

## Perspective

## Fighting COVID-19: Integrated Micro- and Nanosystems for Viral Infection Diagnostics

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The pandemic of coronavirus disease 2019 (COVID-19) highlights the importance of rapid and sensitive diagnostics of viral infection that enables the efficient tracing of cases and the implementation of public health measures for disease containment. The immediate actions from both academia and industry have led to the development of many COVID-19 diagnostic systems that have secured fast-track regulatory approvals and have been serving our healthcare frontlines since the early stage of the pandemic. On diagnostic technologies, many of these clinically validated systems have significantly benefited from the recent advances in micro- and nanotechnologies in terms of platform design, analytical method, and system integration and miniaturization. The continued development of new diagnostic platforms integrating micro- and nanocomponents will address some of the shortcomings we have witnessed in the existing COVID-19 diagnostic systems. This Perspective reviews the previous and ongoing research efforts on developing integrated micro- and nanosystems for nucleic acid-based virus detection, and highlights promising technologies that could provide better solutions for the diagnosis of COVID-19 and other viral infectious diseases. With the summary and outlook of this rapidly evolving research field, we hope to inspire more research and development activities to better prepare our society for future public health crises.

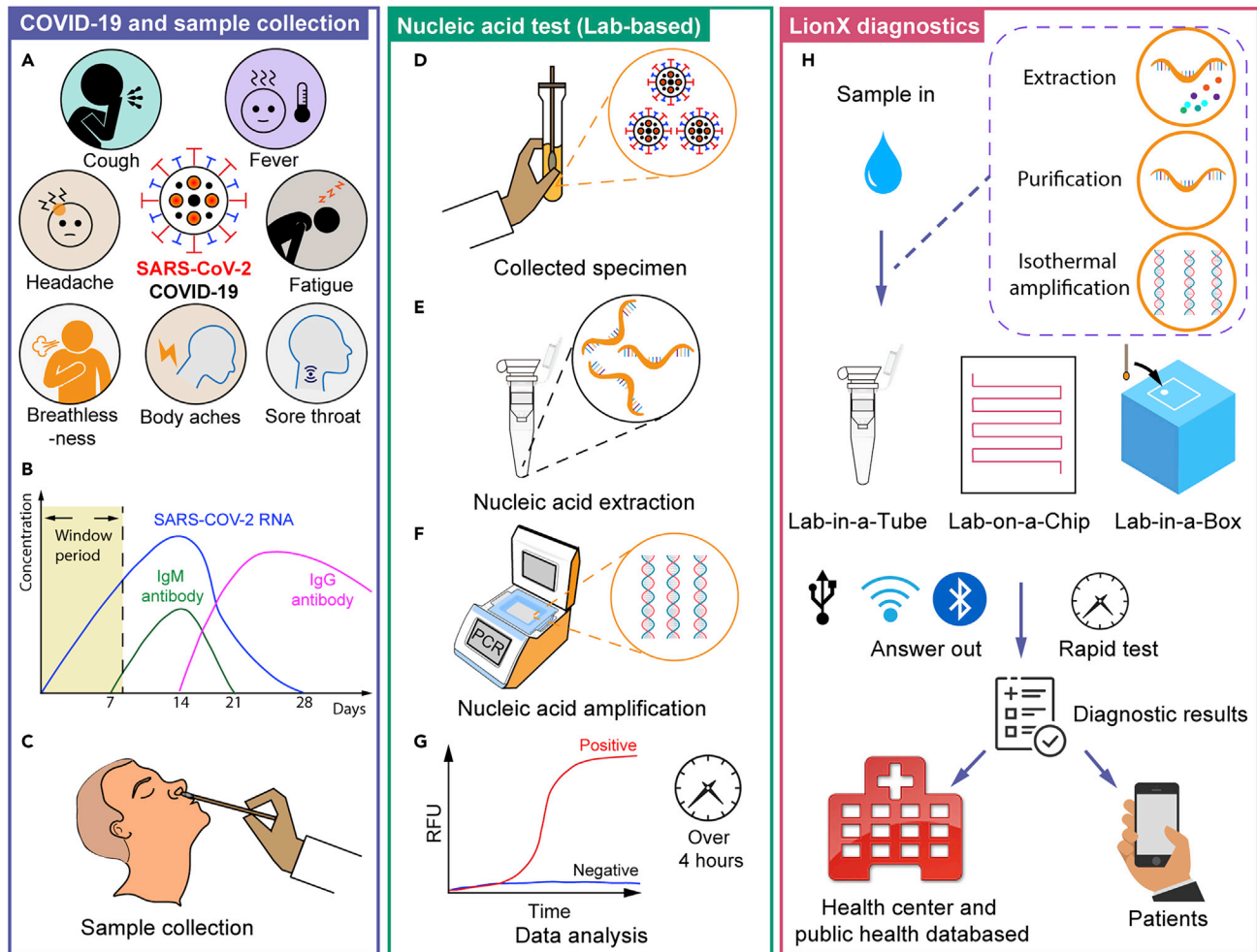
## INTRODUCTION

The current pandemic of coronavirus disease 2019 (COVID-19) has affected 217 countries and territories around the world<sup>1</sup> and has significantly affected everyone's life as well as the global economy. As of June 5, 2020, 6.52 million people have been confirmed infected and 387,000 people have died because of COVID-19.<sup>2</sup> Caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the COVID-19 spreads rapidly and causes deadly symptoms (10-fold more deadly than the 2009 swine influenza).<sup>3</sup> The reported infection cases share similar initial symptoms such as fever, cough, myalgia, or fatigue (Figure 1A), and many cases further developed pneumonia and severe respiratory diseases such as acute respiratory distress syndrome.<sup>4</sup> Considering the disease's high transmissibility ( $R_0$ : 1.4–5.5),<sup>5,6</sup> the early testing of SARS-CoV-2 infection is essential; it is also highly critical to trace cases and implement necessary measures against the spread of COVID-19. Patients infected with SARS-CoV-2 express nonspecific symptoms similar to those of other types of respiratory infections;<sup>6</sup> therefore, suspected cases must be screened through specific diagnostic methods.

There have been three main diagnostic methods widely used in the current pandemic: nucleic acid (NA)-based testing, computed tomography (CT) chest scan, and serological testing. NA-based testing, primarily based on RT-PCR, directly targets signature regions

## Progress and Potential

The development of integrated molecular diagnostic platforms based on state-of-the-art micro- and nanotechnologies has greatly improved both laboratory-based and point-of-care testing of the coronavirus disease 2019 (COVID-19). Enabled by recent advances in molecular biology, analytical chemistry, microfluidics, nanotechnology, and biomedical instrumentation, these integrated micro- and nanosystems hold significant potential to provide better solutions for diagnosis of COVID-19 and other viral infectious diseases in epidemic and pandemic situations. By summarizing the state-of-the-art molecular diagnostic systems and highlighting the promising technologies that need further development, this Perspective intends to spark further research and development activities in this critical research area.



**Figure 1. The Diagnosis of COVID-19 with Laboratory-Based and POC Tests**

(A) The symptoms of SARS-CoV-2 infection.

(B) Schematic illustration of SARS-CoV-2 and the antibody concentrations within an infected human body changing with time.<sup>7–9</sup>

(C) Nasopharyngeal sample collection from a patient.

(D) The collected specimen stored in a sample tube.

(E) Virus RNA extraction.

(F) PCR-based RNA amplification in a thermal cycler.

(G) Schematic illustration of the fluorescent signal readout during the amplification process.

(H) The testing process with lab-in/on-an-X (LionX) systems for diagnosis of COVID-19.

of the SARS-CoV-2 sequence and could, in principle, provide high sensitivity and specificity. Thus, NA-based detection of SARS-CoV-2 has become the gold standard for COVID-19 diagnostics.<sup>10–12</sup> The CT chest scan has also been used for COVID-19 diagnosis in China. It provides relatively high sensitivity (86%–98%) but low specificity (~25%) because the symptomatic features of the CT scan are similar to those of other types of viral pneumonia.<sup>12</sup> Serological tests detect antibodies generated by our immune system against SARS-CoV-2, which can confirm infection or previous exposure to the virus.<sup>13</sup> However, after SARS-CoV-2 infection, it usually takes a week or two for the body to produce a detectable amount of antibodies (Figure 1B). Thus, serological testing is not effective for early diagnosis of COVID-19 but more suitable for assessing infected populations. Until now, the serological testing is only recommended by the World Health Organization for research purposes but not for clinical diagnostics.<sup>14</sup>

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For NA-based testing (Figures 1C–1G), the rapid genome sequencing of SARS-CoV-2 strains has led to the development of many RT-PCR kits with 11 approved by the United States Food and Drug Administration (FDA) and more than 100 products under registration.<sup>10</sup> The commercial RT-PCR kits for COVID-19 testing are summarized in the recent literature<sup>4,13</sup> and online resources.<sup>15</sup> There has been a growing list of commercial COVID-19 diagnostic systems regulatorily approved by different countries for both laboratory-based and point-of-care (POC) testing.<sup>16,17</sup> Table S1 lists the commercial COVID-19 diagnostic products approved by the US FDA (through Emergency Use Authorization)<sup>17</sup> and Health Canada.<sup>16</sup> The commercial diagnostic systems greatly benefit practices of first responders, aid in immediate risk evaluation of on-site events, and thus play a crucial role in fighting the COVID-19 pandemic.<sup>18,19</sup>

In public health crises such as the current pandemic, an ideal diagnostic platform is expected to be fully integrated, autonomous, and high-throughput, and to be able to provide accurate results in short turnaround time (TAT).<sup>20,21</sup> For POC testing, the diagnostic platform may also need to be portable, easy to operate, and resource/infrastructure independent. The commercial diagnostic systems (Table S1) possess many of the aforementioned features such as high throughput, high integration level, and short TAT;<sup>22</sup> however, there are still shortcomings of these commercial products that prevent them from fully meeting the diagnostic needs during the pandemic, such as the involvement of manual operations, insufficient sensitivity, and the dependence on critical consumable supplies (e.g., nasal swabs and RNA extraction columns).<sup>23,24</sup> In the past two decades, intensive research efforts have been spent on developing integrated micro- and nanosystems for disease diagnostics.<sup>25,26</sup> Leveraging interdisciplinary expertise in molecular biology, analytical chemistry, microfluidics, nanotechnology, and biomedical instrumentation, significant advances have been made to develop new types of diagnostic platforms with many unique merits that promise better solutions for COVID-19 diagnostics.

