹ | 25min | Syringe | Electro osmosis | Paper | 2019 | [151] |
| | *Yersinia pestis* EV76 | 1.2 x 10⁷ CFU mL⁻¹ | 5min | Capillary force | Paper | Nitrocellulose membrane | 2020 | [153] |
| | COVID-19S1 protein | 16.9 ng mL⁻¹ | 10 min | Capillary force | Paper | Nitrocellulose membrane | 2021 | [131] |
| | COVID-19N protein | 32 TCI/mL⁻¹ | 12min | Magnetism | Air pressure | Pubmed | 2021 | [333] |
| | COVID-19 total antibodies | – | 60min | Magnetism | Air pressure | Pubmed | 2021 | [333] |
| | hproBP | – | 16 min | Laser cutting | POE GMA brush | | 2021 | [343] |
| | CRP | – | – | Laser cutting | POE GMA brush | | | |
| | NT-proBNP | – | – | Laser cutting | POE GMA brush | | | |
| | MYO | – | – | Laser cutting | POE GMA brush | | | |
| | D-dimer | – | – | Laser cutting | POE GMA brush | | | |
| | PCT | – | – | Laser cutting | POE GMA brush | | | |
| | CK-MB | – | – | Laser cutting | POE GMA brush | | | |
| **Heterogeneous Chemi-luminescence immunoassays** | Hb | 8.8 g dL⁻¹ | 25min | Centrifugal force | Capillary force | Injection molding | PET membrane | 2020 | [164] |
| | HbA1c | 0.65 g dL⁻¹ | 45min | Pump | Capillary force | Injection molding | Soft lithography | MBs | 2015 | [174] |
| | Ferritin | 2.55 ng mL⁻¹ | 12min | Pump | Capillary force | Injection molding | Soft lithography | MBs | 2016 | [175] |
| | cTnI | 0.40 pg mL⁻¹ | 20min | Pump | Capillary force | Injection molding | Soft lithography | MBs | 2016 | [175] |
| | cTnI | 0.50 pg mL⁻¹ | – | Pump | Capillary force | Injection molding | Soft lithography | MBs | 2016 | [175] |
| | cTnI | 0.044 ng mL⁻¹ | 12min | Magnetism | Capillary force | Injection molding | MBs | 2022 | [194] |
| | IgE | 2.4 ng mL⁻¹ | 27min | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | CRP | 1.87 pg mL⁻¹ | 70min | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | PCT | 0.17 ng mL⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | IL-6 | 49.75 pg mL⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | CEA | 0.89 ng mL⁻¹ | 20min | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | AFP | 1.72 ng mL⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | CA125 | 3.62 U mL⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | CA19-9 | 1.05 U mL⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | DSG3 | 0.10 fg mL⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | VEGF-A | 0.20 fg mL⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | VEGF-C | 0.20 fg mL⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | β-Tub | 0.20 fg mL⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2021 | [190] |
| | PSA | 0.07 ng mL⁻¹ | 20min | Pump | Capillary force | 3D printing | Chitosan | 2018 | [201] |
| | anti-HIV1 | 0.03 ng mL⁻¹ | 20 min | Pump | Capillary force | 3D printing | Chitosan | 2018 | [201] |
| | anti-HA | 2.8 nmol L⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2018 | [201] |
| | anti-DEN | 7.1 nmol L⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2018 | [201] |
| | anti-HA | 19.3 nmol L⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2018 | [201] |
| | anti-DEN | 4.0 nmol L⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2018 | [201] |
| | IL-2 | 75.4 μg mL⁻¹ | – | Pump | Capillary force | 3D printing | Chitosan | 2018 | [201] |
| | GM-CSF | GM-6 | 320 amol mL⁻¹ | – | Pump | Silicon micromachining | | | |
| | IL-6 | 350 amol mL⁻¹ | – | Pump | Silicon micromachining | | | |
| | 12 circulating cytokines | 0.1–5 pg mL⁻¹ | 40min | Pump | Soft lithography | Fluorescent beads | 2019 | [240] |
| | IL-6 | 12 fmol mL⁻¹ | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | IL-2 | 1.2 pg mL⁻¹ level | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | IL-10 | 40 pg mL⁻¹ | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | TNF-α | 0.015 ng mL⁻¹ | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | IFN-γ | 0.099 pg mL⁻¹ | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | IL-2 | 40 pg mL⁻¹ | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | SARS-CoV-2 IgG protein | 30 amol mL⁻¹ | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | 20 amol mL⁻¹ | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | 0.015 ng mL⁻¹ | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | 0.099 pg mL⁻¹ | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | More than 1h | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | Gravity | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | Vacuum suction | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
| | Blu-ray | – | Pump | Soft lithography | Fluorescently encoded beads | 2021 | [261] |
capture region, forming fluorescent microsphere immune-complexes. Then the complexes were immobilized by dAbs on the test region and control region. Next, dispersed liquid in the channel was thrown into the waste chambers by centrifugation for 10 s without washing. Finally, the fluorescence detection results were read and obtained from the analyzer. With matrix nanopatterning, parallel repeats were performed for stable and reliable results. As for CLIA, Shenzhen Watmind Medical Co Ltd has designed a series of products, including M2/M5 microfluidic chemiluminescence immunoassay analyzer and microfluidic chips for COVID-19 antigen and antibody tests [366,367]. The microfluidic chip channel was a two-layer structure with reagents pre-stored. The upper channel was mainly for sample introduction, filtration, and reaction with dAbs. The lower channel was for washing twice and forming MB-cAb-analyte-dAb immune-complex, including a reading chamber for the test. With analyzers involved, results were obtained in 13–15 min after the sample was added. Moreover, analyzers were built-in communication modules: GPS, 4G, Wireless Fidelity, Bluetooth, iCloud enable connecting with each other for long-distance monitoring [368]. As for digital immunoassays, Walt’s group designed a digital ELISA platform, namely a single-molecule array (Simoa), for detecting serum proteins at subfemtomolar concentrations [369]. And then matched Simoa HD-1 analyzer was developed for automated analysis [370]. The immune-complexes linked to the magnetic beads were captured and sealed separately in the spiral-sized micropores on the Simoa disc composed of 24 arrays consisting of 216,000 40 fl-sized wells to achieve digital ELISA with much lower detection and higher sensitivity (Fig. 12C). This device was also adapted for COVID-19 detection with LOD of 0.015 ng mL\(^{-1}\) for IgG and 0.099 pg mL\(^{-1}\) for N protein [371–374].

