Rapid Antigen Diagnostics as Frontline Testing in the COVID-19 Pandemic

Jiang Xu,* Liam Kerr, Yue Jiang, Wenhao Suo, Lei Zhang, Taotao Lao, Yuxin Chen,* and Yan Zhang*

1. Introduction

The ongoing global COVID-19 pandemic, caused by the SARS-CoV-2 virus, has resulted in significant loss of life since December 2019. Timely and precise virus detection has been proven as an effective solution to reduce the spread of the virus and to track the epidemic. Rapid antigen diagnostics has played a significant role in the frontline of COVID-19 testing because of its convenience, low cost, and high accuracy. Herein, different types of recently innovated in-lab and commercial antigen diagnostic technologies with emphasis on the strengths and limitations of these technologies including the limit of detection, sensitivity, specificity, affordability, and usability are systematically reviewed. The perspectives of assay development are looked into.

J. Xu
Department of Systems Biology
Blavatnik Institute
Harvard Medical School
Boston, MA 02115, USA
E-mail: jiang_xu@hms.harvard.edu

J. Xu
Department of Molecular Virology
Viorgen Biotech Ltd.
3800 Wesbrook Mall, Vancouver, BC V6S 2L9, Canada

L. Kerr
Department of Mechanical Engineering
Center for Intelligent Machines
McGill University
Montreal, QC H3A0C3, Canada

Y. Jiang
China-Australia Institute for Advanced Materials and Manufacturing
Jiaxing University
Jiaxing 314001, China

W. Suo
Dana-Farber Cancer Institute
Harvard Medical School
Boston, MA 02215, USA

W. Suo
Department of Pathology
The First Affiliated Hospital of Xiamen University
55 Zhenhai Road, Xiamen 361003, China

L. Zhang
Department of Chemical Engineering
Waterloo Institute for Nanotechnology
University of Waterloo
200 University Avenue West, Waterloo, ON N2L3G1, Canada

T. Lao
Department of Molecular Diagnostics
Boston Molecules Inc.
564 Main Street, Waltham, MA 02452, USA

T. Lao
Center for Immunology and Inflammatory Diseases
Massachusetts General Hospital
Harvard Medical School
Charlestown, MA 02114, USA

Y. Chen
Department of Laboratory Medicine
Nanjing Drum Tower Hospital
Nanjing University Medical School
Nanjing, Jiangsu 210008, China
E-mail: yuxin.chen@nju.edu.cn

Y. Zhang
Tianjin Key Laboratory for Modern Drug Delivery and High-Efficiency Collaborative Innovation Center of Chemical Science and Engineering School of Pharmaceutical Science and Technology
Tianjin University
Tianjin 300072, China
E-mail: yan.zhang@tju.edu.cn

Y. Zhang
Frontiers Science Center for Synthetic Biology (Ministry of Education)
Tianjin University
Tianjin 300072, China

The ongoing global COVID-19 pandemic, caused by the SARS-CoV-2 virus, has resulted in significant loss of life since December 2019. Timely and precise virus detection has been proven as an effective solution to reduce the spread of the virus and to track the epidemic. Rapid antigen diagnostics has played a significant role in the frontline of COVID-19 testing because of its convenience, low cost, and high accuracy. Herein, different types of recently innovated in-lab and commercial antigen diagnostic technologies with emphasis on the strengths and limitations of these technologies including the limit of detection, sensitivity, specificity, affordability, and usability are systematically reviewed. The perspectives of assay development are looked into.

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/smsc.202200009.

© 2022 The Authors. Small Science published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

DOI: 10.1002/smsc.202200009
also been recorded in longitudinal cohort studies.\(^5,^6\) Since the outbreak of the COVID-19 pandemic in December 2019, dozens of variants with distinct strain clades have arisen globally (Figure 1a), seven of which were labeled and classified as variants of concern or interest by World Health Organization (WHO)\(^7\) as of April 12, 2022 (Figure 1b). Despite the recent development of COVID-19 vaccines,\(^8,^9\) the lower-than-expected vaccination rates,\(^10,^11\) fast waning vaccine effectiveness,\(^12\) and ascending demand of booster doses\(^13,^14\) make the progression of the pandemic difficult to predict. Furthermore, despite their effectiveness in reducing the rates of severe diseases and mortality, none of the available vaccinations have been demonstrated to be effective in stopping the spread of COVID-19. Therefore, timely detection of COVID-19 is extremely important for monitoring the scale of infection and for ensuring early treatment.

This perspective focuses on the antigen test because it offers distinct advantages over other diagnostic approaches such as the nucleic acid (NA) test and the antibody test. NA tests in general employ polymerase chain reaction (PCR) technology, requiring repetitive temperature control steps for signal amplification, and thus are time-consuming and instrument dependent.\(^15\) In contrast, antigen tests are quick and in expensive, with some of them simple to operate, making them suitable for the purposes of use in-home, at the point of care (POC), and even self-testing.\(^16\) So far antigen tests have been broadly used and proven to be able to greatly alleviate the testing demands when PCR resources are saturated due to prematurely relaxing lockdown measures in many countries.\(^17\) In addition, antigen tests are effective in detecting asymptomatic infections and provide valid results prior to symptom onset for symptomatic infections, which is in contrast to antibody tests that are 1–2 weeks delayed in response (Figure 2).