This Perspective aims to summarize the state of the art of integrated micro- and nanosystems for NA-based virus detection, and highlight promising diagnostic platforms and technologies that could significantly facilitate the diagnostic practices during and after the current pandemic, and, more importantly, better prepare our healthcare systems for future crises. There have been several timely reviews and editorial articles recently published on diagnostics, therapeutics, and vaccine development of COVID-19.<sup>4,13,20,27–29</sup> This Perspective focuses on the state-of-the-art platform technologies integrating micro- and nanocomponents for the diagnosis of viral infections such as COVID-19. Targeting a high level of hardware integration and automation, many diagnostic systems have been developed and conceptually presented in forms such as “lab-on-a-chip,” “lab-in-a-tube,” and “lab-in-a-box”. We focus our review on these “lab-in/on-an-X” or “LionX” platforms and discuss all technical components (e.g., sample preprocessing and NA extraction, amplification, and signal detection) required to realize NA testing on these integrated systems (Figure 1H). Given the current situation and long-term projection of the COVID-19 pandemic, we hope that this Perspective will provide useful information on the integrated micro- and nanosystems applicable to virus detection, and further accelerate the translation of these LionX diagnostic platforms into practical frontline tools to tackle COVID-19 and other viral infectious diseases.

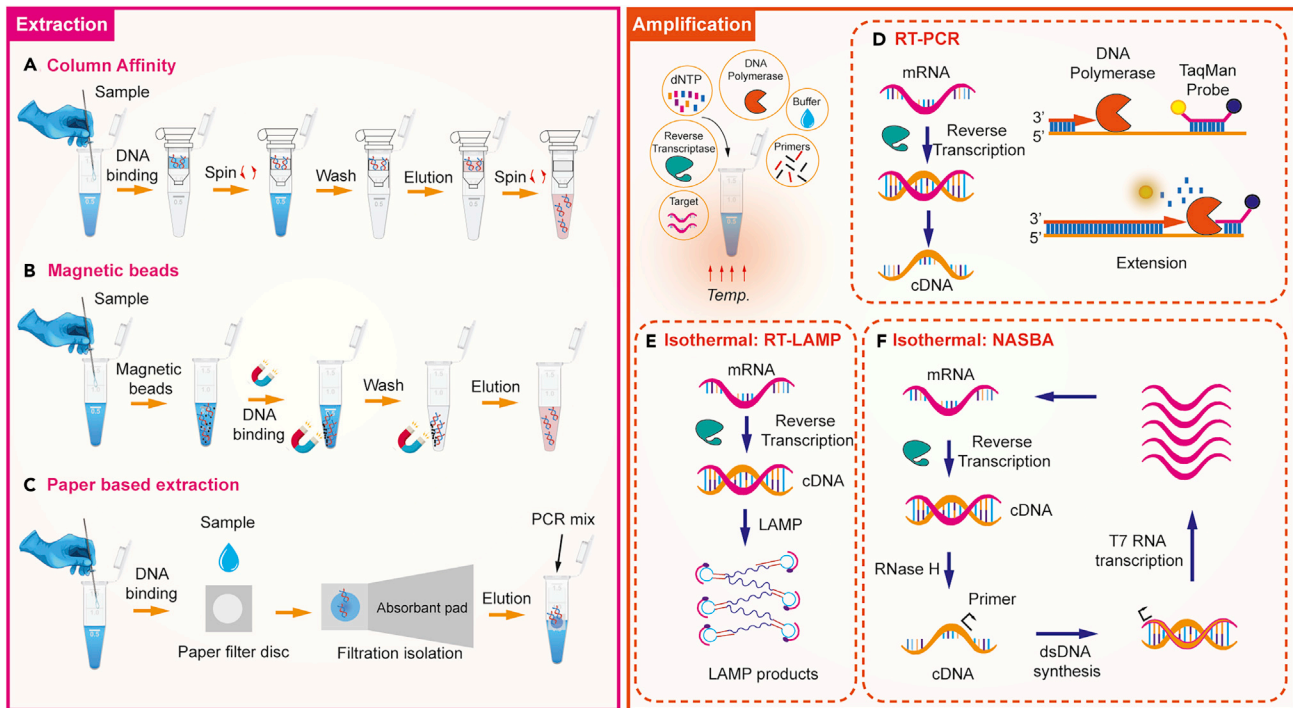
## SAMPLE COLLECTION AND NUCLEIC ACID EXTRACTION

The SARS-CoV-2 has a large positive-sense single-stranded RNA genome (26–35kb)<sup>30,31</sup> and contains four structural proteins including the spike protein (S),

membrane protein (M), envelope protein (E), and nucleocapsid protein (N). The spike protein is critical for binding to host cell receptors to facilitate viral entry into the host cell, while proteins M, E, and N are structural proteins that are necessary for general coronavirus functions.<sup>31,32</sup> The NA-based testing of SARS-CoV-2 includes four major steps: sample collection, RNA extraction, amplification, and signal detection. The integrity and quantity of viral RNA extracted from patient samples are essential for downstream NA amplification and detection.<sup>33</sup> Quantitative RT-PCR (qRT-PCR) is presently the gold standard laboratory test for routine confirmation of suspected acute respiratory infection cases.<sup>15,22,23,34</sup> For COVID-19 sample collection, the SARS-CoV-2 can be isolated from respiratory samples such as sputum, nasal, and throat swabs/washes,<sup>18</sup> with typical viral load ranging from 641 to  $1.34 \times 10^{11}$  copies/mL, with a median of  $7.99 \times 10^4$  copies/mL in throat samples,  $7.52 \times 10^5$  copies/mL in sputum, and  $1.69 \times 10^5$  copies/mL in nasal samples.<sup>34,35</sup> The collected specimen is then purified via a multi-step sample preparation process including virus lysis, inhibition of ribonuclease enzymes, and NA binding, washing, and elution to ensure the RNA of interest is free of contaminants such as proteins, undesired NAs, and other cellular components.<sup>36,37</sup>

For NA extraction, many chemical and mechanical methods have been developed.<sup>38,39</sup> Phenol-chloroform extraction is a low-cost laboratory technique frequently used for NA extraction.<sup>40</sup> However, this method is based on delicate and tedious steps and is associated with hazardous waste by-products that can directly affect the PCR results and lead to a decrease in assay sensitivity. The clinical demand for efficient, rapid, and simpler NA purification techniques has led to the development of many solid-phase extraction methods such as silica columns and aluminum oxide membrane-based platforms.<sup>41–52</sup> The majority of these NA extraction methods rely on high centrifugal force with multiple liquid handling and centrifugation steps that are required for elution of bound NAs from a column/membrane support (Figure 2A). Another NA extraction method based on functionalized silica-paramagnetic microbeads has been widely used in many LionX systems for NA testing. These magnetic microbeads, which are commercially available, can be used to covalently bind and isolate NAs through magnetic control (Figure 2B). The magnetic microbead method<sup>53–56</sup> eliminates the need for column separation and repeated centrifugation. Although this method still requires additional incubation and multiple washing steps, these manipulation steps can be readily streamlined on a LionX system (see details in the section [Integrated Lab-in/on-an-X Systems](#)).<sup>57,58</sup>

Recent literature also reported different types of paper-/membrane-based extraction methods that do not require complex device fabrication and the use of laboratory equipment for the centrifugation, separation, and elution steps (Figure 2C). For example, filtration-based isolation of NAs has been developed by using cellulose paper discs (Fusion 5 filters)<sup>59–61</sup> and the commercial Whatman Flinders Technology Associates (FTA) cards.<sup>62–64</sup> In systems involving these new NA extraction methods, the extracted NA is often directly amplified on the paper/membrane disc, which is particularly useful for developing POC LionX systems. In contrast, the solid-phase extraction method is not suitable for NA testing at the POC due to its multiple sample manipulation steps and heavy dependence on laboratory equipment. To simplify the system operation and enable highly sensitive molecular detection platforms for POC diagnostics, microfluidic systems that integrate the solid-phase-based NA extraction, amplification, and detection into a single reaction chamber were recently designed and adopted by existing LionX platforms.<sup>43,44,64–67</sup> In addition, microfluidic paper-based analytical devices ( $\mu$ PADs)<sup>68–73</sup> represent another promising



**Figure 2. Schematic Illustration of Major Methods for NA Extraction and Amplification**

- (A) Column-affinity, solid-phase-based NA extraction.
- (B) Magnetic bead NA extraction.
- (C) Paper-based NA extraction by filtration-based isolation.
- (D) RT-PCR amplification method.
- (E) RT-LAMP amplification method.
- (F) NASBA amplification method. dsDNA, double-stranded DNA.

platform suitable for POC diagnostics that could successfully integrate the main processing steps involved in infectious disease diagnosis.<sup>59,74–78</sup>

