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Microfluidic-based technologies for diagnosis, prevention, and treatment of COVID-19: recent advances and future directions

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Abstract
The COVID-19 pandemic has posed significant challenges to existing healthcare systems around the world. The urgent need for the development of diagnostic and therapeutic strategies for COVID-19 has boomed the demand for new technologies that can improve current healthcare approaches, moving towards more advanced, digitalized, personalized, and patient-oriented systems. Microfluidic-based technologies involve the miniaturization of large-scale devices and laboratory-based procedures, enabling complex chemical and biological operations that are conventionally performed at the macro-scale to be carried out on the microscale or less. The advantages microfluidic systems offer such as rapid, low-cost, accurate, and on-site solutions make these tools extremely useful and effective in the fight against COVID-19. In particular, microfluidic-assisted systems are of great interest in different COVID-19-related domains, varying from direct and indirect detection of COVID-19 infections to drug and vaccine discovery and their targeted delivery. Here, we review recent advances in the use of microfluidic platforms to diagnose, treat or prevent COVID-19. We start by summarizing recent microfluidic-based diagnostic solutions applicable to COVID-19. We then highlight the key roles microfluidics play in developing COVID-19 vaccines and testing how vaccine candidates perform, with a focus on RNA-delivery technologies and nano-carriers. Next, microfluidic-based efforts devoted to assessing the efficacy of potential COVID-19 drugs, either repurposed or new, and their targeted delivery to infected sites are summarized. We conclude by providing future perspectives and research directions that are critical to effectively prevent or respond to future pandemics.

Keywords COVID-19 detection · Vaccine · Drug development · Microfluidics · Organ-on-chip

1 Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) belongs to the *betacoronavirus* genus with two known human coronaviruses, severe acute respiratory syndrome (SARS) (Cheng et al. 2007) and the Middle East respiratory syndrome (MERS) (Zumla et al. 2015), that led to regional epidemics in the early 2000s. The genetic material of SARS-CoV-2 is reserved in a single-stranded RNA with a glycoprotein-spiked envelope that gives the virus its crown-like appearance (Schmidt and Leach 2003; V'kovski et al. 2021). The virus contains four main structural proteins categorized as: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins (Udugama et al. 2020). SARS-CoV-2 is known to affect the respiratory tract and several organ systems in the body with symptoms varying between patients. It has caused the COVID-19 outbreak around the world, threatening public health since December 2019. Pyrexia, dry cough, ageusia, anosmia, shortness of breath, and respiratory distress are the most common manifestations of the disease caused by SARS-CoV-2 (Merad et al. 2022). Symptomatic cases can result in severe illness or death, including acute respiratory syndrome (Xu et al. 2020), neurologic manifestations (Mao et al. 2020), acute kidney injury (Chen et al. 2020), gastrointestinal symptoms (Galanopoulos et al. 2020) and cardiovascular complications (Lo et al. 2022). Along with the asymptomatic cases that are believed to contribute to the transmission of the

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disease, SARS-CoV-2 has resulted in a total of 607 million confirmed cases and 6.5 million reported deaths worldwide so far (JHU 2022).

To date, there have been many studies aimed to develop diagnostic, therapeutic, and preventive technologies against COVID-19. The need for the development of innovative health technologies has become more urgent with the emergence of SARS-CoV-2 variants because the immunity gained against the ancestor virus with vaccines or previous infection might not be effective in fighting the new coronavirus variants such as B.1.1.7 (Alpha), B.1.617.2 (Delta), 1.351 (Beta), P.1 (Gamma) and more recently newly described B.1.1.529 (Omicron) (Andrews et al. 2022; Tao et al. 2021).

The development of new diagnostic tests that are capable of detecting COVID-19 variants is critical to control the spread of the virus and to treat COVID-19 complications before they become life-threatening. In general, the current methods for the detection of SARS-CoV-2 can be categorized as direct or indirect based on the target molecule(s). Direct diagnostic techniques are based on detecting the genetic material of the virus and viral antigens which are predominantly N proteins. Reverse transcription-polymerase chain reaction (RT-PCR) is the most common technique used for the RNA-based detection of SARS-CoV-2 in nasopharyngeal, oropharyngeal, and nasal swab samples of patients (Gupta et al. 2021). Along with the RT-PCR, other nucleic acid amplification techniques such as loop-mediated isothermal amplification (LAMP) (Augustine et al. 2020), clustered regularly interspaced short palindromic repeats (CRISPR) (Hou et al. 2020) and reverse transcription rolling polymerase amplification (RT-RPA) (Lau et al. 2021) can also be used in the detection of SARS-CoV-2. It is also possible to detect viral antigens via sandwich enzyme-linked immunosorbent assay (ELISA) by using anti-SARS-CoV-2 antibody-coated microwell plates that capture the viral antigens. Similarly, fluorescence-labeled secondary antibodies allow the detection of viral antigens present in patient samples (Carter et al. 2020). Indirect diagnostic techniques for SARS-CoV-2 are mainly based on the detection of host antibodies (IgG/IgM) produced against the viral antigens through serological tests. ELISA-based immunoassays are among the most commonly used techniques for detection of the antibodies and for the prognosis of post-COVID-19 infections (Carter et al. 2020). In addition, lateral flow immunoassay (LFA) (Wen et al. 2020) and automated chemiluminescence immunoassay (CLIA)(Infantino et al. 2020) are commonly used for the detection of SARS-CoV-2 antibodies in patient samples.

The rapid spread of COVID-19 and its serious threat to public health has called for the development of effective therapeutic and preventive strategies for pandemic management. Initially, the focus was on isolating COVID-19 patients from the rest of the community and providing symptomatic treatments (such as respiratory support and nutritional supplementation) to reduce discomfort and improve wellbeing (Brugliera et al. 2020; Ñamendys-Silva 2020). Considering the long and labor-intensive timeline of the drug development process (i.e., 10–15 years), initial drug development efforts have been directed towards repurposing existing drugs that are proven effective in the treatment of diseases with similar mechanisms as COVID-19 (Zhou et al. 2021b), rather than developing completely new ones. Along with the repurposing of the existing drugs, studies that are focused on developing novel drug molecules that effectively target COVID-19 are also ongoing. In both cases, there are two main goals: preventing the viral binding and the entry to the host cell and/or blocking one or more steps in the viral replication process within the host cell (Kruse 2020). The first strategy is to hinder the binding of the virus to the cell via saturating the receptor binding domain (RBD) of the spike protein or blocking the binding site of the Angiotensin Converting Enzyme-2 (ACE2) receptor on the membrane of the host cell using either soluble RBD proteins, antibodies or antibody fragments and soluble ACE2 (Larue et al. 2021; Monteil et al. 2020; Tai et al. 2020; Wu et al. 2020). Manipulating the viral RNA is another possible solution to keep the virus from invading the host cells and replicating its genetic material (Idris et al. 2021; Medeiros et al. 2021). Although these strategies are promising for the treatment of COVID-19, the most important remaining roadblock that hinders the development of effective therapeutic options for COVID-19 is the low solubility of conventional or newly developed therapeutic formulations in the blood, which limits the transfer of these molecules to the target organs, tissues and cells. To overcome this barrier, there are studies focused on targeted drug delivery methods using nanoparticles as drug carriers (Chowdhury et al. 2021). Besides, convalescent plasma therapy is another therapeutic approach where the plasma of recovered patients is used for utilizing the antibodies produced against SARS-CoV-2 (Wang et al. 2021b). In some patients, overstimulation of the immune system by alveolar macrophages is shown to cause excessive cytokine production called cytokine storm (Nitulescu et al. 2020). Anti-inflammatory agents such as monoclonal antibodies and immune mediators can hold down the effect of cytokines that usually cause inflammation and may lead to acute respiratory distress syndrome (ARDS) or even multi-organ failure (Esmaeilzadeh and Elahi 2021; van de Veerdonk et al. 2022).

Antiviral vaccines are preventive technologies developed to create an immunological memory in patients to alleviate the symptoms at the next encounter with the virus, to reduce the propagation rate of the virus, and to lower the mortality rates around the world (Liang et al. 2021; Sette and Crotty 2022). Viral antigens or their products are the major components of the vaccines along with other supportive materials such

as adjuvants, antibiotics, preservatives, stabilizers, and inactivators (Meyer and Zepp 2022). Various types of vaccines (such as whole inactivated-virus, live attenuated, protein-based, viral vector, and nucleic acid (mRNA and DNA) vaccines) are already developed, being tested in clinical trials and approved (or waiting for approval) for use in specific/general populations. Whole inactivated virus vaccines contain killed SARS-CoV-2 with inactivated genetic material (Ndwandwe and Wiysonge 2021). Inactivated vaccines do not cause infection with the virus but they can trigger the protective immune response. Inactivation can be done by chemicals, radiation, or heat (Hadj Hassine 2022). Live attenuated vaccines contain weakened SARS-CoV-2 virus with reduced pathogenicity (Kumar et al. 2021). They can still replicate their genome and stimulate the immune system in a way to produce a protective immune response. Protein-based vaccines are classified as subunit vaccines and virus-like particle vaccines (Ndwandwe and Wiysonge 2021). Subunit or recombinant protein vaccines contain the viral antigens or proteins such as spike protein or its RBD produced using a vector (Heidary et al. 2022). Virus-like particles are composed of viral components without the genetic material and they cannot replicate but still induce immunity (Mohsen and Bachmann 2022). Viral vectors are classified as non-replicating and replicating virus vectors, both of which can produce viral proteins and result in an immune response (Callaway 2020). DNA and RNA vaccines are designed to transmit a genetic message into the host cell, usually within a nanocarrier (Chauhan et al. 2020). The gene carried in DNA or RNA contains the information that encodes a SARS-CoV-2 antigen. The genetic code is then translated into the viral protein within the host cell and subsequently stimulates the immune pathway (Chavda et al. 2021).