The omicron variant has become the prevalent strain in most nations since the start of year 3 of the COVID-19 pandemic. It was fortunate that the measured case fatality rate had fallen considerably, most likely due to the protection of vaccination and the efficacy of new antiviral drugs. As the public is weary and longs for a return to normal, many countries have lifted the lockdown ignoring the fact that omicron is a highly transmissible variant with an average relative \(R_0\) (basic reproduction number) to Delta as 2.5.\(^18\) As a result, the number of infected people soared, quickly overwhelming the NA testing capacity at many places. Under such backgrounds, commercial antigen test kits especially self-testing kits were approved for emergency use around the world. More recently excess mortality rate was used to evaluate the damage to the community by Omicron,\(^19,^20\) highlighting the importance of tighter pandemic control and more frequent testing of potential viral exposure. Up to the date of this manuscript

![Figure 1. Timeline of the emergence of SARS-CoV-2 variants and those of greater concern.\(^116\) a) Timeline of the emergence of SARS-CoV-2 variants. All variants are presented with corresponding first-identified date, pango lineage, and first-identified location, ten of which (labeled in orange) have been named by WHO using letters of the Greek Alphabet as of December 2021. b) Date of designation of variants of concern by WHO.](image-url)
in preparation, more than 1000 commercial antigen test kits have been available in Europe and the United States. We have performed a thorough review of these commercial kits devoting attention to their targets and testing principles, statistically comparing their performance and application scenarios, hoping to guide the choice and use of antigen tests and more importantly to direct future development of pandemic control policy and testing technology.

2. Targets of SARS-CoV-2 Antigen Detection

The genome size of SARS-CoV-2 is 29.6 kilobase, which only shares 79.0% sequence identity to SARS-CoV\(^{[21]}\) and thus contributes to the unique pathogenicity of SARS-CoV-2.\(^{[22]}\) The SARS-CoV-2 contains 16 nonstructural proteins (NSP), 9 other accessory factors, and 4 structural proteins.\(^{[23]}\) Four structural proteins, including spike (S), envelope (E), membrane (M), nucleocapsid (N), are composed of 1273, 75, 222, and 419 amino acids, respectively.\(^{[24]}\) The S protein of SARS-CoV-2 is a trimeric glycoprotein consisting of two subunits (S1 and S2) upon cleavage by the host protease, responsible for attachment and fusion of viral and cellular membranes through angiotensin-converting enzyme 2 (ACE2) of the host cells, respectively\(^{[25]}\) (Figure 3). The important physiological role and abundance of S protein potentiate a good target in antigen diagnostic tests. However, it was soon realized that the S protein contains mutation hotspots. These mutations even caused significant changes of the overall protein structure\(^{[26,27]}\) that are in turn responsible for altered antigenic properties. Taken together, it is not practical to develop an anti-S antibody-based antigen test kit that could be applied to detect all variants from Alpha to Omicron and the next new variants (Figure 3). To date, most COVID-19 test kits, especially commercial ones, target the N protein, which is the most conserved protein among all four structural proteins and is more abundant than the E and M proteins\(^{[28]}\) (Table 1).

3. Principles of SARS-CoV-2 Antigen Tests

Based on the testing principles, the existing Sars-CoV-2 antigen tests can be categorized into lateral flow assay (LFA), enzyme-linked immunosorbent assay (ELISA), chemiluminescence assay (CLIA), electrochemical assay, and surface plasmon resonance (SPR) assay (Figure 4).
4. Lateral Flow Assay (LFA)

LFA is a multilayered paper-like substrate with functional components including a sample well, a conjugate pad, and a nitrocellulose membrane featured with testing and control lines (Figure 5). In a typical LFA test, buffer solution contains lysing components (i.e., Triton X-100) to decompose the viruses in a collected sample down to small antigen fragments (Figure 5 Step 1), which reduces steric hindrance of target antigen sites and thus facilitates subsequent antigen binding. Sample solution is added to the sample pad and flows toward the conjugated pad (Figure 5 Step 2), where gold nanoparticles conjugated to a specific COVID-19 antibody (Ab 1) are embedded. The antigens in a positive sample bind to the Ab-1-conjugated nanoparticles and form complexes (Figure 5 Step 3), which continue to migrate and are immobilized by another antibody (Ab 2) at the test line (Figure 5 Step 4). An irrelevant antibody pair is often employed with one conjugated to gold nanoparticles and the other at the control line (Figure 4 Step 5 and 6).

The limit of detection (LoD) of LFAs are reported to be around $10^3$–$10^4$ viral copies mL$^{-1}$ (equivalent to a Ct value in the range of 20–30 in qPCR assay) [29–31], which is considered to be a viral load with relatively low risk of transmission.[32] Fluorescent dyes are also commonly used for higher sensitivity of LFAs.[33] SARS-CoV-2 LFA is valuable as an alternative solution to NA testing for large-scale screening due to its easy operation, low cost, and fast readout. Its accuracy has also been recognized by certain regions in the implementation of public health and travel policies.[34–36] The major disadvantage of LFA lies in its relative low sensitivity compared with NA testing. In addition, color appearance and intensity is based on subjective perception and thus a significant difference in test sensitivity was found between professional and self-trained users.[37] Smartphones[38–40] and artificial intelligence[41,42] have been employed to overcome this limitation by improving result interpretation and data collection.

5. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is a common assay in analytical biochemistry, first described by Engvall and Perlmann in 1971,[43] particularly for soluble protein targets like peptides,[44] antibodies,[45] and hormones.[46] ELISA detects analyte through a specific antigen–antibody interaction and a subsequent color or fluorescence signal generated from enzyme–substrate reaction[47] (Figure 6). The sandwich ELISA method is particularly suitable for the detection of unconcentrated targets in solution and is therefore the most

---

Table 1. Mutated amino acid count of Omicron variant (BA.1) by comparing with Wuhan-Hu-1 (NCBI Reference Sequence: NC 045512.2) reference sequence.[120,121]

| Structural proteins | Mutational counts | Mutation rates [normalized by length] |
|---------------------|-------------------|--------------------------------------|
| Spike (S)           | 32                | 2.51%                                |
| Envelope (E)        | 1                 | 1.33%                                |
| Membrane (M)        | 3                 | 1.35%                                |
| Nucleocapsid (N)    | 4                 | 0.95%                                |

---

Figure 4. Common SARS-CoV-2 viral antigen diagnostic methods based on different mechanisms. This figure was created with BioRender.com.
widely used ELISA method for detection of COVID-19 antigens.\cite{48} ELISA test can either qualitatively or quantitatively identify viral copies on the scale of $10^3$ viral copies mL$^{-1}$.\cite{48} Such sensitivity is close to the PCR-based NA tests\cite{48} and better than typical LFAs.\cite{49} Moreover, the signal intensities (absorbance or fluorescence) of ELISA are well correlated with the concentrations of the analyte.\cite{50,51} Thus, it has been used as a standard tool for antibody screening\cite{52,53} and methodology evaluation.\cite{54,55} Nevertheless, the excellent LoD and sensitivity of ELISA are equipment dependent requiring a fluorescent/color signal reader,\cite{56} which inevitably increases the complexity of operation and time to result (typically 1–5 h\cite{57}), restricting its application in point of care testing (POCT) scenarios.\cite{55}

6. ChemiLuminescent Immunoassay (CLIA)

Chemiluminescence makes use of a special type of chemical reaction, in which when the intermediates return from their excited state to their stable ground state, a photon is released and can be detected by the luminescent signal instrument.\cite{58,59} Based on such a mechanism, chemiluminescence immunoassays (CLIA) is developed for quantitative antigen detection.\cite{59} In a typical CLIA, magnetic beads coated with anti-COVID-19 antibodies (Ab1) (Figure 7a) can specifically bind to SARS-CoV-2 viral antigens in clinical samples (Figure 7b). The viral antigen then binds to another antibody (Ab2) conjugated with either luminophore markers (such as acridinium and ruthenium esters) or enzyme

---

**Figure 5.** Schematic of testing workflow and mechanism of a typical LFA, created with BioRender.com.

**Figure 6.** Basic setup and procedures of sandwich ELISA and principles of direct, indirect, and competitive ELISAs. This figure was created with BioRender.com.

---
markers (such as alkaline phosphatase and horseradish peroxidase with luminol) and later forms an Ab 1-antigen-Ab 2 immune complex (Figure 7c). The light emission is often times initiated by adding the pretrigger and/or trigger solutions (Figure 7d). SARS-CoV-2 antigens could be calibrated by the intensity of luminescence. The compatibility of multiplex tests for various biomarkers and the potential for high-throughput automation are two of CLIA’s primary advantages over traditional LFA. CLIA may also be adaptable to a variety of test formats and is fast in data collecting with minimum noise interference.

7. Electrochemical Assay

In recent years, electrochemical sensors have been rapidly developed for detection of biological markers. Electrochemical signals can be classified as voltametric, impedimetric, and amperometric (Figure 8). The electrical signals generated by the redox reactions between their recognition groups and target molecules on the electrode surface endow electrochemical sensors high specificity and sensitivity, advantages of both chemical reaction and electrochemical conduction. Unlike LFA and ELISA employing multiple antibodies, the electrochemical sensor-based platform requires no complex labeling reagent. This technology has been used in detection of COVID-19 antigen with great performance manifested by low LoD and high specificity. Electrochemical sensors that are capable of distinguishing various forms of SARS-CoV-2 spike proteins have been reported. Compared with traditional ELISA and NA tests, electrochemical sensors have much faster readouts (within a few minutes) and lower manufacturing costs. Furthermore, electrochemical assays can be easily integrated with digital analysis and big data collection using cloud-connected mobile apps. Other advantages of the electrochemical assay include portability, less reagent consumption, and less preprocessing, with all well suited for POCT.

Figure 7. Mechanistic illustration of CLIA detecting SARS-CoV-2 antigen. This figure was created with BioRender.com.