6. Conclusion and future outlook

In this review, we have summarized luminescence immunoassays based on microfluidic chips for urgent POCTs of COVID-19. The typical examples are listed in Table 4 to provide a comparative discussion. Although many significant applications have been reported in the fields of luminescence immunoassays on microfluidic systems, they still need to be improved in specificity, dynamic range, sensitivity, throughput, portability, cost, and reproducibility in the future. Besides, false negative rates of immunoassays must not be ignored, particularly in the window period and early stage. Available immunoassay platforms for COVID diagnosis cannot meet the clinical needs with relatively high rates of missed and misdiagnoses. Excitingly, the emerging novel enzyme and nucleic acid-based signal enhancement strategies have significantly improved analytical performance of luminescence immunoassays, such as polymerized catecholamines depositing around HRP for cascade amplification [375,376], nucleic acid chains as labels for cascade amplification [377–381], fluorescent-labeled aptamers based thermophoretic enrichment assays [192,382–386] and clustered regularly interspaced short palindromic repeat (CRISPR) based amplification [387,388]. In addition to methodology, the other perspective is miniaturization of equipment and intellectualization of performance, benefiting from the rapid development of the Internet, such as smartphones and 5G technology [313,331,389–393]. Finally, other than endpoint testing, real-time detecting systems, such as wearable devices, with continuous monitoring of processes can better meet the needs for POCTs on the progress of the immune response [394–398].

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.

Data availability

No data was used for the research described in the article.

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