Flexible application of technologies that can promote rapid and effective utilization is very important in the (1) management of pandemics such as COVID-19, (2) prevention of their spread by implementing novel diagnostic tools, and (3) treatment of subsequent damages (Lamprou 2020; Zhang et al. 2021). Microfluidic methods allow versatile applications in different biomedical domains, including those that are related to pandemic outbreaks and their consequences (Maged et al. 2022; Sharma and Sharma 2022). Microfluidics is generally defined as the manipulation, processing, and control of liquids with micro-scale chips, channels, or devices (Cottet and Renaud 2021; Yang et al. 2020). It involves a wide range of technologies that process and move a very small volume of fluids through microchannels in a controlled manner. By incorporating a network of microchannels into a chip that is linked to the macro-environment through holes, tubing, or pumping adapters, microfluidic systems allow the miniaturization, automation, and acceleration of complex biological assays (Wang and Li 2011). These systems provide new tools to assess the vital functions of biological cells in their natural microenvironment under physiologically relevant biochemical forces and flow conditions (Wang et al. 2020c).

Microfluidic technologies enable chemical synthesis and reaction, bioprocesses, tissue modeling, drug modeling, biomimetics, diagnosis, monitoring, and treatment of diseases, and hence, already found applications in various fields of life sciences, medicine, and engineering (Kong et al. 2020; Whitesides 2006; Yaman et al. 2018). The integrated use of microfluidics with other technological elements such as sensors, actuators, artificial intelligence, and telemedicine could enable the development of multi-functional and interconnected point-of-care devices that could provide fast, remote, automated, robust, and sensitive analysis tailored according to the needs of specific applications (Galan et al. 2020; Li et al. 2020; Martinez et al. 2008; Riordon et al. 2019; Tarim et al. 2021).

Sustainable prevention of viral infection caused by the SARS-CoV-2 virus has proven to be a crucial step in managing the COVID-19 pandemic, and microfluidic systems could play a leading role in this context. In addition to the widespread use of microfluidic tools for diagnostic purposes, prospective microfluidic methods are also being used for the development of vaccines, drugs, and other biomedical countermeasures. Recent reviews summarize progress in the application of microfluidic- or biosensor-based approaches to COVID-19 diagnostics for readers interested in these aspects (Dong et al. 2021; Kumar et al. 2022; Ramezankhani et al. 2021; Shpichka et al. 2020; Yin et al. 2022a). Although there are reviews focusing on the role of microfluidics in COVID-19 diagnostics or treatment (Jamiruddin et al. 2022; Jiang et al. 2021; Sun et al. 2021; Ziqi et al. 2022), to the best of our knowledge, there is no study that summarizes the existing applications of microfluidics in different COVID-19-related domains with a more holistic approach that allows understanding the current applications, needs, and limitations with a comprehensive view. Here, we take a step forward and compile more recent applications of microfluidics for COVID-19 detection, treatment, and prevention, and discuss the main barriers that are currently hampering the clinical translation of these microfluidic-assisted tools and devices.

2 Microfluidic methods for diagnosis

Ideally, microfluidic solutions developed and used for the detection of viral infections should counter the speed of the virus spread by allowing early detection with their rapid, accurate, easy-to-use, and portable nature. Microfluidic-assisted diagnostic tools can be divided into two broad categories: direct methods that allow the detection of the virus and virus content (nucleic acids, proteins, particles, molecules) and indirect methods that detect the metabolic

changes in the infected organism (Fig. 1). This section covers the recent microfluidics application for the diagnosis of COVID-19 and these applications are summarized according to their methodology, target, sensitivity, specificity, detection time and limits in Table 1.

2.1 Direct detection

There are a wide range of techniques that allow direct detection of COVID-19 (Tarim et al. 2021; Tayyab et al. 2020). Nucleic acid-based detection techniques are widely used for the direct detection of SARS-CoV-2. The polymerase chain reaction (PCR) (Donia et al. 2022; Ho et al. 2022; Nguyen et al. 2022; Shen et al. 2021; Xie et al. 2020; Xiong et al. 2021; Zai et al. 2022), loop-mediated isothermal amplification (LAMP) (Davidson et al. 2021; Jhou et al. 2022; Lim et al. 2022; Malic et al. 2022; de Oliveira et al. 2021; Parker et al. 2022; Soares et al. 2021; Tarim et al. 2023; Tsai et al. 2022; Yang et al. 2021), clustered regularly interspaced short palindromic repeats (CRISPR) (Li et al. 2022b; Liu et al. 2022; Park et al. 2021), and recombinase polymerase amplification (RPA) (Liu et al. 2021; Sun et al. 2022; Yin et al. 2021b) are among the most popular methods for amplification of viral DNA/RNA. In addition to nucleic-acid-based detection techniques, immunoassay-based and microarray systems have been commonly used for the detection of the SARS-CoV-2 proteins (Akarapipad et al. 2022; Bhuiyan et al. 2022; Cui et al. 2022; Kim et al. 2021; Li and Lillehoj 2021; Wu et al. 2022a; Zhang et al. 2022; Zhao et al. 2021).

PCR can work in harmony with microfluidic technologies and is frequently used for virus detection (Basiri et al. 2021). For example, a centrifugal microfluidic platform was developed for rapid multiplex detection of SARS-CoV-2, influenza A, and B using direct RT-qPCR (Ji et al. 2020). Detection was achieved in 1.5 h after the swap sample loading with a 100% consistency rate for SARS-CoV-2 RNA. The platform had a detection limit of 20 copies per reaction in 2 µL of swap sample with fluorescence sensing. Droplet-based assays are of great importance in microfluidic systems for detecting and quantifying SARS-CoV-2 variants. In a recent example, the application of a thin-film-transistor digital-microfluidic system (commercially known as aQdrop™) that was capable of manipulate multiple droplets along various pathways for the molecular diagnosis of COVID-19 was demonstrated (Anderson et al. 2021). The system was shown to detect 80–320 copies of RNA extracted per µL salvia sample. In another example, a droplet-based microfluidic system combined with a rapid digital PCR method using a micro-heater array (Yin et al. 2021a). The designed microfluidic chip handled the generation of > 20.000 droplets with mineral oil and the integrated micro-heater array in the chip allowed the detection of two SARS-CoV-2 specific genes (ORF1ab and N genes) in a RT-PCR reaction. This system could detect 10 copies/µL with small PCR reaction volume

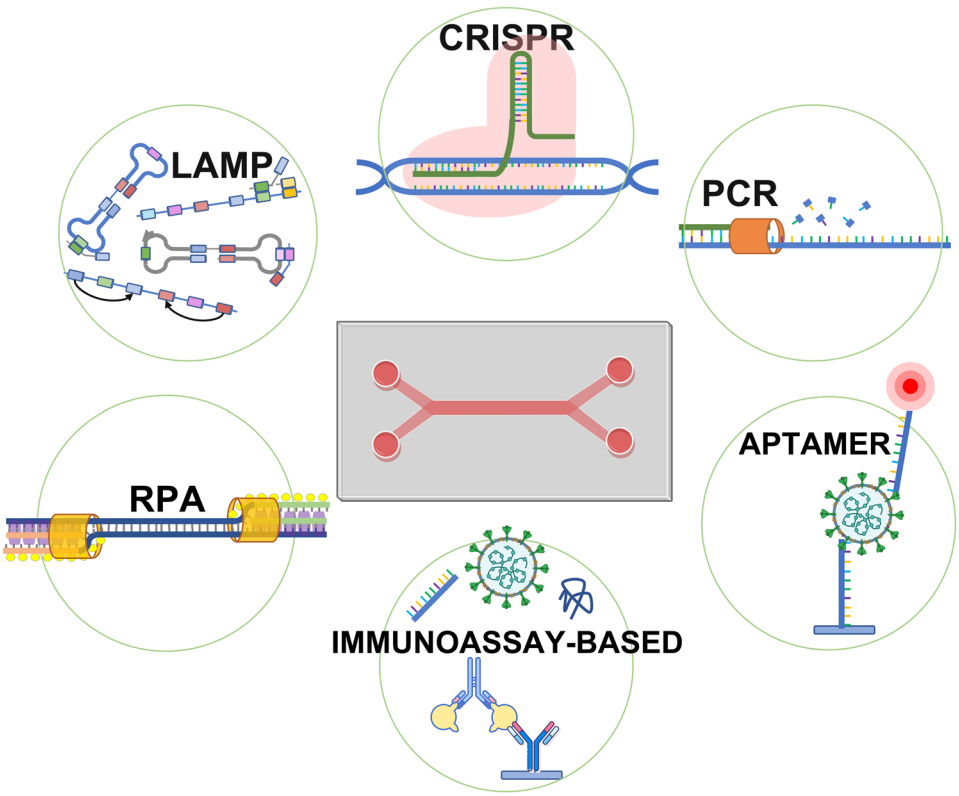

Fig. 1 Overview of the most commonly used microfluidic diagnostic methods for the detection of SARS-CoV-2. These methods use virus, virus contents or virus-related metabolites of the infected organisms for direct/indirect detection

Table 1 A summary of microfluidic-based direct and indirect methods for SARS-CoV-2 detection