Figure 8. Principles of electrochemical techniques used for the detection of SARS-CoV-2 viral antigens. Inputs of antigen–antibody binding can be output in forms of voltametric, impedimetric, and amperometric signals via an electrochemical analyzer. This figure was created with BioRender.com.
electrochemical sensor-based platform is promising not only for COVID diagnosis but also for the detection of many other disease biomarkers.\(^{[67]}\)

8. Surface Plasmon Resonance (SPR) Assay

The SPR assay enables rapid measurements of kinetics and affinity of bimolecular binding in real-time and quantitative fashion.\(^{[73]}\) It is based on the electromagnetic resonance of the collective oscillations of free electrons occurring at a plasmonic metallic interface(i.e., a thin gold film)\(^{[24]}\) (Figure 9a). This resonance intensity signal varies with the extent of the absorption of target molecules to ligands grafted onto the interface, which can be accurately detected and then converted to the quantification of molecules of interest\(^{[75]}\) (Figure 9b). The SPR test can transform tiny amounts of viral antigen into sensitive electrical signals, allowing for in situ and dynamic detection of virus presence and concentration.\(^{[76]}\) The SPR assay, in contrast to sophisticated and expensive NA assays, is a relatively simple and low-cost procedure.\(^{[77]}\) In comparison with LFAs, the SPR assay provides superior LoD and sensitivity,\(^{[78]}\) which can be as low as 100 viral particles mL\(^{-1}\).\(^{[79]}\) This sensitivity is sufficient to classify most clinical samples, given that the median viral load for SARS-CoV-2 in nasal specimens was around 6 \(\times\) 10\(^6\) copies mL\(^{-1}\).\(^{[80]}\) Briefly, as an antigen detection technology, the SPR assay has good simplicity and sensitivity and can be used as an effective alternative screening method to NA tests in evaluating large numbers of samples.\(^{[81]}\) However, its relatively high equipment dependence and reliance on fine processing of agents are currently prohibitive to POCT scenarios and in-home use.\(^{[82]}\)

9. Commercial COVID Antigen Test Kits in Western Countries

The outbreak of COVID-19 has ushered in a period of rapid market expansion in the diagnostics industry. The global market for COVID-19 diagnostic services was valued at $60.3 billion in 2020, with an estimated compound annual growth rate (CAGR) of > 15% during 2021–2027.\(^{[83]}\) Compared with the gold standard NA tests, antigen tests have much simpler operation and faster readout, with more flexible facility and operator requirements. Therefore, the development of novel commercial COVID-19 antigen detection technology to supplement molecular testing is of great significance.

Here we summarized the commercial antigen test kits in the American and European markets. From October 2020 to April 2022, 48 antigen diagnostic tests for SARS-CoV-2, from 28 manufacturers, were granted Emergency Use Authorization (EUAs) by Food and Drug Administration (FDA) (Table 2). Among them, 32 test kits are classical LFAs providing visual readouts, accounting for two-thirds of all tests with EUAs. The remaining tests that require additional instruments to aid in the reading include ten fluorescence LFA test kits, four CLIA test kits, one electrochemical assay, and one SPR assay. The simplicity and rapidity of LFAs may be important reasons as to why they are favored by in vitro diagnostics (IVD) companies. In addition, 47 test kits target N protein, and there are only two products targeting RBD domains of S protein, including 1 N/S hybrid test kit. This is most likely due to the fact that S protein is less abundant and more prone to mutations under selective pressure than N protein.\(^{[84]}\) More importantly, many commercial test kits have satisfactory sensitivities (>90%) and specificities (>95%), with a considerable number of kits even claiming 100% specificity, and most test kits can provide results within 30 min.

Features including LoD, sensitivity, effective time, cost, and operability of test kits in the U.S. market are summarized using a five-star chart model (Figure 10). Generally, LFA is obviously superior to all other test methods in terms of effective time, cost, and operability, with compromised LoD and sensitivity. Among five methods, ELISA and CLIA exhibit intermediate levels of LoD, sensitivity, cost, and operability. Despite relatively longer assay time required, satisfactory parameters make these two methods as standard protocols in R&D labs but much less popular in self-testing and POC scenarios. Electrochemical and SPR assays present excellent LoD and sensitivities but their commercial competitiveness is hampered by their high cost, long reaction time, and complex operations. One should recognize that the LoD and sensitivities of the commercial test kits were not necessarily correlated. A possible explanation is that the LoDs were measured with inactivated viruses while the sensitivities were measured with clinic specimens. Particularly, various manufacturers of inactivated viruses might have different quality control standards, which could subsequently lead to different levels of specific antigens arising from unassembled viral particles and different tissue culture infectious dose (TCID) assay results.\(^{[85]}\) Proteins and
Table 2. Commercial COVID-19 antigen tests issued with U.S. FDA EUAs as of April 6, 2022.