## NUCLEIC ACID AMPLIFICATION METHODS

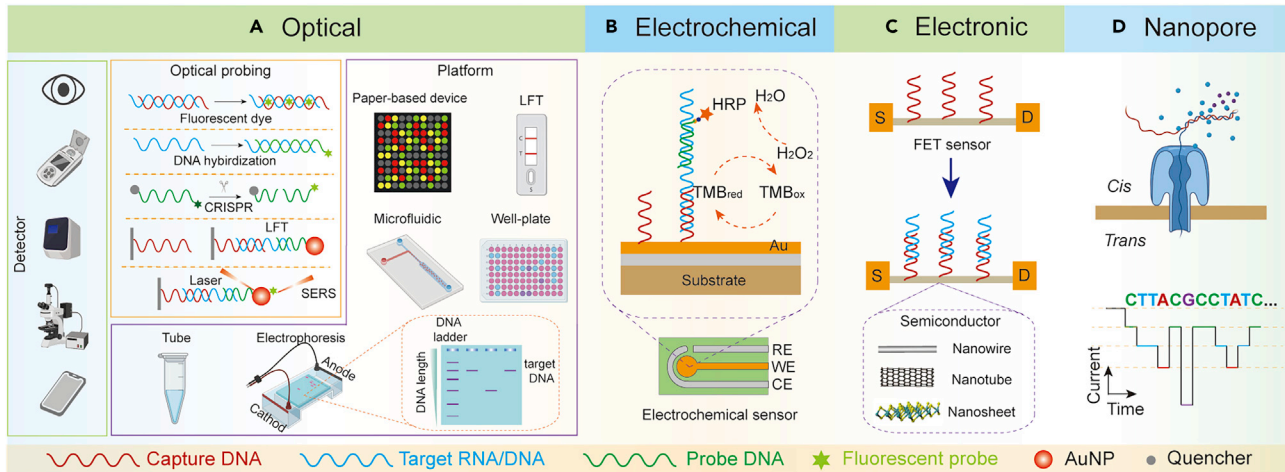
NA-based testing of viral infection has a great advantage over serological testing, with the capability of directly detecting pathogenic DNA or RNA genes in minute quantities with a limit of detection (LOD) down to a single pathogen.<sup>79</sup> During the early acute phase of SARS-CoV-2 infection, the virus replicates rapidly but antibodies produced by our immune system are not yet detectable. Thus, COVID-19 diagnosis should rely on molecular NA amplification, through reverse transcription of the viral RNA into cDNA followed by cDNA amplification (Figure 2D). Among many amplification technologies, qRT-PCR is considered to be highly sensitive<sup>80,81</sup> and is thus commonly used in clinical testing. This reliable test has displaced the conventional northern blotting-based assays<sup>82,83</sup> and has become the gold standard for early clinical diagnosis of pathogenic infections. The qRT-PCR testing has already demonstrated satisfactory sensitivity and specificity in amplifying and detecting a variety of viral RNAs, such as the ones from SARS-CoV,<sup>84,85</sup> acute Middle East respiratory syndrome (MERS)-CoV,<sup>86,87</sup> influenza,<sup>84,88</sup> and Zika viruses.<sup>89–91</sup> Presently, the majority of COVID-19 suspected cases are screened via qRT-PCR tests.<sup>10,11,18,92,93–96</sup> Note that the qRT-PCR tests typically have a lengthy laboratory workflow requiring multiple solution operation steps and relying on sophisticated equipment for thermal cycling and optical signal detection. It may not be a viable option for the screening of COVID-19 at locations where well-equipped

laboratories and highly trained technicians are absent (e.g., at ports of entry and in remote regions).

To circumvent the demanding thermal cycling of the RT-PCR-based amplification, new methods have been developed for enabling exponential NA amplification at constant and relatively low temperatures.<sup>97–99</sup> A practical alternative for cost-effective and rapid RNA amplification is reverse transcription loop-mediated isothermal amplification (RT-LAMP).<sup>100,101</sup> This method (Figure 2E) was designed to directly amplify specific reversely transcribed cDNA through incubation at a fixed temperature (65°C) through only one-step reverse transcription and achieved an efficiency of 10<sup>9</sup> amplicons of genomic DNA in less than 1 h. It has been demonstrated that RT-LAMP can provide a sensitivity 10-fold higher than that of qRT-PCR.<sup>102</sup> Recently, RT-LAMP-based assays have been deployed for rapid and specific detection of pathogenic avian influenza and respiratory viruses (including SARS-CoV-2<sup>103–105</sup> and other infectious diseases in humans,<sup>102–104,106–109</sup> and have also been successfully integrated into several LionX platforms.<sup>110–114</sup>

In contrast to RT-LAMP that utilizes only one enzyme such as DNA polymerase for target NA amplification, other commonly used isothermal amplification methods require several enzymes and proteins for the amplification process. For instance, recombinase polymerase amplification (RPA) requires DNA polymerase and recombinase enzymes.<sup>115,116</sup> It can reach a similar amplification efficiency within 30 min at a comparably low temperature (37°C) and has been implemented on LionX systems.<sup>117,118</sup> The RPA has also been applied to SARS-CoV-2 detection.<sup>119</sup> Helicase-dependent amplification (HDA) utilizes an additional helicase enzyme unwinding the target DNA at a constant temperature (37°C) and allows elimination of the heat-induced denaturation step of the PCR method for viral amplification and detection.<sup>120</sup> The exponential amplification reaction (EXPAR)<sup>121</sup> is unique among these isothermal methods because it does not require primers and performs NA amplification through only one specifically designed target template. NA sequence-based amplification (NASBA) was specifically designed to amplify any single-stranded NA at a relatively low temperature (41°C) for efficient molecular viral detection (Figure 2F) and can achieve up to 10<sup>9</sup> genomic amplicons in 1.5–2 h.<sup>122,123</sup> Quantitative NASBA (QT-NASBA) is highly sensitive and can achieve an amplification performance comparable with that of the primary qPCR method.<sup>122</sup> All the isothermal amplification methods described above eliminate the need for an expensive high-precision thermal cycler used in conventional PCR-based methods and possess the potential of providing simple, rapid, and inexpensive molecular tools for use by LionX systems for detection of infectious diseases in POC settings. Such LionX systems that integrate NA extraction, isothermal amplification, and signal quantification have been successfully developed,<sup>58,74,76,78,124,125</sup> which will be further discussed in the section [Integrated Lab-in-on-an-X Systems](#).

The commercial LionX systems have employed both PCR and isothermal methods for SARS-CoV-2 virus detection, as summarized in [Table S1](#), which target different detection fragments of ORF1ab, S, ORF8, and N genes. These gene sequences code for the ORF1ab polyprotein, surface glycoprotein, ORF8 protein, and nucleocapsid, respectively, and have been employed for amplification primer design for the LionX system COVID-19 assays.<sup>29</sup> Among them, ORF1ab regions of SARS-CoV-2 have shown the least similarity with other Coronavirus sequences such as SARS-CoV-1 and MERS-CoV.<sup>31</sup> The appropriate selection of the target gene and primer design will highly affect the detection performance of the assay.<sup>36</sup> Two genes are commonly amplified and detected with either real-time RT-PCR or LionX systems



**Figure 3. Schematic Illustration of Commonly Used Signal Detection Methods in NA Testing**

(A) Optical detection. LFA, lateral flow assay; LFT, lateral flow test; SERS, surface-enhanced Raman scattering.  
 (B) Electrochemical sensing. HRP, horseradish peroxidase; TMB, tetramethylbenzidine; RE, reference electrode; WE, working electrode; CE, counter electrode.  
 (C) Electronic sensing. FET, field-effect transistor.  
 (D) Nanopore-based sequencing.  
 AuNP, gold nanoparticle.

to confirm the infection of the patient while a single target gene assay can easily miss the low-level positive samples and the mutant virus.<sup>29,126</sup>

### SIGNAL DETECTION METHODS

During or after NA amplification, the amount of amplified NA needs to be quantified to provide the final results of virus detection. A variety of signal detection methods have been employed in NA-based testing, and some of them have been commonly used in existing COVID-19 diagnostic systems (see Table S1). This section summarizes the typical signal detection techniques (Figure 3) commonly used in NA analysis, including optical detection, electrochemical sensing, electronic sensing, and nanopore-based sequencing.

#### Optical Detection

Optical detection of target NAs or NA compositions is generally achieved by sensing optical signals generated by optical probes conjugated on the sensing targets, and typical optical signals include optical absorbance, fluorescence emission, optical reflection, and plasmon resonance. The readout of these optical signals can be achieved using naked eyes, fluorescence readers, plate readers, microscopes, and smartphones on various reaction platforms such as microcentrifuge tubes, microwell plates, lateral flow test (LFT) strips, and microfluidic devices (Figure 3A).

Among these optical detection strategies, fluorescent detection is the most thriving and commonly used method in NA detection. As summarized in Table S1, 80% (12 out of 15) of the recently approved COVID-19 diagnostic systems are based on fluorescent signal readout for quantifying the PCR products. Fluorescent probes are key to fluorescent detection systems, which can be conjugated onto target amplicons nonspecifically by intercalating fluorescent dye molecules (such as SYBR green<sup>4</sup>) with any double-stranded DNAs present in the sample, or specifically by binding single-stranded oligonucleotides labeled with fluorescent reporters to target amplicons with complementary sequences. The specific target probes designed by

incorporating fluorophores and quenchers promise higher specificity and sensitivity in NA recognition.<sup>127</sup> As a result, combining mature amplification tools (such as RT-PCR,<sup>128</sup> LAMP,<sup>114</sup> and NASBA<sup>129</sup>) and specific target probing technology is the predominant strategy for detecting viruses such as SARS-CoV and SARS-CoV-2.<sup>11,130</sup> For example, the detection of SARS-CoV by fluorescent detection has been reported with an LOD of 1 copy RNA per reaction by RT-PCR.<sup>131</sup> The detection of COVID-19 as low as 100 copies/mL by RT-PCR has also been realized by two of the commercial systems listed in [Table S1](#). Besides, synthetic biology-based techniques have also been integrated with optical detection systems to improve the limitation of conventional PCR-based methods. In particular, the emerging clustered regularly interspaced short palindromic repeats (CRISPR)-based tools have been reported to detect target RNAs with extremely high specificity by effectively cleaving DNA fragments modified with fluorescent probe-quencher pairs.<sup>132–134</sup> Working protocols specifically for COVID-19 diagnosis were also reported,<sup>135–138</sup> providing another promising avenue for quick diagnosis of COVID-19 based on fluorescence detection.