Reference	Target	Type	Method	Limit of Detection	Assay Time	Sensitivity	Specificity
(Qi et al. 2022)	N protein	Direct	Aptamer/ impedance change	3.16 fg/mL	NR	NR	99.99%
(Zakashansky et al. 2021)	S1 protein	Direct	Aptamer/ electrochemical	1 ag/mL	1 h	NR	NR
(Ge et al. 2022)	N protein	Direct	Aptamer-antibody sandwich method/ fluorescence	33.28 pg/mL	45 min	NR	76.71%
(Sundah et al. 2021)	RNA (S and N gene)	Direct	CATCH/ nucleic acid immobilization	~8 RNA copies/µL	< 1 h	100%	92%
(Liu et al. 2022)	RNA (N and orf1ab gene)	Direct	CRISPR/ fluorescence	50 copies/mL	< 40 min	98.5%	100%
(Ramachandran et al. 2020)	RNA	Direct	CRISPR/ fluorescence	10 copies/mL	35 min	NR	NR
(Ackerman et al. 2020)	RNA	Direct	CRISPR/ fluorescence	1 copies/µL	30 min	NR	92.4%
(Park et al. 2021)	RNA	Direct	Digital CRISPR/Cas-assisted/ fluorescence	1 genome equivalent/ µL	30 min	99.07%	100%
(Shen et al. 2021)	synthetic DNA (N gene)	Direct	Digital PCR/ fluorescence	10 copies/µL	NR	99.00%	NR
(Bhuiyan et al. 2022)	N protein	Direct	ELISA/ colorimetric	13 pg/mL	NR	NR	99.50%
(Zhao et al. 2021)	RNA (S gene)	Direct	Enzyme–DNA nanostructures/ electrochemical	7 copies/µL	< 20 min	92.30%	87.50%
(Li and Lillehoj 2021)	N protein	Direct	Immunoassay/ electrochemical	50 pg/mL	< 1 h	NR	NR
(Akarapipad et al. 2022)	N protein	Direct	Immunoassay/ flow profile	10 pg/mL	15 min	89%	NR
(Cui et al. 2022)	SARS-CoV-2 and/or antigen	Direct	Immunoassay/ microwave	4000 copies/mL	90 min	NR	NR
(Wu et al. 2022a)	N protein	Direct	Immunoassay/ colorimetric	0.1 pg/mL	40 min	NR	NR
(Wang et al. 2020a)	RNA (N, E, and Orf1a gene)	Direct	Immunoassay/ LFA/ fluorescence	500 copies/mL	57 min	100%	99%
(Gao et al. 2022)	S and N proteins	Direct	Immunoassay/ FET	8 fg/mL	20 min	98%	NR
(Zhang et al. 2022)	N-gene mRNA	Direct	Nucleic acid hybridization/ chemiluminescent	1.0 pM	20 min	Differ with respect to concentration	NR
(Crevillen et al. 2022)	RNA (N gene)	Direct	Nucleic acid hybridization/ electrochemical	15×10^{-9} M	7 min	NR	NR
(Ho et al. 2022)	RNA (N gene)	Direct	RT-qPCR/ fluorescence	10 copies/µL	42 min	NR	NR
(Kim et al. 2021)	RNA (orf1ab gene)	Direct	RCA/ flow profile	0.7 aM	15 min	NR	NR
(Yin et al. 2021b)	RNA (N, E, and orf1a gene)	Direct	RPA and LAMP/ colorimetric	100 genome equivalent/mL	NR	NR	NR
(Sun et al. 2022)	RNA (N gene)	Direct	RPA-Cas12a/ fluorescence	1.3 copies/µL	30 min	100%	100%
(Liu et al. 2021)	RNA (N gene)	Direct	RPA/LFA/ colorimetric	1 copy/µL	30 min	97%	100%
(Li et al. 2022b)	RNA	Direct	RPA/CRISPR/LFA/ colorimetric	100 copies	NR	94.10%	100%
(Soares et al. 2021)	RNA	Direct	RT-LAMP/ fluorescence	10 copies/µL	< 1 h	96.60%	100%
(Shi et al. 2021)	RNA (N gene)	Direct	RT-LAMP/ fluorescence	10^3 copies/µL	> 60 min	NR	NR
(Davidson et al. 2021)	RNA (orf7a, orf7b and orf1ab gene)	Direct	RT-LAMP/ colorimetric	200 genomic copies/µL	60 min	97%	100%
(Yang et al. 2021)	RNA (M and N genes)	Direct	RT-LAMP/ colorimetric	0.5 copy/µL	< 2 h	NR	NR

Table 1 (continued)

Reference	Target	Type	Method	Limit of Detection	Assay Time	Sensitivity	Specificity
(Malic et al. 2022)	RNA (E and N genes)	Direct	RT-LAMP/ colorimetric	0.5 copies/μL	60 min	100%	NR
(Nguyen et al. 2022)	RNA (N gene)	Direct	RT-LAMP and RT-PCR/ colorimetric	10 copies/μL	30 min	95%	100%
(de Oliveira et al. 2021)	RNA (orf1a gene)	Direct	RT-LAMP/ fluorescence	10^{-3} copies/μL	10 min	NR	NR
(Tsai et al. 2022)	RNA (RdRp, E, and N genes)	Direct	RT-LAMP/ fluorescence	200 copies/ μL	82 min	NR	NR
(Parker et al. 2022)	RNA (ORF1ab gene)	Direct	RT-LAMP/ fluorescence	NR	NR	NR	NR
(Lim et al. 2022)	RNA (N and S genes)	Direct	RT-LAMP/ fluorescence	≥10 copies/μL	30 min	>90%	100%
(Jhou et al. 2022)	RNA (E, N, and RdRp genes)	Direct	RT-LAMP/ fluorescence	200 copies/μL	90 min	NR	NR
(Zai et al. 2022)	RNA (N and orf1ab gene)	Direct	RT-qPCR/ fluorescence	200 copies/mL	30 min	97.60%	98%
(Xiong et al. 2021)	RNA (N and orf1ab gene)	Direct	RT-PCR/ fluorescence	10 copies/μL	15 min	91.82%	100%
(Anderson et al. 2021)	RNA	Direct	RT-PCR/ fluorescence	20 copies/μL	NR	99.73%	NR
(Yin et al. 2021a)	RNA (N and orf1ab gene)	Direct	RT-PCR/ fluorescence	10 copies/μL	15 min	99.95%	NR
(Xie et al. 2020)	RNA (N gene)	Direct	RT-qPCR/ cycle of quantification	1 copy/μL	NR	94.50%	NR
(Donia et al. 2022)	RNA (N and orf1ab gene)	Direct	RT-qPCR and RT-LAMP/ colorimetric	NR	NR	85.70%	NR
(Tan et al. 2020)	S1 IgG	Indirect	ELISA/ chemiluminescent	2 ng/mL	15 min	NR	NR
(González-González et al. 2021)	anti–SARS-CoV-2 IgG	Indirect	ELISA/ colorimetric	NR	<2 h	NR	NR
(Carrell et al. 2020)	anti-N IgG	Indirect	ELISA/ colorimetric	2.8 ng/mL	20 min	NR	NR
(Wang et al. 2021a)	IgG/ IgM and N and S antigens	Indirect	Immunoassay/ fluorescence	0.3 pg/mL	<10 min	NR	NR
(Swank et al. 2021)	anti–SARS-CoV-2 IgG	Indirect	Immunoassay/ fluorescence	1 nM	NR	98%	100%
(Rodriguez-Moncayo et al. 2021)	S, S1, RBD and N IgG/IgM	Indirect	Immunoassay/ fluorescence	1.6 ng/mL	6.6 min/ assay	95%	91%
(Rajsri et al. 2022)	ant-RBD and anti-S IgG	Indirect	Immunoassay/ fluorescence	47 ng/mL	15 min	NR	NR
(Lin et al. 2020)	IgG/IgM/Antigen	Indirect	Immunoassay/ fluorescence	NR	15 min	NR	NR
(Heggestad et al. 2021)	anti-S1, anti-RBD, and anti-N antibody	Indirect	Immunoassay/ fluorescence	NR	60 min	78.9% (anti-S1), 89.5% (anti-RBD), 78.9% (anti-N), 100% (after 2 weeks from symptom onset)	100%
(Lee et al. 2021)	anti-SARS-CoV-2 IgG/IgM	Indirect	Immunoassay/ LFA/ fluorescence	NR	5 min	91.67%	100%
(Song et al. 2021)	IL-6, TNF-α, IL-1β, IL-10	Indirect	Immunoassay/ fluorescence	0.191 pg/mL (IL-6), 0.198 pg/mL (TNF-α), 0.350 pg/mL (IL-1β), 0.377 pg/mL (IL-10)	30 min	NR	NR
(Huang et al. 2020)	SARS-CoV-2 IgM	Indirect	Immunoassay/LFA/ colorimetric	NR	15 min	100%	93.30%
(Cognetti et al. 2021)	anti- RBD, anti-S1 and anti-S2 IgG	Indirect	Immunoassay/ photonic spectra	NR	1 min	77.80%	100%
(Funari et al. 2020)	anti-SARS-CoV-2 S antibody	Indirect	Immunoassay/ LSPR	0.08 ng/mL	30 min	NR	NR

Table 1 (continued)

Reference	Target	Type	Method	Limit of Detection	Assay Time	Sensitivity	Specificity
(Xu et al. 2021)	anti-SARS-CoV-2 IgG	Indirect	Immunoassay/ Fresnel reflection	0.82 ng/mL (IgM), 0.45 ng/mL (IgG)	< 7 min	NR	NR
(Ali et al. 2021)	anti-S1 antibody and anti-RBD antibody	Indirect	Immunoassay/ electrochemical	2.8 fM (S1 antibody), 16. fM (RBD antibody)	11.5 s	NR	NR
(Mattila et al. 2022)	anti-S protein IgG	Indirect	Immunoassay/ particle accumulation	4 AU/mL	< 10 min	NR	NR
(Wu et al. 2022b)	anti-spike and anti-RBD IgG	Indirect	Immunoassay/particle accumulation	13.3 ng/mL (sensitive mode), 57.8 ng/mL (rapid mode)	20–70 min	NR	NR
(Gao et al. 2021)	IL-1β, IL-2, IL-6, IL-10, TNF-α, and IFN-γ	Indirect	Immunoassay/ nanoplasmonic	0.91 pg/mL (IL-1β), 0.47 pg/mL (IL-2), 0.46 pg/mL(IL-6), 1.36 pg/mL (IL-10), 0.71 pg/mL (TNF-α), and 1.08 pg/mL(IFN-γ)	60 min	NR	NR
(Bordbar et al. 2022)	Urine metabolites	Indirect	Colorimetric sensor array	NR	7 min	NR	NR

NR Not Reported

(22.4 pL per droplet) within 15 min. In another study, microwell array and droplet microfluidic chips were used for the qualitative and quantitative detection of SARS-CoV-2 using RT-qPCR (Yin et al. 2022b). The detection limits of both chips were below 10 replicates per 20 µL reaction sample test with a detection time of 15 min.