| Product name                        | Entity                  | Date EUA issued or last updated | Mechanism | Target       | Readout | PPA+ NPA        | Authorized settings | Effective time |
|-------------------------------------|-------------------------|---------------------------------|-----------|--------------|---------|-----------------|--------------------|-----------------|
| Pilot COVID-19 At-Home Test         | SD Biosensor, Inc.      | 04/04/2022                      | LFA       | N protein    | Visual  | 95.3%/100%     | Home, H, M, W      | 20–30 min      |
| iHealth COVID-19 Antigen Rapid Test | iHealth Labs, Inc.      | 04/04/2022                      | LFA       | N protein    | Visual  | 94.3%/98.1%    | Home, H, M, W      | 15–30 min      |
| BinaxNOW COVID-19 Antigen Self Test | Abbott Diagnostics      | 04/04/2022                      | LFA       | N protein    | Visual  | 84.6%/98.5%    | Home, H, M, W      | 15–30 min      |
| MaximBioClearDetect COVID-19 Antigen Home Test | Maxim Biomedical, Inc. | 03/30/2022                      | LFA       | N protein    | Visual  | 86.9%/98.9%    | Home, H, M, W      | 15–30 min      |
| iHealth COVID–19 Antigen Rapid Test | iHealth Labs, Inc.      | 03/29/2022                      | LFA       | N protein    | Visual  | 94.3%/98.1%    | Home, H, M, W      | 15–30 min      |
| BD Veritor At-Home COVID-19 Test    | Becton, Dickinson       | 03/25/2022                      | LFA       | N protein    | Digital | 84.6%/99.8%    | Home, H, M, W      | >20 min        |
| CareStart COVID-19 Antigen Home Test | Access Bio, Inc.       | 03/25/2022                      | LFA       | N protein    | Visual  | 87.0%/98.0%    | Home, H, M, W      | 10–15 min      |
| CelltrionDiaTrust COVID-19 Ag Home Test | Celltrion USA, Inc.   | 03/23/2022                      | LFA       | N & S protein| Visual  | 86.7%/99.8%    | Home, H, M, W      | 15–20 min      |
| CLINITEST Rapid COVID-19 Antigen Self-Test | Siemens Healthineers | 03/23/2022                      | LFA       | N protein    | Visual  | 86.5%/99.3%    | Home, H, M, W      | 15-20 min      |
| Ellume COVID–19 Home Test           | Ellume Limited          | 03/18/2022                      | LFA (fluorescence) | N protein | Digital | 96.0%/100%     | Home, H, M, W      | 15 min         |
| INDIKAID COVID-19 Rapid Antigen At-Home Test | PHASE Scientific International, Ltd. | 03/16/2022                      | LFA       | N protein    | Visual  | 81.7%/99.4%    | Home, H, M, W      | 20–25 min      |
| Atellica IM SARS-CoV-2 Antigen (CoV2Ag) | Siemens Healthcare Diagnostics, Inc. | 03/11/2022                      | CLIA      | N protein    | Digital | 85.1%/100%     | H, M               | >24 min        |
| ADVIA Centaur SARS-CoV-2 Antigen (CoV2Ag) | Siemens Healthcare Diagnostics, Inc. | 03/11/2022                      | CLIA      | N protein    | Digital | 85.1%/100%     | H, M               | >24 min        |
| Clip COVID Rapid Antigen Test       | Luminostics, Inc.      | 03/04/2022                      | LFA (fluorescence) | N protein | Digital | 96.9%/100%     | H, M, W            | >30 min        |
| SCoV-2 Ag Detect Rapid Test         | InBios International, Inc. | 03/03/2022                      | LFA       | N protein    | Visual  | 86.7%/100%     | Home, H, M, W      | 20–25 min      |
| SCoV-2 Ag Detect Rapid Self-Test    | InBios International, Inc. | 03/03/2022                      | LFA       | N protein    | Visual  | 85.7%/100%     | H, M               | 20–25 min      |
| ASSURE-100 Rapid COVID-19 Test      | Oceanit Foundry LLC     | 02/28/2022                      | LFA       | N protein    | Visual  | 89.0%/100%     | H, M               | 20–30 min      |
| INDIKAID COVID-19 Rapid Antigen Test | PHASE Scientific International, Ltd. | 02/22/2022                      | LFA       | N protein    | Visual  | 86.7%/97.2%    | H, M               | 20–25 min      |
| Flowflex COVID-19 Antigen Home Test | ACON Laboratories, Inc  | 02/18/2022                      | LFA       | N protein    | Visual  | 92.0%/100%     | Home, H, M, W      | 15–30 min      |
| LumiraDx SARS-CoV-2 Ag Test         | LumiraDx UK Ltd.       | 02/17/2022                      | LFA       | N protein    | Digital | 97.6%/96.6%    | H, M, W            | ~12 min        |
| LIAISON SARS-CoV-2 Ag              | DiaSorin, Inc.         | 02/16/2022                      | CLIA      | N protein    | Digital | 84.4%/99.5%    | H, M               | 120-180 min    |
| BinaxNOW COVID-19 Ag Card           | Abbott Diagnostics      | 02/04/2022                      | LFA       | N protein    | Digital | 84.6%/98.5%    | Home, H, M, W      | 15–30 min      |
| BinaxNOW COVID-19 Ag Card Home Test | Abbott Diagnostics      | 02/04/2022                      | LFA       | N protein    | Digital | 84.0%/98.3%    | H, M               | 15–30 min      |
| Nano-Check COVID-19 Antigen Test    | Nano-Ditech Corp.      | 02/01/2022                      | LFA       | N protein    | Digital | 90.3%/100%     | H, M, W            | 15–20 min      |
| IntelSwab COVID-19 Rapid Test Rx    | OraSure Technologies, Inc. | 01/27/2022                      | LFA       | N protein    | Visual  | 85.0%/98.0%    | Home, H, M, W      | 30–40 min      |
| IntelSwab COVID-19 Rapid Test       | OraSure Technologies, Inc. | 01/27/2022                      | LFA       | N protein    | Visual  | 85.0%/98.0%    | Home, H, M, W      | 30–40 min      |
Table 2. Continued.