Fluorescent signals are generally detected by photodetectors or photomultipliers completely compatible with integrated electronics, which greatly facilitates the integration of fluorescence measurement modules into diagnostic systems. Under resource-limited situations, handheld UV flashlights, cellphones, and cameras can also be employed for constructing sample-in-answer-out (SIAO) diagnostic platforms,<sup>139,140</sup> thus making the optical detection module more portable and multi-functional. For example, by using a smartphone fluorescence reader, a detection limit of  $2.8 \times 10^3$  DNA copies was achieved.<sup>57</sup>

Another type of optical readout for NA testing is the colorimetric signal, which can be observed on LFT strips. Conventionally, the LFT is used for rapid testing of protein markers. Because of its simple device structure and autonomous sample/reagent manipulation (through a one-time lateral flow), the LFT strip has also been used for NA detection. The rapid visual readout of color intensity changes on an LFT strip completely meets the requirements of POC NA testing. Generally, detection of target amplicons is achieved by hybridization of gold nanoparticle (AuNP)-conjugated target single-stranded RNAs with single-stranded capture DNAs immobilized at the test line of a strip and reading of the color change of the test line by naked eyes, a portable colorimetric reader, or simply a cellphone camera.<sup>110</sup> The colorimetric detection can also be performed in aqueous solutions. For example, well-dispersed AuNPs conjugated with capture DNA are red in color; when target DNA presents, AuNP conjugates aggregate due to hybridization of the target DNA with the capture DNA, resulting in a color change of the AuNP-DNA conjugates from red to blue due to the increment of particle size and coupling of the plasmon band.<sup>13</sup> As a result, the DNA testing result can be evaluated by the color change. However, the NA hybridization design works well only for single-stranded target DNAs/RNAs (e.g., the product of NASBA method).<sup>129</sup> For double-stranded DNAs produced by PCR, RPA, and LAMP, the hybridization between the target DNA and capture probe is not feasible, and the detection of double-stranded target DNA is usually realized by streptavidin-biotin conjugations.<sup>76</sup> Quantum dot barcode-based diagnostic sensors have also been developed for high-throughput detection of DNA markers,<sup>33,141</sup> whereby a smartphone was used for taking photographs of the barcode sensor with a custom-written program for image processing and colorimetric signal quantification. Note that the sensitivity of the above optical sensing techniques for NA without amplification is usually not high enough, and amplification-free testing of NA samples using optical detection methods may cause false-negative or false-positive results.<sup>142</sup>



To enhance the sensitivity of optical detection, metallic nanomaterials such as AuNPs and silver NPs were applied to modify the detection substrate or target molecule to achieve surface plasmon resonance (SPR) or surface-enhanced Raman scattering (SERS).<sup>143</sup> For example, an LOD of 4.8 pg/mL was achieved on a portable SPR biosensor designed for the Enterovirus 71 test.<sup>144</sup> It was also reported that the fluorescence signal of a single dye probe bounded on a Zika viral RNA can be enhanced by orders of magnitude by creating a hotspot between optical antennas and metallic nanoparticles because of the SERS effect.<sup>145</sup> SERS-based rapid identification of virulence determinants in influenza viruses with 100% sensitivity and 100% selectivity has also been achieved.<sup>146</sup> Through signal enhancement strategies, the sensitivity of biosensors increases dramatically; samples without molecular amplification may be detected. Optical signal enhancement is a promising candidate for amplification-free DNA analysis,<sup>143</sup> but these optical enhancement methods usually require complicated, time-consuming processes for sample substrate preparation.<sup>147</sup>

The traditional gel electrophoresis is also a popular method for visualization of amplified DNA molecules, and has been widely used in lab sample analysis due to its simplicity and reliability. However, it is a tedious and time-consuming process and involves a bulky gel bed setup; thus, it is not commonly used in clinical NA testing.<sup>128</sup> Since the 1990s, integration and parallelization of NA analyses on capillary electrophoresis-based microfluidic systems have been widely investigated, which enables efficient on-chip RNA/DNA testing;<sup>148</sup> however, these systems are generally complicated when both precise flow control and optical detection are needed,<sup>149</sup> hampering its usability in practical applications. In summary, optical detection methods are generally suitable for use on integrated diagnostic systems for NA-based virus testing, and can provide merits such as high testing throughput, satisfactory sensitivity and specificity, and good cost efficiency. However, it is still challenging to realize some specific NA testing functions, such as amplification-free NA detection and real-time NA sequencing of RNA/DNA solely based on optical detection methods. Other NA detection methods based on electrochemical and electrical readouts have also been investigated to expand the diversity of NA testing methods.

### Electrochemical Sensing

Besides optical detection methods, electrochemical sensing is another widely used category of signal detection methods for NA testing,<sup>150–152</sup> which provides many valuable merits such as high sensitivity and specificity, simple device structure, and excellent compatibility with micro- and nanoengineering platforms. A typical electrochemical DNA sensor includes three contact electrodes, a working electrode (WE), a counter electrode, and a reference electrode, all inside a reaction reservoir, with the WE surface-functionalized with capture DNA probes for target DNA segments (Figure 3B). Detection of the target DNA is based on measuring changes of electric signals such as current, potential, and impedance from the three electrodes, which are caused by hybridization of the target DNA in the sample and the capture probe on the WE and the related chemical reactions.<sup>153</sup> Figure 3B illustrates the typical sandwich-hybridization-based DNA electrochemical sensing, where a DNA segment conjugated with an electrochemical reporter (horseradish peroxidase [HRP]) serves as the label for reacting with its substrate and producing a current signal.

To achieve specific testing, the complementary capture DNA is immobilized on the WE through physical absorption or covalent bonding. Thin-film gold is one of the

most popular WE materials, and stable covalent immobilization of capture DNA on gold substrates can be obtained through organic linkers such as alkanethiol, cyclic disulfides, phosphorothioated adenine tags, and functionalized self-assembled monolayers.<sup>151</sup> A variety of other WE materials and biofunctionalization strategies have also been employed to improve the uniformity and stability of the DNA probes on the WE. For instance, carbon-nanomaterial-based WEs have been fabricated to take advantage of their high surface-to-volume ratio and high electrical conductivity, and COOH-functionalized polymers were used for covalent bonding of the amine-terminated DNA via carbodiimide chemistry.<sup>154</sup> Electrodes with wrinkled nano-/microstructures were applied to prevent aggregation of DNAs, which usually happens on flat surfaces.<sup>155</sup>

Compared with optical detection methods, electrochemical sensing has several advantages. For instance, amplification-free NA detection can be realized by employing functional nanomaterials for redox modification or functionalization.<sup>143</sup> By using electrospun semi-conductive manganese(III) oxide (Mn<sub>2</sub>O<sub>3</sub>) nanofibers as sensing electrodes, the detection of Dengue DNA hybridization with an LOD as low as  $120 \times 10^{-21}$  M was demonstrated.<sup>156</sup> Direct detection of microRNAs in serum samples was also demonstrated with high sensitivity and specificity.<sup>157</sup> In addition, label-free NA detection can be realized through electrochemical sensing with high sensitivity. An example of label-free detection of Zika viral cDNA amplicons by using disposable three-contact electrode sensors was reported, whereby a limit detection of 25 nM was achieved.<sup>158</sup> Diagnosis of hepatitis A virus (HAV) was implemented on a capture DNA-modified gold electrode by monitoring direct hybridization of cDNA, and an LOD of 6.94 fg/ $\mu$ L was reported.<sup>159</sup> Another bonus of electrochemical DNA sensing is that the sensors can be made reusable by regenerating the WE surfaces through strategies such as switchable or looped capture probes and washing-based dehybridization steps,<sup>160,161</sup> which could significantly lower the cost of NA testing.

Upon the COVID-19 pandemic, the US FDA approved an electrochemical sensing system named the ePlex SARS-CoV-2 test (see [Table S1](#)), which provides an LOD of  $10^5$  copies/mL. It is believed that researchers and companies have been racing to develop more electrochemical platforms for COVID-19 diagnostics, especially for POC applications. For instance, the combination of plastic- and paper-based electrochemical sensors<sup>162,163</sup> with sensitive nanomaterial-decorated electrodes<sup>164</sup> may enable amplification-free direct SARS-CoV-2 detection on portable LionX systems with satisfactory sensitivity. Label-free, reusable electrochemical sensors can be implemented on COVID-19 diagnostic systems to shorten the assay TAT and reduce the testing cost.