The LAMP method is a simple method that can readily be integrated with microfluidics for SARS-CoV-2 detection due to its isothermal application property (Zhang et al. 2019). The Epidax® was proposed by the National University of Singapore as a point-of-care testing platform for the detection of SARS-CoV-2 using both RT-PCR and reverse transcription LAMP (RT-LAMP) techniques (Nguyen et al. 2022). The system could sense changes in both fluorescence and colorimetric signals with its image-processing feature. The detection limit of the device was reported to be 25 copies per 10 µL reaction volume, with an average analysis time of 1 h. In another study, the LAMP-based microfluidic system was designed as an integrated on-chip platform to extract, separate, and detect the SARS-CoV-2 RNA in a single device (Rodriguez-Mateos et al. 2021). They used immiscible filtration assisted by surface tension (IFAST) technique for the extraction of the viral RNA using oligo (dT)-functionalized magnetic beads. The amplification and detection occurred with colorimetric RT-LAMP and then the color change was analyzed for the qualitative detection of SARS-CoV-2 RNA. The detection limit of the device was 47 copies/mL and it provided qualitative results within 1 h. An RT-LAMP-based electromechanical point-of-care device was developed to detect SARS-CoV-2 in a microtube (Tarim et al. 2023). The designed colorimetric testing system with embedded image processing algorithms could potentially be used for both qualitative and quantitative detection of viruses remotely, independent of the location, and without the need for sophisticated technical equipment or expertise. This device could be operated with very low sample size (2 µL) with the detection capabilities of 10^3 copies/mL in less than 1 h.

The integration of droplet-based microfluidic systems with the LAMP method has already been successfully tested and demonstrated in several analytical devices for virus detection. Lab-in-a-fiber microfluidic device was developed to generate monodisperse droplets to capture SARS-CoV-2 RNA (Parker et al. 2022). RNA-containing droplets were generated using uniaxial capillary flow focusing, and fluorescent RT-LAMP results were measured with a periscope-type laser-induced fluorescence sensor. In another study, a microfluidic device called the droplet array SlipChip was developed by integrating two chromium and photoresist-coated glass microfluidic plates into a closed system for the generation and manipulation of fluids to detect viral RNA (Lyu et al. 2021). The device was reported to use fluorescence LAMP technology for the detection of SARS-CoV-2 with 553 copies/mL detection limit in nearly 40 min. In another study, a droplet-based

centrifugal microfluidic chip was developed for quantifying the SARS-CoV-2 N gene (Shi et al. 2021). The chip used the fluorescence digital LAMP method for the detection of 10^3 copies/μL N gene in less than 60 min.

CRISPR and RPA methods offer great promise to increase selectivity and sensitivity in microfluidic-based virus detection (Bai et al. 2022; Yin et al. 2021c). A microfluidic chip was integrated with a selective ionic focusing technique called *isotachophoresis* for nucleic acid extraction and purification using combined CRISPR and LAMP (used for the pre-amplification) diagnostic assays (Ramachandran et al. 2020). CRISPR-Cas12 was used as an enzyme complex containing a guide RNA that activated the target DNA. Activated complex cleaved the fluorophore – quencher labeled ssDNA and the subsequent change in fluorescence signal was measured. This technique could conduct measurements in 30–40 min using 0.2 μL of the loaded sample, with a detection limit of 10 copies/μL. In another amplification-free CRISPR/Cas12-based detection study, a simple microfluidic chip was developed (Silva et al. 2021). The cleavage of the Cas12a protein on non-related ssDNA fragments, which specifically recognized the ORF1ab target on SARS-CoV-2 RNA, caused the generation of gas bubbles. The number of bubbles further indicated the viral load. By determining the number of bubbles with the help of a cell phone, this system achieved a detection limit of 50 copies/μL with an operation time of 71 min. The droplet-based Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids (CARMEN) platform was developed for the detection of nucleic acids of 169 human-associated viruses including SARS-COV-2 (Ackerman et al. 2020). Nanolitre droplets containing CRISPR-based nucleic acid detection reagents was paired with the droplets containing the molecules to be tested. The CRISPR-Cas13 platform enabled the detection of 4500 CRISPR RNA-target pairs in nearly 30 min. In another study, the reverse transcription RPA (RT-RPA) and the lateral flow measurement rod were integrated for the detection of SARS-CoV-2 RNA in a microfluidic chip (Liu et al. 2021). The viral RNA was loaded into one of the reservoirs of the microfluidic chip, and then the RT-RPA reaction was performed using specific fluorescein amidite (FAM)-biotin-labeled primers. The FAM-biotin-labeled amplification products were bound to the gold-labeled FAM-specific antibodies at the entrance of the lateral flow rod and turned the gold/amplicon form. These gold/amplicons bound to immobilized biotin-ligand molecules gave the red-colored band. The entire detection process took ~ 30 min and the system had a detection limit of 30 copies in 30 μL of extracted RNA.

Aptamer-based diagnostic methods are also suitable to be used in microfluidics (Lou et al. 2022). A microfluidic chip was designed for the detection of SARS-CoV-2 N protein using the aptamer/antibody switching method (Ge et al. 2022). The developed method involved an aptamer/N-protein/β-galactosidase-linked antibody complex attached to fluorocarbon-modified magnetic beads. The β-galactosidase present in this magnetic bead complex reacted with the fluorescent-di-β-D-galactopyranoside added to the well of the chip. With this reaction, a highly fluorescent product was obtained, enabling the detection of the presence of SARS-CoV-2 N-protein. An aptasensor microelectrode array chip using the N-protein as the target antigen was developed for the direct detection of SARS-CoV-2 (Qi et al. 2022). The aptamer-coated microelectrode array chip employing a solid–liquid interface capacitance was successfully used for the detection of trace SARS-CoV-2 N-protein utilizing the change of capacitance. Measurement was completed in 15 s with a detection limit as low as nanogram per mL levels. An aptamer-based electrochemical assay called Shrinky-Dink© was developed for the detection of the S1 subunit of the SARS-CoV-2 spike protein (Zakashansky et al. 2021). The chip was coated with gold on polystyrene for the immobilization of methylene blue modified aptamers specific to the receptor binding domain and 6-mercaptohexanol as the blocking molecule. This microfluidic chip used Shrinky-Dink wrinkled electrodes for the detection of S1 protein at 1 ag/mL in 10% diluted saliva samples.

Immunoassay-based microfluidic systems are commonly used for the detection of SARS-CoV-2 specific proteins and antigens (Yin et al. 2022a). For instance, the LFA was designed with fluorescent-nanoparticle-labeled monoclonal antibodies bound to DNA probes–RNA double-stranded hybrids where DNA probes were specific to 1ab, envelope protein, and the nucleocapsid regions of SARS-CoV-2 RNA (Wang et al. 2020a). This system had the ability to detect 500 copies of SARS-CoV-2 RNA per mL with 57 min detection time. Furthermore, a field-effect transistor (FET) biosensor with graphene oxide-graphene (GO/Gr) van der Waals heterostructure was used for the detection of spike and N proteins in a microfluidic system (Gao et al. 2022). These proteins were trapped by immobilized SARS-CoV-2 capture antibody on the GO layer on top of the Gr surface of the FET sensor. The developed system could detect SARS-CoV-2 proteins within 20 min with a detection limit as low as ~8 fg/mL.

Single-stranded DNA (ssDNA) probes that targeted specific regions of SARS-CoV-2 RNA were also used for the detection of the virus. An ssDNA-modified genosensor was designed to target the N gene sequence that detected the electro-oxidation of adenines in ssDNA interacting with viral RNA. (Crevillen et al. 2022). The ssDNA was then desorbed from the genosensor surface by electro-oxidation of adenines present in ssDNA, causing the sensor's differential pulse voltammetry signal to decrease. The limit of detection was 1.5×10^{-8} M, and the detection occurred in 7 min. Moreover, catalytic amplification by transition-state molecular switch (CATCH) method bypassing all steps of PCR was used in a microfluidic device that was modified

with oligonucleotides for direct detection of SARS-CoV-2 (Sundah et al. 2021). Catalytic amplification was used with a transition-state molecular switch to leverage DNA-enzyme hybrid complexes. The states of the molecular switch due to polymerase activity generated a fluorescence signal that detected specific binding of SARS-CoV-2 RNA on the modified surface. The limit of detection of the device was ~ 8 RNA copies/µL with a smartphone-based optical sensor, and the detection was completed in nearly 1 h at room temperature through the CATCH method.

There is also an innovative method that used hydrogels to prevent fluid movement in a microfluidic channel with hybridization of SARS-CoV-2 nucleic acids and hydrogel after rolling circle amplification (RCA) (Kim et al. 2021). The amplification of nucleic acids blocked the pores in hydrogel to prevent fluid flow and detection was made by observing the fluid characteristic (flow velocity, incubation time, traveling time) in the microfluidic channel. This system achieved a detection limit of 0.7 aM with 15 min incubation.

2.2 Indirect detection

Although it differs from patient to patient, the body usually starts to produce neutralizing antibodies against the virus after 6th day of PCR confirmation of SARS-CoV-2 (Suthar et al. 2020). While the median value of IgG in clinical samples is 0.32 AU/mL, the median value of IgM was 0.59 AU/mL in COVID-19 patients after 5–9 days of symptom onset (Soleimani et al. 2021). ELISA is the gold standard method in the detection of neutralizing IgG/IgM antibodies in the serum. Although it gives accurate results with high sensitivity, the experimental steps are laborious, reagents are expensive, and the samples collected from patients require transport to qualified test facilities (Van Elslande et al. 2020). Hence, microfluidic platforms to detect anti-SARS-CoV-2 antibodies have been designed and manufactured as rapid, reliable, sensitive, affordable, and easily applicable diagnostic tools. Microfluidic platforms designed for the indirect detection of SARS-CoV-2 antibodies were mostly based on fluorescence, chemiluminescence, colorimetric, electrochemical, and plasmonic detection principles.