| Product namea) | Entity | Date EUA issued or last updated | Mechanism | Target | Readout | PPA+NPA | Authorized settings | Effective time |
|----------------|--------|--------------------------------|-----------|--------|---------|---------|--------------------|----------------|
| InteliSwab COVID-19 Rapid Test Pro | OraSure Technologies, Inc. | 01/27/2022 | LFA | N protein | Visual | 85.0%/98.0% | Home, H, M, W | 30–40 min |
| iHealth COVID-19 Antigen Rapid Test Pro | iHealth Labs, Inc. | 01/14/2022 | LFA | N protein | Visual | 88.2%/100% | H, M, W | 15–30 min |
| Simoa SARS-CoV-2 N Protein Antigen Test | Quanterix Corporation | 12/21/2021 | LFA | N protein | Visual | 83.9%/99.9% | H, M | 10–20 min |
| Sienna-Clarity COVID-19 Antigen Rapid Test Cassette | Salofa Oy | 12/17/2021 | LFA | N protein | Visual | 87.5%/98.9% | H, M, W | 10–20 min |
| BD Veritor System for Rapid Detection of SARS-CoV-2 | Becton, Dickinson and Company | 12/10/2021 | LFA (fluorescence) | N protein | Digital | 84.0%/100% | H, M, W | 15–20 min |
| CareStart COVID-19 Antigen Test | Access Bio, Inc. | 12/02/2021 | LFA | N protein | Visual | 93.4%/99.3% | H, M, W | 10–15 min |
| GenBody COVID-19 Ag | GenBody Inc. | 11/17/2021 | LFA | N protein | Visual | 91.1%/100% | H, M, W | 15–20 min |
| VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack | Ortho Clinical Diagnostics, Inc. | 11/16/2021 | CLIA | N protein | Digital | 80.0%/100% | H, M | >48 min |
| QuickVue SARS Antigen Test | Quidel Corporation | 11/09/2021 | LFA | N protein | Visual | 96.8%/99.1% | H, M, W | 10–15 min |
| Status COVID-19/Flu A&B | Princeton BioMeditech Corp. | 10/27/2021 | LFA | N protein | Visual | 93.1%/100% | H, M, W | 15–20 min |
| QuickVue At-Home OTC COVID-19 Test | Quidel Corporation | 10/21/2021 | LFA | N protein | Visual | 83.5%/99.2% | Home, H, M, W | 10–15 min |
| SPERA COVID-19 Ag Test | Xtrava Health | 10/12/2021 | LFA | N protein | Visual | 91.8%/96.9% | H, M, W | 15–30 min |
| NIDS COVID-19 Antigen Rapid Test Kit | ANP Technologies, Inc. | 09/24/2021 | LFA | N protein | Visual | 95.1%/97.0% | H, M, W | 15–30 min |
| CelltrionDiaTrust COVID-19 Ag Rapid Test | Celltrion USA, Inc. | 09/01/2021 | LFA | N protein | Visual | 93.3%/99.0% | H, M, W | 15–20 min |
| QiAreach SARS-CoV-2 Antigen Test | QIAGEN GmbH | 08/05/2021 | LFA (fluorescence) | N protein | Digital | 85.0%/99.1% | H, M | 2–15 min |
| ellume.lab COVID Antigen Test | Ellume Limited | 07/08/2021 | LFA (fluorescence) | N protein | Digital | 81.8%/100% | H, M, W | 3–15 min |
| Sofia SARS Antigen FIA | Quidel Corporation | 06/11/2021 | LFA (fluorescence) | N protein | Digital | 96.7%/100% | H, M, W | ~15 min |
| Omnia SARS-CoV-2 Antigen Test | Qorvo Biotechnologies, LLC. | 04/13/2021 | SPR assay | N protein | Digital | 89.5%/100% | H, M | 15–20 min |
| BD Veritor System for Rapid Detection of SARS-CoV-2 & Flu A+B | Becton, Dickinson and Company | 03/24/2021 | LFA (fluorescence) | N protein | Digital | 86.7%/99.5% | H, M, W | 15–20 min |
| QuickVue At-Home COVID-19 Test | Quidel Corporation | 03/01/2021 | LFA | N protein | Visual | 84.8%/99.1% | Home, H, M, W | 10–15 min |
| Sampinute COVID-19 Antigen Mia | Celltrion USA, Inc. | 10/23/2020 | Electrochemical assay | S protein | Digital | 94.4%/100% | H, M | >40 min |
| Sofia 2 Flu + SARS Antigen FIA | Quidel Corporation | 10/02/2020 | LFA (fluorescence) | N protein | Digital | 95.2%/100% | H, M, W | ~15 min |

a)Products are listed in a sequence of the date EUA issued or last updated. This table is updated from our published work[90] and the U.S. FDA website[122]. Effective time is counted from the contact of swab sample and buffer. PPA and NPA stand for positive percentage agreement and negative percentage agreement with NA test thus representing sensitivity and specificity of a test, respectively. N protein: nucleocapsid protein. S protein: Spike protein. H: Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) that meet requirements to perform high complexity tests. M: Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) that meet requirements to perform moderate complexity tests. W: Patient care settings operating under a CLIA Certificate of Waiver.
sensitivity. Many European test kits had already been engaged in interpretation tools or essential auxiliary devices to convert visual signals to digital readout, which may improve assay LoD and sensitivity. Many European test kits had already been engaged in such attempts that nearly 70 products utilized semi-automated, automated, or even robot-assisted assays (Figure 11f). Devises or technologies that involve smart phones also facilitate in-home data collection and result interpretation. An alternative approach is to use bispecific monoclonal antibodies to boost up the signal and improve sensitivity. Concerns have been raised whether rapid antigen tests for SARS-CoV-2 can result in false-positive test results and undermine pandemic management for COVID-19. Emerging evidence suggested that the overall rate of false-positive results among the total rapid antigen test screens for SARS-CoV-2 was very low. However, false-positive results can occur, especially when used in situations where the prevalence of infection is low, which is true for all in vitro diagnostic tests.