### Electronic Sensing

Electronic sensors are developed based on semiconductor materials whose electronic properties are sensitive to the binding of biomolecules on the material surface, and they are typically implemented in the form of field-effect transistors (FETs). Very similar to electrochemical sensors, in the FET NA sensor design, capture DNA probes are immobilized on the surface of semiconductor FET channel; the output signal, caused by the hybridization of target NA with the capture DNA, is obtained by monitoring the change of electric current through the FET channel ([Figure 3C](#)). Silicon nanowires,<sup>165</sup> carbon nanotubes,<sup>166</sup> graphene,<sup>167</sup> and two-dimensional transition metal dichalcogenides<sup>168</sup> are popular semiconductor nanomaterials for constructing FET-based NA sensors. As these nanomaterials are highly sensitive to surface DNA hybridization, the FET-based NA sensing can achieve ultra-high sensitivity and thus enables amplification-free virus detection. For instance,

amplification-free detection of DNA lengths by enzyme functionalized CNT-based FET nanocircuits has been demonstrated.<sup>169</sup> A recent study demonstrates the detection of unamplified target DNA via CRISPR/Cas9 immobilized on a graphene-based FET sensor, with an LOD down to 1.7 fM and assay time of 15 min.<sup>170</sup> However, batch fabrication of high-quality FET sensors with consistent analytical performance is still challenging, mainly because of quality variations of the channel nanomaterial and device fabrication. With further advances in nanomaterial synthesis and micro-/nanofabrication, it is expected that more market-viable FET-based LionX platforms will emerge.

### Nanopore-Based Sequencing

Nanopore-based gene sequencing is one of the most promising next-generation sequencing (NGS) technologies.<sup>171</sup> Besides the widely recognized application in whole-genome sequencing, it has also been used for detecting various types of viruses such as porcine epidemic diarrhea-related virus,<sup>147</sup> HIV,<sup>172</sup> and hepatitis B virus.<sup>173</sup> The working mechanism of nanopore-based sequencing, as illustrated in Figure 3D, is based on the real-time, base-by-base electrical reading of a single-stranded long DNA or RNA while it passes through a nanopore driven by an electric field.<sup>174</sup> To date, the most effective nanopore for DNA sequencing is fabricated by self-assembling proteins or peptides, while nanopores fabricated by solid-state materials and two-dimensional nanomaterials such as graphene and MoS<sub>2</sub> have also been investigated.<sup>175</sup>

Oxford Nanopore Technologies is one of the industrial pioneers in NGS, and their sequencing platforms have been successfully applied to sequencing the SARS-CoV-2 strains at the early stage of the COVID-19 pandemic.<sup>31</sup> Because of the limited length (~30,000 nucleotides) of the SARS-CoV-2 RNA genome, the time for sequencing the entire virus genome by the Oxford sequencing platform was reported to be around 1 h.<sup>176</sup> Liu et al.<sup>177</sup> reported the application of nanopore platforms to sequence amplified COVID-19 DNA fragments within a few minutes. Note that the preparation of DNA/RNA samples and the initialization of the sequencing system are still relatively time consuming (~7 h) for COVID-19 RNA sequencing.<sup>178</sup> A useful capability of the nanopore-based sequencing is that its real-time, single-base-resolution readout can identify mutations of the virus immediately during the spread of the pandemic, and thus provide substantial information for further epidemiological analysis.<sup>177</sup> The nanopore sequencing system can also identify various types of viral targets simultaneously, based on the established SARS-CoV-2 GenBank.<sup>179</sup> At its current stage, the nanopore-based sequencing technology is more suitable for virus genome identification amid a pandemic rather than clinical diagnosis.<sup>31</sup> However, with ongoing research efforts on developing nanopore-based biosensing technologies,<sup>180,181</sup> it is highly plausible that the sequencing-based NA detection method will become practical for integration with the LionX systems for viral infection diagnosis.

### INTEGRATED LAB-IN/ON-AN-X PLATFORMS

The LionX diagnostic systems provide multiple choices for both laboratory-based and POC virus detection, which could significantly enrich the COVID-19 diagnostic toolset. The integrated LionX systems often adopt miniaturized fluidic manipulation platforms to reduce the system footprint and automate a streamlined virus detection process including sample preparation, NA amplification, and signal detection to avoid or reduce manual operations and minimize operation errors. An example of typical LionX systems is the Roche cobas Liat PCR System based on a lab-in-a-

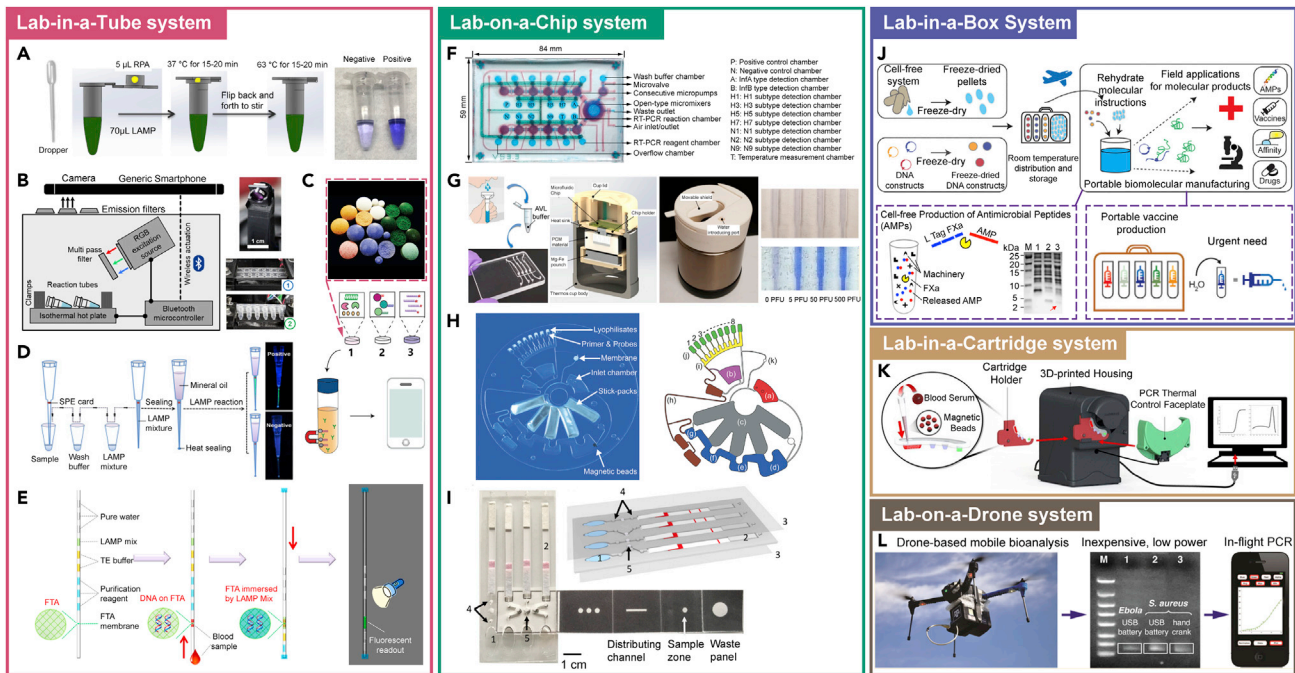
tube design, which has been successfully applied to clinical diagnosis of influenza virus<sup>182,183</sup> and SARS-CoV-2 infections.<sup>184</sup> Before the COVID-19 pandemic, enormous research activities on diagnostic LionX systems led to many promising solutions for testing various viral infections.<sup>25,185</sup> Researchers and companies are now adapting some of these most promising platforms for COVID-19 diagnostics.<sup>186,187</sup> In this section, we summarize the existing LionX systems in the hope of providing useful information for the ongoing and future activities in this area.

### Lab-in-a-Tube Systems

The traditional clinical virus testing procedure, including NA extraction, amplification, and detection, is primarily conducted in various tubes/plates. The lab-in-a-tube platforms integrate new analytical techniques and fluid manipulation functions into the widely used laboratory tubes/plates, which simplifies assay operations and makes it less dependent on laboratory equipment and resources. These merits make the lab-in-a-tube diagnostics particularly promising for use on the site and/or in resource-limited settings. Note that there is another “lab-in-a-tube” concept of using rolled-up microtubes for analytical applications,<sup>188</sup> which is beyond the scope of discussions here. The lab-in-a-tube diagnostics are generally compatible with major laboratory equipment and conceptually easy to adopt by clinicians. It should be pointed out that, given the nature of multi-step operations in virus testing, it is challenging to streamline the entire workflow in unmodified commercial tube/plate architectures in an integrated and automatic fashion. To form integrated lab-in-a-tube systems, customized tube-shaped cartridges (e.g., the Roche cobas Liat PCR System) need to be developed to realize automated multi-step fluid operations. This subsection briefly introduces recent advances in lab-in-a-tube diagnostics, and some of the typical designs are illustrated in [Figures 4A–4E](#).