Detection of antibodies is mostly achieved by the incorporation of serological immunoassays into microfluidic systems. As frequently used detection techniques, fluorescence-based assays rely on the presence of fluorescent-labeled antibodies in the immunoassay system (Lin et al. 2020). For instance, an immunoassay biochip was functionalized with graphene oxide quantum dots (GOQDs) which could detect both the SARS-CoV-2 antigens (S and N antigens) and neutralizing IgG/IgM antibodies with fluorescent agents (Wang et al. 2021a). The detection of IgG/IgM in the serum sample was based on the sandwich immunoassay principle via the binding process of fluorescent-labeled secondary antibodies with the IgG/IgM in the serum sample. The quantitative detection of a total of 60 samples of antigen and/or antibodies could be carried out simultaneously with low sample volume (2 µL), high specificity, and low detection limit (~ 0.3 pg/mL) under 10 min. In another study, a nano immunoassay was developed where the samples were collected with commercial blood test strips to avoid high volumes of blood sampling (Swank et al. 2021). The sampling was limited to finger-prick blood, and the sample volume needed was at the sub-microliter level (0.6 µL). Blood samples were processed and transferred to the microfluidic chip which could detect the anti-SARS-CoV-2 spike IgG antibodies with excellent specificity (100%) and sensitivity (98%) with a limit of detection around 1 nM for IgG. A semiautomatic microfluidic device was developed to comparatively evaluate the IgG and IgM antibody levels in the serum against four SARS-CoV-2 antigens: S, N, S1 subunit, and RBD (Rodriguez-Moncayo et al. 2021). The mechanically induced trapping of molecular interactions (MITOMI) technique was integrated into a microfluidic device. Activation of MITOMI technique using button valves on each microchamber allowed for the fluorescence detection of the binding of antigens immobilized on the surface of the microchambers with antibodies in the serum sample. The device could detect antibody levels in low-volume (6 µL) serum samples with a detection limit of 1.6 ng/mL for RBD protein. An integrated point-of-care design for serological quantitative analysis of circulating anti-SARS-CoV-2 IgG antibodies in the blood could monitor the seroconversion after infection and/or after vaccination (Rajsri et al. 2022). 3D agarose bead sensors, integrated into the microfluidic cartridges provided a larger surface area for immunocomplexes. Agarose beads were functionalized with antigens (recombinant SARS-CoV-2 WA-1 RBD protein) to capture and form immunocomplexes with anti-SARS-CoV-2 IgG antibodies in the sample. Secondary immunofluorescent antibodies were used to bind the immunocomplexes and fluorescent signals and analyzed for the detection of IgG antibodies. The system was able to detect anti-SARS-CoV-2 IgG antibodies in 15 min with a detection limit of 47 ng/mL. As a colorimetric detection technique for immunoassay-based systems, colloidal gold nanoparticle (AuNP)-based LFA was developed for the detection of IgM antibodies against SARS-CoV-2 (Huang et al. 2020). The N protein of SARS-CoV-2 was coated onto the device membrane to capture SARS-CoV-2 IgM using the conjugated AuNP-IgM as a detecting reporter. The complete detection time was 15 min with 100% sensitivity and 93.3% specificity.

Several immunoassay methods have been integrated with optical equipment and sensors for on-site detection (Heggestad et al. 2021; Lee et al. 2021). For instance, the glass surface

of an opto-microfluidic biosensing platform was coated with gold nanospikes which were further functionalized with SARS-CoV-2 S peptide to detect anti-SARS-CoV-2 IgG antibodies (Funari et al. 2020). Then, anti-SARS-CoV-2 IgG antibodies were introduced by a syringe pump into the microfluidic channel. Since antigen–antibody binding changed the peak wavelength of the localized surface plasmon resonance (LSPR) on gold nanospikes, the developed opto-microfluidic platform allowed the quantification of SARS-CoV-2 spike antibodies in the sera. The system was shown to be sensitive to IgG antibodies and had a limit of detection value of ~ 0.08 ng/mL. A microfluidic biosensor based on the Fresnel reflection method was also developed for sensitive and rapid detection of IgG and IgM antibodies against the SARS-CoV-2 spike protein (Xu et al. 2021). This microfluidic biosensor system could quantitatively detect the anti-SARS-CoV-2 IgG and IgM antibodies in 7 min with a limit of detection values of 0.45 ng/mL and 0.82 ng/mL, respectively. Moreover, a disposable bioassay system containing ring resonator photonic sensors made of silicon nitride was fabricated on a polymer micropillar chip and used for the detection of anti-SARS-CoV-2 antibodies by measuring the light intensity change (Cognetti et al. 2021). This microfluidic platform was able to detect total antibodies in only one minute with a sensitivity and specificity of 77.8% and 100%, respectively.

In another study, a 3D-printed COVID-19 test chip (3DcC) platform with an electrochemical cell was proposed (Ali et al. 2021). In this platform, electrodes were prepared by aerosol jet nano printing technology using gold nanoparticle ink. Electrode surfaces were coated with reduced graphene oxide (rGO) nanoflakes which were functionalized with antigens (S1 and RBD proteins) in different sensors. After introducing the serum sample in the microfluidic chamber, the detection of antibodies was conducted by sensing the impedance change on the electrical circuit caused by antigen–antibody binding. The system had a limit of detection values of 2.8×10^{-5} M and 16.9×10^{-15} M for anti-S1 and anti-RBD antibodies, respectively. The advantage of this system was the short detection time and the ease of analysis enabled by a smart phone.

ELISA is an immunoassay-based system that uses enzymes to detect viral traces through indirect methods. For instance, the colorimetric point of care ELISA system was designed for the detection of anti-N IgG of SARS-CoV-2 (Carrell et al. 2020). The N protein of SARS-CoV-2 was coated onto the microfluidic channel. Then, anti-N protein antibodies were bound to the N proteins and the horseradish peroxidase (HRP)-labeled secondary antibodies were captured by the anti-N protein antibodies. The enzymatic reaction between the tetramethylbenzidine substrate and HRP molecules caused the formation of a specific color. This color intensity was calculated for the quantitative detection with a 2.8 ng/mL limit of detection from whole blood. The total detection process took 20 min. A portable microfluidic system based on the chemiluminescent ELISA technique was used to detect the SARS-CoV-2 S1 specific IgG antibody in 15 min from 8 µL sample volume with a limit of detection value of 2 ng/mL (Tan et al. 2020). Another ELISA-based automated lab-on-a-chip platform with 4 microfluidic channels, which were functionalized with SARS-CoV-2 spike proteins, was also presented (González-González et al. 2021). After the introduction of the patient samples into the microfluidic channels, anti-IgG-HRP complexes were loaded to detect IgG in the serum samples. The binding reaction of IgG antibodies and the anti-IgG-HRP complexes with tetramethylbenzidine resulted in a colorimetric change in the medium which was photographed by a smartphone and the acquired images were analyzed.

The detection can also be made by particle accumulation with the help of microfluidics for immunoassay-based methods. The microfluidic "RapidQ" immunoassay chip system was designed for the detection of anti-SARS-CoV-2 S protein IgG antibodies in the serum via visualization of particle accumulation using paramagnetic beads (Mattila et al. 2022). The system was able to detect target antibodies in less than 10 min with a limit of detection value of 4 AU/mL. Similarly, a microfluidic immunoassay chip was designed for the detection of anti-spike RBD IgG antibodies via visual monitoring of the accumulation of magnetic nanoparticles on polystyrene microparticles. (Wu et al. 2022b). This microfluidic system could quantitatively detect the IgG antibodies with a limit of detection value of 13.3 ng/mL and 57.8 ng/mL in sensitive (70 min) and rapid mode (20 min), respectively.

Cytokine storm is one of the indications of SARS-CoV-2 infection and the rapid and sensitive detection of the cytokine levels in the blood is important for monitoring the severity of the disease and deciding on patient-specific therapy (Song et al. 2021). Digital microfluidic sandwich immunoassay using plasmonic nanoparticles could monitor the multiple cytokine-antibody binding in very low sample volume (3 µL) (Gao et al. 2021). Anti-cytokine antibody-coated microarrays on the microfluidic chip captured the cytokines (IL-1β, IL-2, IL-6, IL-10, TNF-α, and IFN-γ) and nanosilver-tagged detection antibodies bound to the cytokines. Bounded plasmonic silver nanocubes produced signals that was then visualized using a dark-field microscope. The signals were analyzed and counted via image processing with a convolutional neural network (CNN) algorithm. The system could detect the six cytokines with a limit of detection values of 0.91 pg/mL, 0.47 pg/mL, 0.46 pg/mL,1.36 pg/mL, 0.71 pg/mL, and 1.08 pg/mL for IL-1β, IL-2, IL-6, IL-10, TNF-α, and IFN-γ respectively. Also, a digital protein microarray called PEdELISA system was developed based on fluorescent immunoassay to detect cytokines (IL-6, TNF-α, IL-1β, IL-10) in the serum

in 30 min with a limit of detection values ranging between 0.19–0.38 pg/mL (Song et al. 2021).

Moreover, metabolic changes due to COVID-19 in the human body were examined using indirect detection (Bordbar et al. 2022). For this purpose, a microfluidic colorimetric sensor array was developed for the detection of the metabolic changes in the urine samples. The collected samples were examined by gold and silver nanoparticles, metalloporphyrins, metal ion complexes, and pH-sensitive indicators. The complete color pattern of the array took 7 min and this color change was calculated by image processing techniques. However, this technique should be tested for other viral and bacterial infections to prove the specificity of COVID-19 infection.

3 Microfluidic methods for vaccine development

Vaccines are biological substances produced to create an immune response against infections or diseases. The use of vaccines has a great impact on world health for preventive and therapeutic purposes, as evidenced by the decreased the mortality rates from both infectious and non-infectious diseases in vaccinated populations (Greenwood 2014). Vaccines are generally classified based on their ability to replicate in the host or the technology used in their production, and these classifications can be subdivided into 3 generations:

- *First-generation vaccines* include vaccine types that contain the whole pathogen in an attenuated or inactivated form,
- *Second generation vaccines* include vaccine types that consist of an immunogenic part of the pathogen instead of the complete pathogen,
- *Third-generation vaccines* include vaccine types that contain the genetic material encoding antigens with immunogenic potential.

Detailed descriptions of various vaccine technologies are outside the scope of this review but are extensively covered elsewhere (Ghattas et al. 2021; Tahamtan et al. 2017). The upstream processing (production), downstream processing (purification), and formulation sections, which are the vaccine development stages followed by the tests on animal models and clinical trial phases, vary considerably based on vaccine type (Josefsberg and Buckland 2012). Microfluidic technologies are mainly used in the formulation phase, with the aim of improving the immunogenicity and stability of the vaccine, especially in the production of lipid nanoparticles and liposomes as vaccine delivery particles (Fig. 2a, b).