The sample loading volumes in most commercial tests are usually low (i.e., several drops), because their capillary or microchannel structures relying on passive diffusion to process samples can be easily saturated by excessive fluid. The intrinsic defect significantly slows detection and increases the possibility of false-negative results. Combining ultrahigh-throughput hydrodynamic filtration and sandwich immunoassay is one promising solution for increasing sample loading volume. Indeed, in our recent study, using a simple handheld injection, the microfluidic test kit was able to process fluid samples on a milliliter scale, a volume that was 1–2 orders of magnitude greater than that of conventional methods resulting in improved sensitivity.

The emergence of SARS-CoV-2 variants has also raised concerns about evasion of detection by rapid antigen diagnostics. Indeed, mutation of N protein in SARS-CoV-2 VoCs such as D399N and T135I may potentially lead to false-negative results in rapid antigen tests, despite a high viral load. Indeed, mutation of N protein in SARS-CoV-2 VoCs such as D399N and T135I may potentially lead to false-negative results in rapid antigen tests, despite a high viral load. [91, 92] Polyclonal anti-N antibodies have been shown sensitive against various mutants including N501Y, H69/V70, D796H, and D614G. [93, 94] When a single monoclonal antibody is used for capturing or labeling, it is of greater concern whether the performance of the test could be altered by emerging strains of SARS-CoV-2. On the other hand, the FDA requests that antigen test manufacturers report surveillance when an existing test becomes invalid due to a new viral variant.

To expedite identification and isolation of infected cases, COVID-19 antigen self-testing has been implemented worldwide. The advantages for antigen testing over NA testing are obvious. Most antigen tests are easy to distribute and could be carried out in home, while NA testing relies on a PCR laboratory, trained professionals, and sophisticated testing procedures, which are further complicated by pooled assays in some highly populated epicenters such as Shanghai. The individuals in such epicenters have to wait in queues for NA tests, taking the risk of being infected on site, while in-home antigen self-testing minimizes the chance of viral exposure. According to studies, more frequent screening reduces the likelihood of an outbreak, and the fast diagnostic turnaround time of an antigen test tends to outweigh the reduced sensitivity of NA testing. Because antigen testing is simple, low cost, and immediate, massive rapid-testing programs have been implemented in public places such as schools, hospitals, prisons, airports, borders, workplaces, tourist attractions, and parks. In those crowded places, we envision flexible transoral robot adopted for COVID-19 swab sampling, reducing the risk of infection while also ensuring accurate swab sampling. The quarantine and isolation policy for individuals who are antigen-tested positive varies by
countries. Authorities in some countries require an immediate NA test to confirm infection. Currently, certified laboratories and testing sites are committed to report all positive cases to the state or local public health departments. An effective report system for positive cases in antigen self-test is important for public health surveillance and to avoid community transmission of COVID-19.

10. Conclusion and Outlook

Antigen detection provides a number of distinct advantages: 1) high speed, low cost, and noninvasive small volumes of sampling;[97] 2) equipment-free interpretation of qualitative results and mild equipment dependence to interpret semiquantitative results;[98] 3) relatively short R&D cycles and quick market-oriented iterations;[99] 4) easy sampling and simple testing procedures;[100] 5) universal test objects and scenarios;[101] and 6) long shelf-life and easily achievable storage conditions.[102]

Antigen tests remain to be further improved in many aspects: 1) inaccurate sample volume affects accuracy (especially in LFA products with manual sample loading);[103] 2) small sample volume can limit LoD and sensitivity;[90] 3) antigen–antibody binding signal barely undergoes a secondary amplification like in NA tests; thus, trace amounts of antigen could become undetectable;[104] 4) time to results is usually uncertain and varies by the volume, viscosity, concentration, etc. of the sample (Table 1); and 5) the fact that antigen–antibody testing relies on macroscopic effects (aggregation, color change, current change, etc.) is problematic due to the intrinsic scarcity of antigen proteins of SARS-CoV-2 viruses.[105] Therefore, the test sensitivity can be uncertain as the virus load gradually drops to a low concentration, which usually occurs in the first 5–7 days
before symptom onset and the late postsymptom period. Nevertheless, daily screening could significantly increase the chances of detection.

We envision the following future technical developments of current COVID-19 antigen diagnostic platforms: 1) acceleration of antigen design and high-throughput screening; 2) combinations of technologies like lab-on-a-chip and machine learning; 3) cloud methods to increase data collection and reduce misinterpretation of results; 4) improvement of quality controls and manufacturing practices to meet the increasing demand for test kits for individuals with disabilities (i.e., color blindness, impaired mobility) and areas with inadequate medical infrastructure.

With its accuracy, cost effectiveness, speed, and simplicity, we look forward to antigen testing continuing to play an important role in the battle against the COVID-19 pandemic.