To enhance the testing throughput, a massively parallel COVID-19 diagnostic assay was developed based on 96/384 well plates, which combines viral RNA extraction, RT-PCR with barcoding, and next-generation sequencing in one workflow and enables testing of up to 19,200 patient samples per run.<sup>200</sup> To simplify reagent handling in tube-based diagnostics, Udugama et al.<sup>190</sup> developed a high-throughput compression method to produce tableting reagents by encapsulating predetermined quantities of reagents in color-coded, thermally stable tablets. During a test, the tableted reagents are readily recognized by color and simply diluted in a tube for reaction. These tableted reagents significantly simplify the reagent storage and assay operation, and have enabled virus detection in remote areas ([Figure 4C](#)).<sup>190</sup>

To avoid tube-to-tube sample transfer, a two-stage isothermal amplification process was integrated into one tube, where the first RPA step was conducted in the tube cap and the subsequent LAMP step was conducted in the tube after closing the tube cap and mixing of the LAMP reagents with the RPA solution in the cap ([Figure 4A](#)). This two-stage-amplification, closed-tube diagnostic assay was demonstrated to test spiked COVID-19 samples with significantly enhanced sensitivity over RT-PCR, and can be conducted in non-laboratory settings (e.g., at home, in clinics, and at ports of entry) with very limited resources.<sup>109</sup> Virus detection processes can also be integrated into single plastic capillaries or pipette tips. For example, integrated microcapillary-based LAMP with all reagents and DNA extraction card preloaded in a capillary was developed to test CYP2C19 gene from crude blood samples without any sophisticated instruments ([Figure 4E](#)).<sup>192</sup> Similarly, Lu et al.<sup>191</sup> developed a pipette tip-based NA test that integrates NA extraction, LAMP, and signal readout in a single pipette tip ([Figure 4D](#)), allowing for detection of Ebola virus with an LOD



**Figure 4. The LionX Systems for NA-Based Virus Detection**

(A–E) Lab-in-a-tube systems. (A) Closed-tube assay for COVID-19 diagnosis.<sup>109</sup> (B) Integration of tube-based diagnostic with a smartphone.<sup>189</sup> (C) In-tube simplified assays by tableting reagents.<sup>190</sup> (D) High-throughput sample-to-answer detection of DNA within micropipette tips.<sup>191</sup> (E) Integration of NA precontraction, amplification, and detection in a microcapillary.<sup>192</sup> (F–I) Lab-on-a-chip systems. (F) Multiplexed integrated microfluidic system for influenza detection.<sup>193</sup> (G) Instrument-free microfluidic platform for Zika virus detection.<sup>194</sup> (H) The integrated centrifugal microfluidic-circular fluorescent probe-mediated isothermal NA amplification system.<sup>195</sup> (I) A paper-based microfluidic platform for malaria diagnosis.<sup>196</sup> (J–L) Other LionX systems. (J) The lab-in-a-box system with the freeze-dried and cell-free techniques.<sup>197</sup> (K) The lab-in-a-cartridge system utilizing the droplet magnetofluidics technique.<sup>198</sup> (L) The lab-on-a-drone system that realized pinpoint deployment of a smartphone-based diagnostic platform.<sup>199</sup>

as low as 2 copies of target NA fragments. High-throughput, simultaneous detection of 40 samples can be achieved by using a multi-channel pipette.

Besides the academic research summarized above, several commercial lab-in-a-tube systems for COVID-19 diagnostics have also been regulatorily approved recently (see Table S1). A typical example is the Roche cobas Liat PCR System, which automatically performs NA extraction, PCR, and signal readout in a customized tube. The system was initially applied to testing of influenza A/B viruses<sup>183,201</sup> and recently adapted to COVID-19 diagnosis.

### Lab-on-a-Chip Systems

Microfluidics and lab-on-a-chip technologies provide miniaturized solutions for on-chip fluid manipulation and analytical testing, and have been extensively investigated for developing integrated diagnostic platforms with all assay steps automatically carried out on a microfluidic chip. The lab-on-a-chip systems have many advantages such as low consumption of samples and reagents, high level of integration, high sensitivity, low cost, rapid reaction, and short TAT; all these features are particularly valuable for POC diagnostics. There have been many reports on developing integrated lab-on-a-chip systems for detection of various pathogens such as bacteria and viruses.<sup>202–205</sup> This subsection only discusses some of the typical lab-on-a-chip systems for virus detection and highlights the recent reports on using microfluidic platforms for the detection of SARS-CoV-2 and other viruses.

For a more comprehensive and detailed review on microfluidic systems for NA testing and pathogen detection, readers are referred to other review articles.<sup>25,202–204</sup>

Based on conventional hollow-channel designs of the microfluidic device, integrated lab-on-a-chip systems have been developed for virus detection.<sup>193,194,206–208</sup> For instance, Shen et al.<sup>193</sup> developed an integrated microfluidic system for influenza A virus detection and subtyping (Figure 4F). The system is capable of automatically conducting virus extraction and purification, RT-PCR-based amplification, and fluorescence signal detection on the same microfluidic chip. Magnetic microbeads were used for virus extraction and washing, and pneumatic microvalves for on-chip fluid manipulation. The system achieved an LOD ranging from 40 to 3,000 copies for different virus subtypes. Song et al.<sup>194</sup> developed an instrument-free microfluidic platform with a single microfluidic device for Zika virus detection (Figure 4G). Although the system can perform on-chip NA extraction/purification/enrichment, RT-LAMP amplification, and colorimetric DNA detection on the same chip, reagents and washing buffers still need to be added manually. Wang et al.<sup>206</sup> developed an eight-channel microfluidic array device integrated with a portable LAMP amplification system for POC screening of respiratory viruses within 1 h, which achieved high sensitivity in clinical sample testing. Recently, an automated CRISPR-based microfluidic chip and its fluid manipulation and signal detection platform were designed to avoid the sample preparation and amplification steps.<sup>209</sup> However, the assay sensitivity and selectivity cannot reach the levels of standard assays without the amplification step. Targeting COVID-19 diagnostics, Sun et al.<sup>207</sup> recently reported a smartphone-based multiplexing NA detection system integrating a silicon microfluidic chip for LAMP amplification and a smartphone for fluorescence signal detection. No NA extraction step was realized on the microfluidic chip, and repeated manual pipetting was required during the assay.

Centrifugal microfluidics, also called compact disk (CD)-based microfluidics, integrates multiple assay steps on a CD-shaped microfluidic chip in which solutions are transported inside microchannels by spinning-induced centrifugal forces.<sup>210,211</sup> A centrifugal microfluidic system uses a motor to spin the chip at different speed profiles to enable multi-step solution mixing and assay reactions, and relies on passive on-chip valves to regulate the fluid transports. The CD chip stores all required reagents, which are added to a reaction microchamber at different assay steps. Thus, such systems are particularly powerful for realizing streamlined multi-step NA testing. A portable centrifugal microfluidic system has been developed to detect influenza A H3N2 virus (Figure 4H),<sup>195</sup> whereby the virus RNA is extracted via a bind-wash-elute protocol on silica-coated magnetic beads. The CD chip can aliquot the preamplified RNA into 13 chambers through centrifugation for multiplexed amplification and detection. Because of its promising features such as high portability and short TAT, the system is being adapted to the SARS-CoV-2 detection with a short TAT of 30–40 min.<sup>212</sup> There are also other centrifugal microfluidic systems for detection of viruses such as African swine fever virus<sup>213</sup> and dengue virus.<sup>214</sup> More discussions on centrifugal microfluidics for pathogen detection can be found in a focused review.<sup>211</sup>

$\mu$ PADs,<sup>215,216</sup> including the widely used LFT strips, represent another promising platform technology for virus detection.  $\mu$ PADs were initially designed for equipment-free POC testing in remote and/or resource-limited settings, and mainly rely on passive capillary flow transport in porous paper-like substrates. Thus, these devices are inherently suitable for assays requiring no or limited fluid manipulations.

For paper-based NA testing platforms, repeated manual pipetting is usually involved for adding reagents and the transfer of intermediate reaction products.<sup>129,217–220</sup> With various fluidic manipulation strategies developed for paper chips,<sup>221</sup>  $\mu$ PADs have also been extended to more complex, multi-step analytical assays including NA testing.<sup>222,223</sup> Reboud et al.<sup>196</sup> reports an integrated paper-plastic hybrid microfluidic device (Figure 4I) for NA-based testing of malaria in finger-prick blood. The device integrates an origami  $\mu$ PAD for DNA extraction and processing, a plastic microfluidic device for LAMP amplification, and four LFT strips for colorimetric signal readout. The assembly of the origami  $\mu$ PAD and the plastic device enables transfer of DNA elutes by paper folding, and finger-pressing pumps on the plastic device allow manual flow control. Rodriguez et al.<sup>224</sup> developed an integrated paper-based NA testing device capable of on-chip DNA extraction, LAMP amplification, and lateral flow colorimetric detection. The device was demonstrated for rapid testing of human papillomavirus in crude cervical specimens for cervical cancer diagnosis. To meet the need for COVID-19 diagnostics, several groups have developed SARS-CoV-2 tests by combining off-chip RNA extraction/amplification and on-chip CRISPR-based NA detection on LFT strips.<sup>136,225,226</sup> The easy operation of the LFT strip greatly facilitates the NA detection step of these assays.

### Lab-in-a-Box Systems

In response to the outbreak of infectious diseases such as COVID-19, the urgent deployment of diagnostic tools in the developing countries or remote areas has brought multiple requirements for diagnostic systems such as high portability, easy operation, low cost, low contamination risk, and short TAT. The lab-on-a-chip systems represent an excellent technical solution to meet these needs. There are several other types of LionX systems that also hold great potential to be applied to virus detection in remote and/or resource-limited settings, including the lab-in-a-box system that integrates all required reagent synthesis and assay tools in a portable box for self-contained and mobile deployment of molecular diagnostics; the lab-in-a-cartridge system that encloses sample processing, amplification and signal detection all inside a cartridge for automated SIAO testing; and the lab-on-a-drone system that realizes pinpoint deployment of diagnostic tools through an unmanned aerial vehicle (UAV). These LionX systems provide self-contained, cost-efficient diagnostic solutions for both laboratory and POC environments, and can be used to quickly equip suburban/remote communities and poor regions with molecular testing capabilities during a public health emergency.

The lab-in-a-box design includes all needed virus testing tools in a portable box and aims for rapid deployment of molecular diagnostic capacity to any location with basic resources. Such a system can be used to quickly equip a resource-limited laboratory or clinic to meet the local diagnostic needs. The design emphasizes more the self-containment and rapid deployment of the boxed system rather than miniaturization and automation of the assay platform. One lab-in-a-box system is currently under development by Pardee's group at the University of Toronto to implement rapid, low-cost COVID-19 diagnosis in low-resource settings.<sup>227,228</sup> The portable box is about twice the size of a standard cupboard moving box and can serve as a "pop-up" diagnostic laboratory deployable to any place in need, providing a total capacity of 14,000 COVID-19 tests. Importantly, this platform also possesses the capability of on-site reagent synthesis based on synthetic biology and thus is not limited to the disruption of global supply chains.