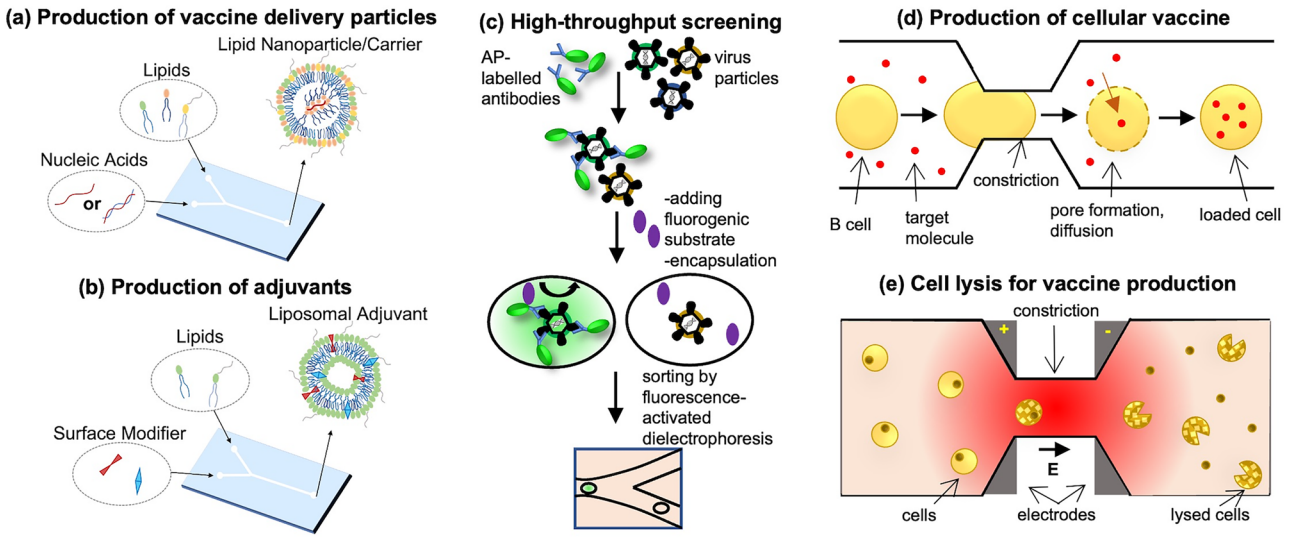

Fig. 2 Microfluidic applications for vaccine development. **(a)** Production of lipid nanoparticles or carriers for vaccine delivery. The microfluidic device basically consists of inlets for lipids and nucleic acid solutions. Two solutions are mixed together to synthesize nucleic acid-loaded lipid nanoparticle/carrier. **(b)** Production of adjuvants. In the synthesis of liposomal adjuvants, surface modifiers used for enhancing antigen adsorption on liposome/liposome stability, the mixture of lipid solution and surface modifiers are injected into the microfluidic device. **(c)** High-throughput screening of virus particles. Virus particles are incubated with alkaline phosphatase (AP)-labeled broadly neutralizing antibody. The particles are encapsulated individually in droplets, together with a fluorogenic substrate in the droplet-based microfluidics platform. Highly fluorescent droplets are sorted by fluorescence-activated droplet sorting. Based on (Chaipan et al. 2017). **(d)** Production of cellular vaccines. Microfluidic cell squeezing device consisting of parallel channels to pass single cells through narrow constrictions provides a robust method for antigen uptake into B-cells as cellular vaccines. Based on (Szeto et al. 2015). **(e)** Cell lysis for vaccine production. Electrical cell lysis micro-device provides high-throughput and continuous production of cell-free viruses by micro-electrodes and constriction nodes. Based on (Won et al. 2021)

Nucleic acids such as mRNA, small interfering RNA (siRNA), and DNA are used for gene-therapy and therapeutic agents in vaccine development (Hou et al. 2021; Yin et al. 2014). For the vaccination of COVID-19, mRNA-based vaccine studies have become increasingly important due to their ease of production, induction of better immunogenic response, and adaptability to new variants (Fang et al. 2022) However, negatively charged mRNA molecules need a carrier for delivery and strong protection from enzymatic activity during extracellular and intracellular transportation (Mendes et al. 2022). For this purpose, a wide range of carriers (e.g., nanoparticles, liposomes, polymer complexes, micelles, and cationic peptides) have been developed for delivery of mRNA vaccines (Chaudhary et al. 2021; Li et al. 2022a).

Vaccines and therapeutic agents can be produced, manipulated, transported, and transferred on droplet-based chips, where the necessary chemicals and molecules are trapped and act as a carrier reactor (Misra et al. 2021). Moreover, droplet-based systems can be applied to a broad range of molecular virology applications such as virus-host sequencing, virus-host culture, viral enrichment and sequencing, and fusion kinetics for enhancing specific vaccine studies (Jing and Han 2022). In a related study, a library of locally matched antibodies from two different donors was generated using a microfluidic droplet-based platform. B cells were maintained individually in droplets containing oligo-dT beads and lysis buffer (Zhou et al. 2021a). The mRNA capture beads were then separated from the droplets and new droplets were obtained, providing a medium for PCR amplification. The products formed as a result of the amplification were determined by adding enzyme sites specific to the target transport sites.

Lipid nanoparticles (LNPs)-based nucleic acid vaccines have a great potential for acquiring world-wide immunity to COVID-19 due to their rapid and efficient production (Papi et al. 2022). For vaccine studies, LNPs having controllable and homogeneous particle size distribution are generated using microfluidic mixing technologies, such as staggered herringbone micromixer (SHM), high-pressure micromixer, flow-focusing micromixer, and invasive lipid nanoparticle production microfluidic system (iLiNP)(Prakash et al. 2022). For instance, < 100 nm of LPNs with homogeneous size distribution were produced with over 100-fold production rate in a single microfluidic chip including SHM arrays for siRNA and mRNA delivery. During this process, rapid and controllable mixing (< 10 ms) enabled high reproducibility and high encapsulation rate (Shepherd et al. 2021b). In another study, mRNA loaded-LNPs were synthesized with iLiNP microfluidic device. The flexibility of different synthesis parameters such as lipid concentrations, flow rate ratio, and total flow rate provided the formation of the desirable size of LNPs (> 200 nm) (Okuda et al. 2022).

Liposomes, as self-assembled lipid bilayers in aqueous media, are preferred in gene therapy and vaccine developments due to their structural similarity to the human cell membrane (Balazs and Godbey 2011; Felgner et al. 1994). Using microfluidic flow focusing platform, liposomes in the nano scale (diameter range 50–150 nm) are formed with a controlled flow rate and convective diffusion of liquid solution (Jahn et al. 2010). In a similar study, plasmid DNA-cationic liposome complexes with desirable physicochemical and gene delivery properties were continuously produced with a simple straight hydrodynamic flow focusing device (Balbino et al. 2013). Moreover, the microfluidic flow focusing method allows the sensitive control of both particle size and polydispersity. For example, utilizing a vertical flow-focusing device (VFF), 80–200 nm size ranges of uniform liposomes were automatically produced, and VFF-generated liposomes displayed lower distribution populations, originated from low polydispersity and high aspect ratio (Hood and Devoe 2015). The protein-loaded liposomes are also important for therapeutic and vaccine purposes. In order to enhance lymphatic targeting, ~ 90 nm size of biotinylated liposomes was formed with a SHM (Khadke et al. 2019).

Another important field among vaccine delivery technologies is controlled vaccine release, both to overcome the difficulty of repeated administration and to boost immunization. Unlike standard techniques such as emulsion-template methods, microfluidic technology provides the advantage of customizable size distribution and release rate. For example, uniform core–shell alginate microparticles were produced with a simple microfluidic system containing four inlets, and two strategies were defined to increase protein retention efficiency (Yu et al. 2019). Microparticles were coated with oppositely charged polymers as a diffusion barrier and small particles were added inside the core to block the pores of the alginate network. Another microfluidic setup allowed the fabrication of poly(DL-lactide-*co*-glycolide) (PLGA)-based core–shell microspheres that could adjust the lag time to 3–7 weeks to provide delayed pulsatile antigen release (van der Kooij et al. 2021). Besides vaccine delivery, microfluidic technologies have the potential to be used for the production of liposomal adjuvants that induce an immune response to the vaccine (Schmidt et al. 2020). Compared to traditional batch-scale methods, microfluidic-based liposomal adjuvants have similar immune activity, and physicochemical and pharmacokinetic properties with the traditional methods such as the hydration method and high shear mixing (Roces et al. 2019). Microfluidic-prepared 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) nanoparticles (< 100 nm) was considered as a promising vaccine adjuvant as it greatly enhanced the T-cell response (Haseda et al. 2020). The adjuvant activity of cationic lipid DOTAP was enhanced both by producing it in small sizes that could not be achieved by conventional methods and by combining it with immune potentiator Type-A CpG oligodeoxynucleotide.

Microfluidic technologies also have the potential to be applied in the design and production stages of vaccines

(Fig. 2c-e). A single-virus droplet-based microfluidics platform was described to screen and sort millions of viral particles based on the expression of epitopes recognized by neutralizing antibodies for optimal antigenic features of vaccine candidates (Chaipan et al. 2017). Although fluorescence-activated cell sorting is a standard technique for analyzing surface epitopes, novel techniques are still needed for single-virus measurements due to very small size of viral particles and the low amount of surface proteins. This microfluidics platform allowed viral particles displaying neutralizing epitopes to be enriched ~ 1900-fold in a single sort, providing a new opportunity to screen virus libraries for vaccine candidates. Another example of the application of microfluidic technology in vaccine production is intracellular antigen loading into B cells by passing single cells through narrow constrictions for use as a cellular vaccine (Szeto et al. 2015). While other techniques, such as specific receptor targeting for endocytic uptake, are often affected by changes in B-cell state and by the inability to individually adjust antigen loading, the microfluidic cell squeezing device enables robust loading of antigens to the cytosol of resting or activated B-cells via mechano-poration. There is also an attempt to apply microfluidic technology at the stage of cell lysis at the beginning of the downstream process (Won et al. 2021). Since even standard techniques such as sonication are, in most cases, not sufficient for obtaining cell-free virus, a microfluidic cell lysis system containing microchannel with micro-electrode arrays has been developed to disrupt large numbers of virus-infected cells with high-throughput.

Microfluidic devices and technologies, that have recently been applied in various stages of vaccine production, promise size-controlled and highly reproducible delivery of vaccine particles, allowing better immunogenicity and stability of the vaccine components, high-throughput vaccine production, and candidate screening. To date, there are approximately 300 COVID-19 vaccines that are in the development stage, ranging from nucleic acid to virus-like particles, and it is anticipated that more and more benefits will be gained from the aforementioned advantages of microfluidic technologies in several stages of development.

4 Microfluidic methods for therapeutics

In this section, we summarize the current and potential use of microfluidic devices for accelerating the discovery of antiviral drugs and controlling their site-specific delivery and release, with a special focus on COVID-19 (Fig. 3).

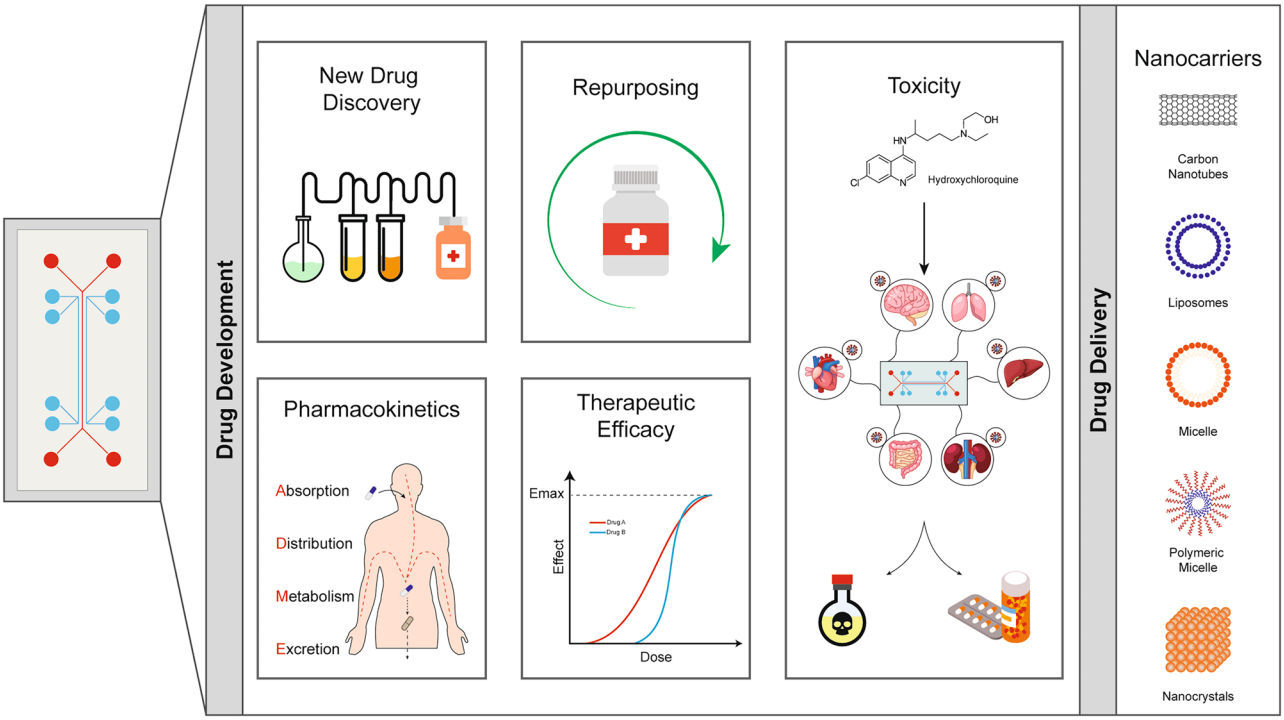

Fig. 3 Microfluidics as an enabling technology for drug development and delivery. The role of microfluidics in pharmacological applications such as identifying and testing potential drug molecules for their therapeutic efficacy, toxic effects, interactions with biological sites and distribution in the body following administration, as well as their controlled delivery and release to infected tissues using nanoparticles as transporters (so-called nanocarriers)

4.1 Microfluidic devices in new drug discovery and repurposing

Microfluidic systems are well suited for high-throughput experimentation and automated multiplexed applications due to their small size, portability, and low production cost. In particular, organ-on-a-chip systems can help lower the cost and time requirements of the drug discovery and development processes in line with the *fail early, fail cheaply* strategy adopted by the pharmaceutical industry. They provide various advantages at specific stages of the antiviral drug discovery pipeline when compared to traditional cell culture systems that often fall short in accurately representing and modeling cell behavior due to the absence of mechanical forces that drive cellular responses (Convery and Gadegaard 2019). While the use of microfluidic cell culture devices in the highly regulated health industry has witnessed both successes and setbacks, the promise of developing new antiviral drugs with the use of microfluidic-assisted technologies remains mostly conceptual rather than practical.

Unlike previously known coronaviruses that are capable of causing respiratory tract infections, the most recent strain of coronavirus (SARS-CoV-2) that causes COVID-19 is suspected of causing neuronal (Baig 2020) and muscular system malfunctions (Gusev et al. 2021). In particular, severe alterations of left ventricular function and serious heart problems were observed in COVID-19 patients with no previous history of cardiac disease (Inciardi et al. 2020). Biopsy findings of COVID-19-infected patients showed clinically meaningful associations between the infection and endothelial dysfunction (Rovas et al. 2021). Persistent multisystem abnormalities were observed in severe cases of COVID-19 illness and death (Morrow et al. 2022; Zaim et al. 2020). Such multi-organ involvement in COVID-19 complicates the early drug development process which needs to be tailored according to the stage of disease and the signs of organ dysfunction. High-throughput systems that allow parallel experimentation are urgently needed for the rapid development of new COVID-19 therapeutics and the repurposing of existing drugs for COVID-19. Drug repurposing that describes alternative uses of existing drugs for new therapeutic purposes allows bypassing a large portion of the time and cost associated with traditional drug development and ultimately, delivers safe and effective treatment to patients faster and at a lower cost.

The potential of microfluidic chips for speeding up the antiviral drug development process has already attracted huge interest from the scientific community and the pharmaceutical industry. For example, Zhou and co-workers developed a lipid bilayer-based lab-on-a-chip device integrated with embedded ACE2 receptors and tested its performance for screening the effects of two drugs, HD5 peptide, and hexapeptide, on blocking the binding of SARS-CoV-2 spike protein to ACE2 (Zhou et al. 2022). A microfluidic-based cardiac microphysiological system was developed for drug screening applications (Mathur et al. 2015). With the emergence of COVID-19, they shifted their focus on predicting cardiotoxicity associated with two particular drugs, hydroxychloroquine, and azithromycin, that are currently being evaluated as potential treatments for COVID-19 (Charrez et al. 2021).

A human airway chip was developed and tested for its potential use in identifying existing drugs that could serve as antiviral therapeutics against COVID-19 (Si et al. 2021a, 2021b, 2020). Their initial tests with drugs suggested that the antimalarial drug amodiaquine significantly reduced infection by a pseudotyped SARS-CoV-2 virus (Si et al. 2020). In their follow-up study, they tested the potential use of eight approved drugs as SARS-CoV-2 entry inhibitors and reported that the active metabolite of amodiaquine, desethylamodiaquine, reduced the entry of SARS-CoV-2 pseudoparticles by about 60% when administered in the human airway chip (Si et al. 2021b). It should be emphasized that these studies can be viewed as a proof-of-concept only since they prove activity against pseudotyped SARS-CoV-2 virus, not the real one, and significant work remains before using organ chips for testing existing drugs with repurposing potential against COVID-19.

4.2 Microfluidic devices in antiviral drug delivery

One of the major problems in drug delivery systems that are currently used in clinical practice is that drugs are administered in a way to interact with the entire body, not only the infected tissues, causing the distribution of administered drugs to unintended organs. The next generation of delivery approaches focuses on ensuring the 'safe' travel of drugs within the body by controlling the delivery of a certain amount of drugs (therapeutic dose) to specific parts of the body (Bae and Park 2011; Singh and Lillard Jr 2009). The ability of microfluidic devices to precisely direct liquid flows and replicate the functionalities of living organs makes them suitable for advanced drug delivery applications. Unlike conventional drug delivery methods that suffer from limited targeting, microfluidic platforms maximize the concentration of drugs that reaches the desired tissue by shortening intake pathways (Nguyen et al. 2013; Sanjay et al. 2018; T Sanjay et al. 2016). The main role of microfluidics in drug delivery systems is the development of nanocarriers for personalized and generic therapeutics.

Nanocarriers are multifunctional nanoparticles that are loaded with drugs and decorated with a targeting ligand that binds to the receptor on the surface of infected cells. As a drug delivery system, nanocarriers offer several advantages such as improving the solubility and stability of encapsulated drugs, facilitating transport across bio-barriers, and enabling cell-specific targeting (Chamundeeswari et al.

2019; Girdhar et al. 2018). Despite the potential benefits of nanoparticle-based drug carrier systems, the clinical translation of the first generation of nanocarriers (liposomes and lipid nanoparticles) was very limited due to the rapid clearance from the body after getting trapped in the liver and/or spleen (Sercombe et al. 2015). In response to the short circulation time in blood, liposomes were incorporated with the hydrophilic polymer polyethylene glycol (PEG) to achieve steric stabilization and prolonged circulation time (Gref et al. 1995; Lasic et al. 1999). Later on, to further improve the performance of liposomal drug carriers, nanoparticles were functionalized with targeting molecules such as monoclonal antibodies, peptides, and carbohydrates (Sercombe et al. 2015). As described in Sect. 3, ionizable lipid nanoparticles are successfully engineered and modified in a way to ensure the effective and safe delivery of COVID-19 mRNA vaccines (Nogueira et al. 2020; Polack et al. 2020; Sahin et al. 2014). While the most prominent technological advances have been made in the field of RNA delivery, the same approach can potentially be adapted for the targeted delivery of COVID-19 therapeutics to the infected cell and tissues. Droplet-based nanocarriers that are produced in microfluidic systems can potentially be used to encapsulate therapeutic agents and to deliver the encapsulated molecules to the target area through the controlled flow of the carrier liquid (Escobar and Xu 2022; Kopp et al. 2020). Droplet-based techniques also help us to understand the prevention of airborne transmission of COVID-19, its infectiousness and viral load rebound (Mohammad Sadeghi et al. 2020; Bisag et al. 2020).