This lab-in-a-box platform is built on several previous studies on synthetic biology-based diagnostics. Pardee et al.<sup>218</sup> reported a new method for long-term (>1

year), room-temperature storage of synthetic gene circuits by freeze-drying commercially available cell-free expression systems on cellulose filter paper, which opened up a new avenue of synthetic-biology-based diagnostics on paper. This approach enables the inexpensive, sterile, and abiotic distribution of gene circuit-based reagents without refrigeration, and was demonstrated for field testing of strain-specific Ebola virus.<sup>218</sup> This method was later combined with a synthetic RNA sensor called toehold switches<sup>229</sup> to perform low-cost POC testing of Zika virus.<sup>129</sup> The use of freeze-dried, cell-free systems with long-term stability at room temperature could alleviate the restrictions of both live-cell biosynthesis and cold-chain distribution requirements. Pardee et al.<sup>197</sup> also demonstrated the use of this paper-based synthetic platform for production of a diverse range of functionally active products, including antimicrobial peptides, vaccines, affinity conjugates, and small molecules (Figure 4J). This technique will allow on-site reagent synthesis for COVID-19 diagnostics on the lab-in-a-box system.

### Lab-in-a-Cartridge Systems

A highly integrated diagnostic system usually prefers to integrate sample preparation, NA extraction, and amplification into a single disposable cartridge. Besides the lab-on-a-chip approach that provides a miniaturized fluidic system for process integration, plastic cartridges with integrated fluid channels/chambers also represent a market-viable solution. The lab-in-a-cartridge systems employ plastic cartridges for streamlined fluid manipulation and multi-step NA testing. Shin et al.<sup>198</sup> presented a lab-in-a-cartridge qRT-PCR system (Figure 4K) for chronic hepatitis C virus (HCV) diagnosis in a SIAO fashion. By using droplet magnetofluidics (DM) for sample processing, the extruded-well design of the cartridge enables precise thermal control of the reagents at suitable ranges for PCR thermal cycling and melt curve analysis. The DM-based manipulation enables the necessary purification of NA targets from clinical samples to obtain quantitative and consistent assay results. The system showed high analytical sensitivity down to 4,500 IU/mL on HCV RNA-positive samples and a high correlation with laboratory-based viral quantification and benchtop PCR. Liu et al.<sup>230</sup> proposed a disposable self-heating cartridge containing computer numerical control (CNC)-milled fluid channels and a chemical heater for isothermal NA amplification and fluorescent signal detection with keychain-mounted UV light. The three-dimensional cartridge architecture also provides a larger space for more flexible channel design for fluid manipulation and better component arrangement. Besides the academic research, a commercial lab-in-a-cartridge system, the Cepheid GeneXpert System, has been successfully applied to COVID-19 diagnostics.<sup>231</sup> Its integrated Xpert Xpress SARS-CoV-2 cartridge realizes automated fluid transport through plunger rod-operated valve rotation and sonication-based virus lysis through an external sonic horn. Xu et al.<sup>232</sup> reported a lab-in-a-cartridge system that integrates RNA extraction and one-color, three-chamber pseudo-multiplexing RT-PCR for rapid influenza virus detection within 2.5 h. The cartridge embedded with microfluidic channels provides precise dispensing of aliquots for multi-chamber RT-PCR testing. The performance of the integrated RNA extraction, custom-built thermal cycler, and optical detection module is comparable with that of manual RNA purification and commercial thermal cyclers.

### Lab-on-a-Drone Systems

A lab-on-a-drone diagnostic system integrates a compact, lightweight lab-in-a-box module on a UAV (drone), and thus can provide pinpointed deployment of diagnostic tools for real-time feedback. Although this is a less explored concept, we believe it is worth introducing to highlight its rapid deployment capability that could be highly useful for handling public health emergencies. Priye et al.<sup>199</sup> developed a portable NA testing platform carried by a consumer-class quadcopter drone



(Figure 4L). The system leverages convective thermocycling to isothermally perform PCR using a single heater and only requires a 5-V USB power source (through battery, solar, or hand crank action) for operation. A smartphone was used for time-lapsed fluorescence detection. A unique design of this system is the use of the four onboard rotors for standard centrifugation-based sample preparation. This lab-on-a-drone system makes full use of the high mobility of commercial drones and extends its function to mobile molecular diagnostics.

## SUMMARY AND OUTLOOK

In the past decades, our global community has experienced several public health emergencies and crises caused by viral infectious diseases such as SARS, Ebola, Zika, MERS, and COVID-19. The spread of these viruses in large populations requires rapid and accurate diagnostics that can be conducted in a streamlined and integrated fashion. This Perspective reviewed the research activities on developing integrated micro- and nano-systems for NA-based virus testing. Many of these systems have been conceptually presented and implemented in the forms of LionX platforms. We summarized the state-of-the-art technologies for NA extraction, amplification, and signal detection that have been adopted in the existing LionX systems, and discussed the typical LionX diagnostic systems that have been applied to diagnosis of viral infectious diseases including COVID-19. We hope that this Perspective can inspire more research and development activities to help combat the current COVID-19 pandemic and prepare our healthcare systems for future public health emergencies and crises.

A variety of LionX systems have been developed for virus detection, all of which provide viable solutions for different diagnostic scenarios including laboratory testing and POC diagnosis. The laboratory-based PCR-based assays, which are the major force fighting the COVID-19 pandemic, still faces certain challenges.<sup>20</sup> For instance, the conventional PCR-based assays require multi-step fluid manipulations that are realized either through manual or robotic pipetting, and take at least several hours to complete. In a global pandemic, there are many occasions that demand rapid virus testing with satisfactory throughput outside a laboratory, for instance at the ports of entry or in remote regions. The LionX systems streamline operation steps of the NA-based virus testing, reduce or completely eliminate manual operations, often shorten the assay TAT, and lower the contamination risk. These systems have been successfully applied to the diagnosis of different viral infections. These systems could greatly contribute to the COVID-19 pandemic by providing alternative diagnostic solutions to meet the significant demands for testing and tracing infection cases. The importance of rapid development of LionX systems for the diagnosis of SARS-CoV-2 infections cannot be overemphasized.

Besides the LionX systems and its associated techniques we have introduced, there are several emerging trends worth mentioning. Firstly, CRISPR-based NA detection has provided a highly promising alternative method to PCR-based assays.<sup>233–235</sup> This has been applied on hollow-channel<sup>209</sup> and paper-based<sup>136</sup> microfluidic platforms for viral NA detection. The diagnostic assays based on CRISPR techniques can produce accurate results within as short as 5 min without solid-phase extraction.<sup>209</sup> Secondly, the isothermal NA amplification techniques such as LAMP have been increasingly used in LionX systems.<sup>236</sup> The LAMP reaction amplifies DNA with high specificity and efficiency under isothermal conditions, and has been confirmed to be effective for SARS-CoV-2 detection.<sup>114,186</sup> The simple setup of the isothermal amplification-based platforms such as the Abbott ID Now System has significantly reduced the system weight and size and reduced the assay

TAT.<sup>237</sup> It is anticipated that more compact systems with shorter TAT will appear for COVID-19 diagnostics by combining microfluidic device designs and isothermal testing. Thirdly, smartphone-based testing could further reduce the size and increase the integration level of LionX systems. In combination with a portable integrated system, a smartphone with a customized analytical application can efficiently quantify the assay result without the specialized knowledge of a medical professional.<sup>207,238</sup> The state-of-the-art smartphone, together with its integrated optics, computation power, and communication functions, have been demonstrated to provide sufficient sensitivity, making it a powerful alternative to the professional analytical readers.<sup>27</sup> Along with the remarkable progress of COVID-19 assay and detection system development, the practical detection performance is required to be constantly monitored given the potential for the virus to mutate, which could increase the risk of false-negative results<sup>13</sup> and is an emerging challenge for the integrated LionX diagnostic systems.<sup>239</sup> A recent work identified 198 filtered recurrent mutations in the SARS-CoV-2 genome based on up-to-date existing 7,710 assemblies, and estimated the mutation rate to be  $\sim 6 \times 10^{-4}$  nucleotides/genome/year for SARS-CoV-2, which is, fortunately, largely unremarkable for an RNA virus.<sup>240</sup> The ability to detect different emergent strains of SARS-CoV-2 may become necessary as the COVID-19 pandemic evolves.

### SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.matt.2020.06.015>.

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### AUTHOR CONTRIBUTIONS

Z.Q. and X.L. conceived the theme and designed the manuscript structure. Z.Q., R.P., I.K.B., and X.L. wrote the manuscript.

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**Matter, Volume 3**

**Supplemental Information**

**Fighting COVID-19: Integrated Micro- and  
Nanosystems for Viral Infection Diagnostics**

**Zhen Qin, Ran Peng, Ilina Kolker Baravik, and Xinyu Liu**

## Supplementary Information:

**Table S1 Commercial COVID-19 RNA detection systems approved by FDA and Health Canada (accessed on May 27, 2020)**

No.	Products	Micro/Nano technique	Sample treatment (lysis and extraction)	Nucleic acid amplification	Detection signal	LOD	Assay time	Multiplexing	Highlights
1	BioFire COVID-19 Test, BioFire (US)	Lab-in-a-Cartridge (Pouch format with nest array spots)	Lysis by agitation. Extract and purify by magnetic beads.	Multiplex PCR	Endpoint melting curve	330 copies/mL	45 minutes	No. One patient sample per device (pouch)	<ul style="list-style-type: none"> <li>• Work with high-throughput BioFire FilmArray Torch System. Two step injection for sample and hydrogen solution.</li> <li>• Reagents pre-loaded and freeze-dried inside the pouch. Realize three independent and non-overlapping assays targeting two SARS-Cov-2 open reading frame sequences (ORF1ab and ORF8).</li> <li>• Clinical performance: 100% PPA for 30 samples and 100% NPA for 66 samples. No cross reaction to six close viruses.</li> </ul>
2	ID NOW platform, Realtime SARS-Cov-2 assay, Abbot (US)	Lab-in-a-Box (Cartridge)	Elution and lysis in sample receiver using buffer. Transfer cartridge manually to test base, comprising two sealed reaction tubes with each containing a lyophilized reagent pellet.	Isothermal amplification	Fluorescence intensity	125 copies/mL	5 ~ 13 minutes	No. One patient sample per device (cartridge)	<ul style="list-style-type: none"> <li>• Lightweight (6.6 pounds) and portable (the size of a small toaster), suitable for POC settings.</li> <li>• Predicted inclusivity of 100 % for COVID-19 tests. No significant combined homologies with human genome and other coronaviruses.</li> <li>• Two tubes containing amplification reagents and internal control.</li> </ul>
3	m2000™ RealTime SARS-CoV-2 EUA test, Abbot (US)	Lab-in-a-Plate (Sealed Abbott 96-Well Optical Reaction Plate)	Microparticle-based protocol and reagents	real-time RT-PCR	Realtime fluorescence intensity	100 copies/mL	24 hours	Yes, 24-96 samples per run	<ul style="list-style-type: none"> <li>• Work with m2000™ RealTime System. Scalable, automated process up to 470 patient samples in 24 hours.</li> <li>• Dual target assay for RdRp and N genes.</li> <li>• Primers and probes had 100% homology to all the available circulating SARS-CoV-2 sequences. No significant cross-reactivity or microbial interference.</li> </ul>
4	Xpert Xpress SARS-CoV-2 test, Cepheid, (US)	Lab-in-a-Cartridge	Cell lysis by sonication	RT-PCR	Light intensity with LED illumination	250 copies/mL	45 minutes	No. 1 sample per cartridge.	<ul style="list-style-type: none"> <li>• Less than a minute of hands-on time to prepare the sample.</li> <li>• Single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process, minimizing the cross-contamination between samples.</li> </ul>
5	Panther Fusion® SARS-CoV-2 Assay, Hologic (US)	Lab-in-a-Tube	Lysis in a specimen transport tube before testing. Purification and Elution based on magnetic separation method.	Multiplex RT-PCR	Fluorescent intensity	$1 \times 10^{-2}$ TCID <sub>50</sub> /mL	2.4 hours (1150 corona virus tests in	Yes. Multiple samples with random access feature.	<ul style="list-style-type: none"> <li>• Fully automated system operation and high-throughput system with small footprint. 32 assays at a time and up to 60 days of onboard reagent stability.</li> <li>• Test for SARS-CoV-2 and other common respiratory viruses with similar symptoms. Target two conserved regions of ORF1ab gene of SARS-CoV-2.</li> </ul>

							24 hours)		<ul style="list-style-type: none"> <li>Eluted nucleic acid is transferred to a Panther Fusion reaction tube which contains oil and reconstituted mastermix reagents.</li> </ul>
6	Roche's Cobas SARS-CoV-2 Test, Roche (US)	N/A	Lysis by proteinase and lysis reagent. Purification based on magnetic separation method, followed by elution at elevated temperature.	real-time RT-PCR	Fluorescent signal measurement	0.009 TCID <sub>50</sub> /mL	3 hours (96 results) and 8 hours (384 results) for cobas 6800 system	Yes. Multiple samples per run.	<ul style="list-style-type: none"> <li>Work with Cobas 6800/8800 systems, 695/132 installed respectively worldwide.</li> <li>Fully automated sample preparation followed by PCR amplification and detection in enclosed and high-throughput system. 3-hour manual interaction and up to 8 hours of walk-away time.</li> <li>Amplification targets at ORF 1 a/b non-structural region that is unique to SARS-CoV-2 virus. Require 0.6 mL sample.</li> </ul>
7	BioGX SARS-CoV-2 Reagents for, Becton, Dickinson (BD) (US)	Lab-on-a-Chip	Nucleic acids are isolated and purified using BD MAX ExK TNA-3 kit from samples in tubes based on magnetic beads separation method.	real-time RT-PCR	Fluorescent signal measurement	40 copies/mL	~hours	Yes. Up to 24 Samples per run.	<ul style="list-style-type: none"> <li>Work with fully automated BD MAX™ System.</li> <li>Amplify the virus nucleocapsid phosphoprotein gene (N1 and N2 genes) in microfluidic PCR cartridge.</li> <li>Less than 1.5 mins hands-on time per specimen.</li> </ul>
8	Accula SARS-Cov 2 Test, Mesa Biotech Inc. (US)	Lab-on-a-Chip (Lateral flow strip in cassette)	Extraction in buffer in a tube without purification.	RT-PCR	Colorimetric test line on strip (blue)	100 copies	30 minutes	No. One sample test per run.	<ul style="list-style-type: none"> <li>Suitable for POC settings. Use with Accula Dock system, which is affordable, easy to use platform.</li> </ul>
9	ePlex SARS-CoV-2 Test, GenMark Diagnostics, Inc. (US)	Lab-on-a-Chip (Cartridge integrates electrowetting digital microfluidics and electrochemical sensor)	Extracted and purified via magnetic solid phase extraction on digital microfluidic chip inside cartridge.	RT-PCR	Electrochemical signal	1×10 <sup>5</sup> copies/mL	90 minutes	Yes. System support multi-cartridges running although One sample testing per cartridge.	<ul style="list-style-type: none"> <li>Work with ePlex system, which automates all aspects of nucleic acid testing including extraction, amplification and detection in a single-use cartridge.</li> <li>Less than 2 minutes hands-on time.</li> <li>Minimum 200 µL sample required.</li> </ul>
10	ARIES SARS-CoV-2 Assay, Luminex (US)	Lab-in-a-Cartridge (Cassette)	Lysis, isolation and purification in cassette with magnetic capture particles.	RT-PCR	Fluorescent intensity	7.5 ×10 <sup>4</sup> copies/mL	2 hours	Yes. One sample per cassette but one system holds up to six cassettes per run.	<ul style="list-style-type: none"> <li>Work with ARIES magazine system. high-throughput, scalable and cost-effective option.</li> <li>One system for 144 tests per day.</li> </ul>
11	NxTAG CoV Extended	Lab-in-a-Plate	Extract nucleic acids with bioMérieux® easyMAG® and	Multiplex RT-PCR	Fluorescent intensity	5×10 <sup>3</sup> copies/mL	4 hours	Yes. Up to 96 patient samples can be	<ul style="list-style-type: none"> <li>Work with MAGPIX instrument. Target genes are ORF1ab, N and E genes for SARS-CoV-2.</li> </ul>

	Panel plate, Luminex (US)	(Lyophilized bead reagents pre-plated well)	EMAG® Systems utilizing magnetic silica.					run in four hours.	
12	QIAstat-Dx Respiratory SARS-CoV-2 Panel, QIAGEN GmbH (Germany)	Lab-in-a-Cartridge	Lysis using mechanical method (high speed rotor) and chemical means, purification based on membrane.	RT-PCR	Fluorescent intensity	500 copies/mL	1 hour	No. One sample per cartridge per test.	<ul style="list-style-type: none"> <li>• Work with QIAstat-Dx Analyzer system. Fully automated assay in the cartridge including sample preparation and assay testing. Less than one-minute hands on time.</li> <li>• First syndromic testing solution to be deployed in epidemic can differentiate novel coronavirus from 20 other serious respiratory infections.</li> </ul>
13	NeuMoDx SARS-CoV-2 Assay, NeuMoDx Molecular, Inc. (US)	Lab-on-a-Chip	Lysis with buffer in extraction plate wells, containing dry affinity coated paramagnetic beads and plate heating for lysis and binding of NA to beads.	RT-PCR	Fluorescent intensity	150 copies/mL	1 hour	Yes. 12 individual analytical lanes in microfluidic cartridge.	<ul style="list-style-type: none"> <li>• Work with NeuMoDx™ 288 Molecular System or NeuMoDx™ 96 Molecular System.</li> <li>• Target on Nsp2-gene FAM and N gene HEX.</li> <li>• NeuDry Technology instead of traditional reagents requiring lyophilization.</li> </ul>
14	Simplexa COVID-19 Direct assay, DiaSorin Molecular LLC (US)	Lab-on-a-Chip	Lyse by high temperature and centrifugation on disc; No RNA extraction required.	RT-PCR	Fluorescent intensity	500 copies/mL	1 hour	Yes. 96 samples in one hour per run.	<ul style="list-style-type: none"> <li>• Work with the LIAISON MDX system.</li> <li>• Assay targets at SARS-CoV-2 genome, ORF1ab and S gene. Only use a maximum of 10 µL reagent.</li> </ul>
15	Spartan Cube COVID-19 system (CA)	Lab-in-a-Box	N/A	RT-PCR	Fluorescent intensity	N/A	1 hour	No. One sample per test.	<ul style="list-style-type: none"> <li>• Approved by Health Canada.</li> </ul>

Note: N/A: Information not available; copies/mL: Viral genomic copies per milliliter; TCID<sub>50</sub>/mL: 50% tissue culture infectious dose per milliliter; LOD: Limit of detection; RT-PCR: Reverse transcription polymerase chain reaction.