To further increase the efficiency of nanoparticle formulations in delivering antiviral drugs to the infected area, microfluidic platforms can be used for the controlled synthesis and modification of lipid-based nanocarriers. An example of this approach demonstrated the use of an in-house microfluidic device for the controlled synthesis of lipid nanoparticles with pre-defined physical characteristics and high drug encapsulation efficacy (Shepherd et al. 2021a). Besides, a microfluidic system was developed that enabled size-controlled and RNA-loaded synthesis of lipid nanocarriers (Maeki et al. 2022). In a similar study, a microfluidic mixer system was used for the controlled synthesis of lipid nanoparticles and demonstrated its use for the co-delivery of a model cancer drug, doxorubicin, and small interfering RNA (Butowska et al. 2022).

A custom-built microfluidic device was used for fabricating inhaled ACE2-engineered microspheres and demonstrated its use for the treatment of COVID-19 (Wang et al. 2022). They engineered vesicular microspheres from ACE2-expressing cells and macrophages treated with lipopolysaccharide and interferon-γ and demonstrated its effectiveness in the neutralization of SARS-CoV-2. A new microfluidic-based technology was developed by infusing oxygen into a liquid solution which was then turned into micro-bubbles, got coated with a membrane, and delivered to the bloodstream intravenously (Vutha et al. 2022). Although not tested on humans yet, this proof-of-concept study suggests that such microfluidic-assisted systems can help reduce severe COVID-19-led symptoms such as hypoxemia (where oxygen levels are too low) experienced by patients on ventilators. While the use of microfluidic-assisted drug delivery systems for COVID-19 has not turned into a clinical reality yet, multifunctional nanocarriers and similar therapeutic interventions will soon become a part of routine antiviral therapy and clinical care.

5 Discussion

The COVID-19 pandemic reminded us of the importance of rapid diagnostic testing to reduce the burden of laboratories and to provide timely treatment for patients with positive test results. Diagnostic assays for SARS-CoV-2 can be classified into two groups: direct detection methods that assess the presence of viral components in the tested sample and indirect methods that look for SARS-CoV-2-specific immune responses and metabolic changes at the time of the test. Microfluidic devices are well-suited for fast and low-cost detection of viral infections and hence, have been extensively applied for the detection of SARS-CoV-2. They play an integral role in existing COVID-19 detection platforms. Microfluidic-enabled diagnostic tests for COVID-19 are summarized in Table 1, together with the type, assay time, genetic/protein target, sensitivity, and specificity of each technique. As can be seen from Table 1, the time to perform a diagnostic test generally varies between 30 min to 1 h. While the detection time can be lower than 15 min for some tests, it is not possible to link the assay time with particular tests as it seems to vary depending on multiple factors. In some studies, the lowest concentration of detectable target could be as low as a few femtograms to nanograms (or few nucleic acid copies for nucleic acid-based detections) per microliter (Table 1). This suggests that even a small amount of relevant biomarkers could be sufficient to detect infected people. In addition, the increase in the detection signal and the linearity of this increment depending on the sample concentration was clearly shown in the studies. This linear relationship can be the evidence that quantitative measurements and high linearity show the power of quantification. The issue of whether these techniques provide quantitative or qualitative results is rather complicated due to the absence of common terminology and criteria related to what is considered ‘quantitative enough’ in the diagnostic domain. Although indirect methods do not offer as sensitive diagnosis as direct methods, the sensitivities and specificities of most of those techniques are as high as 90–100%.

When the direct methods are compared according to all criteria, it is observed that CRISPR is more advantageous compared to other methods with respect to the limit of detection and sensitivity. Hence, its use could expand in the future for direct methods (Chertow 2018; Feng et al. 2021; Kaminski et al. 2021; Otten and Sun 2020). However, CRISPR applications often require fluorescence detection and complex setups that are not practical for point-of-care applications. Since electrochemical assays provide sensitive detection for the direct detection of SARS-CoV-2 proteins, they are proven methods in point-of-care applications. However, complex surface preparation steps and assay protocols can limit their use in certain applications (Simoska et al. 2021). Moreover, LAMP allows cost-effective, isothermal, simple, and naked-eye detection which makes this method well-suited for point-of-care applications (Agbaje 2022). Since automation and standardization of LAMP protocols are not fully achieved, further developments are necessary before the potential of point-of-care testing approaches can truly be realized in clinical settings.

In indirect methods, a low amount of IgG/IgM can be detected using LSPR methods as seen in Table 1. However, LSPR methods are complex and require costly detection schemes (Xu and Geng 2021). LFA, on the other hand, is one of the most useful methods for point-of-care applications, particularly in immunoassay detection (Danks and Barker 2000; Hsiao et al. 2021). The EU commission has recently compared the commercially available microfluidic-based test devices/kits and reported the use of LFA-based immunoassay methods to be quite popular (European Commission 2022). Moreover, cytokines (Wang et al. 2020b) or metabolic changes (Bordbar et al. 2022) could also be potentially used for COVID-19 detection. However, their selectivity and sensitivity need to be studied further.

The performance of microfluidic-assisted diagnostic instruments is highly dependent on the fabrication process (chip material, fabrication methodology, etc.), sample handling, and assay components/steps (sample extraction, fluid operations, heating, etc.) (Clime et al. 2019; Nielsen et al. 2020; Park et al. 2020; Zhao et al. 2020). This dependency further highlights the importance of standardization and optimization of these instruments to maximize their utilization and performance. The majority of the existing microfluidic diagnostic tools except LFA are still at the R&D stage, and not yet commercialized, due to the need for skillful operation and the absence of standardized microfluidic operations (Tayyab et al. 2020). Considering the potential benefits these instruments can provide when fighting against pandemics, their clinical use for point-of-care applications is likely to expand in near future.

Microfluidic technologies may be applied to improve vaccine effectiveness and production efficiency in many stages, from the vaccine design to vaccine stability and immune response-enhancing formulation stage. Microfluidic technologies provide rapid and high-throughput production of carriers, based on controlled physical parameters such as flow rate (Okuda et al. 2022; Shepherd et al. 2021b). Furthermore, using a small volume of reagents and automation in microfluidics could enable easy scale-up (Walsh et al. 2014). Future research may focus on the utilization of microfluidic technologies for the (1) screening of diverse virus libraries for potent vaccine candidates, (2) delivery of complex mixtures of proteins into cells for novel cellular immunotherapies, (3) efficient production of cell-free virus in a continuous manner, (4) discovery of novel adjuvants and delivery particles, and (5) their reproducible production.

Microfluidic-based methods could also be used for measuring the effectiveness of the vaccine based on antibody reactivity, and antibody and protein levels in serum after vaccine administration, as well as the vaccine development stages (Cognetti and Miller 2021; Rodriguez-Moncayo et al. 2021). Hence, the indirect methods presented in Table 1 could be good candidates for analyzing vaccine efficacy. Besides, nanotechnology allows us to better mimic the natural interaction between virus and immune system at the nano level and is effectively applied in vaccine and drug delivery. For instance, nanocarriers and adjuvants for mRNA and DNA vaccines (Entos Pharmaceuticals 2022; Rauch et al. 2021), and the self-assembly of protein nanoparticles for subunit and epitope-based vaccines (Joyce et al. 2021), could be used for COVID-19 vaccine development.

The ability of microfluidic systems to allow parallel experimentation makes them highly suitable for the drug development process. In particular, they can be used for the rapid development of novel COVID-19 therapeutics or repurposing of the existing ones. While the potential use of microfluidic platforms at different stages of drug discovery and development has received considerable attention over the past decade, their clinical translation and use have not been fully optimized. Similarly, the use of microfluidic-assisted drug delivery solutions is still very limited beyond the academic community. Nevertheless, as with all new technologies, microfluidic systems come with new challenges that the most important stakeholders in modern-day health care, the pharmaceutical industry, and the clinicians, need to adapt. With the advances in biomedical microfluidics, it is only a matter of time before these platforms make their way to routine clinical practice.

Resolving the current COVID-19 pandemic and future threats requires the development of novel platforms that integrate advances in materials science, microfabrication techniques, detection schemes, and data sciences (Kumar et al. 2022; Tang et al. 2020). The recent advances in medical informatics, artificial intelligence (AI), control systems, internet of things (IoT), decision support systems, and data processing integrated with microfluidics will ultimately support the efficiency and effectiveness of diagnostic tools

and treatment strategies, and vaccine development (Chen and See 2020; Egorov et al. 2021; Kumar et al. 2020). Literature findings confirm that more precise and robust results in diagnostic studies can be obtained with the support of AI technologies (Bhuiyan et al. 2022). With robotic systems, automation, and flow profiles have become highly controllable and manipulatable for microfluidics (Egorov et al. 2021; Karakuzu et al. 2022). Thanks to wireless communication technologies, all data communication can be provided remotely (Guo et al. 2021). IoT devices and sensor technology could be integrated with microfluidics to provide remote and instantaneous on-site monitoring (Alyafei et al. 2022; Escobedo et al. 2020). With these features, the harmonized use of microfluidics and information technologies will help us to develop smarter systems and fight infectious diseases more confidently in the future. Standardized and automated sampling, handling, manipulation, fabrication, and measurement procedures will be implemented for further applications to avoid the problem of standardization and automation in microfluidics (Yin et al. 2022a). Advancing microelectronics technologies, nanotechnology, and the use of microfluidics in current practices will make us more vigilant in the fight against the pandemic for the foreseeable future (Chen et al. 2021; Rahman et al. 2022; Weiss et al. 2020).

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Declarations

Competing interests The authors declare no competing financial interest.

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