Acknowledgements
J.X. and T.L. thank the Rapid Acceleration of Diagnostics (RADx) program from National Institutes of Health (grant nos. RADx 3343 and RADx 7748) for funding. Y. C. was supported by the Clinical Trials from the Affiliated Drum Tower Hospital, Medical School of Nanjing University (2021-LCYJ-001); X.C. was supported by the Clinical Trials from the Affiliated Drum Tower Hospital, Medical School of Nanjing University (2021-LCYJ-002). The authors declare no conflict of interest.

Conflict of Interest
The authors declare no conflict of interest.

Keywords
antigen tests, COVID-19, in vitro diagnostics, immunoassays

Received: January 30, 2022
Revised: April 25, 2022
Published online: July 5, 2022

[1] B. Zhou, W. Zhao, R. Feng, X. Zhang, X. Li, Y. Zhou, L. Peng, Y. Li, J. Zhang, J. Luo, Pathog. Dis. 2020, 78, faa026.
[2] Q. Liu, Y. Shi, J. Cai, Y. Duan, R. Wang, H. Zhang, Q. Ruan, J. Li, L. Zhao, Y. Ping, Nat. Sci. Rev. 2020, 7, 1658.
[3] S. Li, L. Jiang, X. Li, F. Lin, Y. Wang, B. Li, T. Jiang, W. An, S. Liu, H. Liu, JCI Insight 2020, 5, 12.
[4] A. F. Rendiero, H. Ravichandran, Y. Bram, V. Chandar, J. Kim, C. Meydan, J. Park, J. Fox, T. Hether, S. Warren, Nature 2021, 593, 564.
[5] L. Huang, Q. Yao, X. Gu, Q. Wang, L. Ren, Y. Wang, P. Hu, L. Guo, M. Liu, J. Xu, Lancet 2021, 398, 747.
[6] Q. Xiong, M. Xu, J. Li, Y. Liu, J. Zhang, Y. Xu, W. Dong, Clin. Microbiol. Infect. 2021, 27, 89.
[7] F. Konings, M. D. Perkins, J. H. Kuhn, M. J. Pallen, E. J. Alm, B. N. Archer, A. Barakat, T. Bedford, J. N. Bhiman, L. Caly, Nat. Microbiol. 2021, 6, 7.
[8] M. W. Tenforde, MMWR 2021, 70, 1355.
[9] J. L. Bernal, N. Andrews, C. Gower, E. Gallagher, R. Simmons, S. Thelwall, J. Stowe, E. Tessier, N. Groves, G. Dabrera, N. Engl. J. Med. 2021, 385, 585.
[10] P. Sah, T. N. Vilches, S. M. Moghadas, M. C. Fitzpatrick, B. H. Singer, P. J. Hotez, A. P. Galvani, E Clin. Med. 2021, 35, 100865.
[11] J. Rodriguez; M., Paton; J. M., Acurra, medRxiv 2021, 2020.10.12.2021094.
[12] K. B. Pouwels, E. Pritchard, P. C. Matthews, N. Stoesser, D. W. Eyre, K-D. Vihta, T. House, J. Hay, J. I. Bell, J. N. Newton, J. Farrar, D. Crook, D. Cook, E.ourke, R. Studley, T. Peto, I. Diamond, A. S. Walker, Prepr. Univ. Oxford 2021.
[13] E. H. Livingston, JAMA 2021, 325, 859.
[14] CDC, https://www.cdc.gov/coronavirus/2019-ncov/vaccines/recommendations/immuno.html 2021 (accessed: June 2022).
[15] M. N. Esbin, O. N. Whitney, S. Chong, A. Maurer, X. Darzacq, R. Tjian, RNA 2020, 26, 771.
[16] V. Agulló, M. Fernández-González, V. O. de la Tabla, N. Gonzalo-Jiménez, J. A. García, M. Masiá, F. Gutiérrez, J. Infect. 2021, 82, 186.
[17] T. Rawson, T. Brewer, D. Veltcheva, C. Huntingford, M. B. Bonsall, Front. Public Health 2020, 8, 262.
[18] Y. Liu, J. Rocklov, J. Travel Med. 2022, 29, 3.
[19] C. Maslo, R. Friedland, M. Toubkin, A. Laubscher, T. Akalo, B. Kama, JAMA 2022, 327, 583.
[20] S. Madhi, G. Kwatra, J. E. Myers, W. Jassat, N. Dhar, C. K. Mukendi, A. Nana, L. Blumberg, R. Welch, N. Ngorima-Mabhena, MedRxiv 2021.
[21] S. Jiang, L. Du, Z. Shi, Emerg. Microbes Infect. 2020, 9, 275.
[22] A. Wu, Y. Peng, B. Huang, X. Ding, X. Wang, P. Niu, J. Meng, Z. Zhu, Z. Zhang, J. Wang, Cell Host Microbe 2020, 27, 325.
[23] M. Uddin, F. Mustafa, T. A. Rizvi, T. Loney, H. Al Suwaidi, A. H. H. Al-Marzouqi, A. Kamal Eldin, N. Alsabeeha, T. E. Adrian, C. Stefanini, Viruses 2020, 12, 526.
[24] S. Wang, M. Trilling, K. Sutter, U. Dittmer, M. Lu, X. Zheng, D. Yang, J. Liu, Virol. Sin. 2020, 35, 676.
[25] X. Ou, Y. Liu, X. Lei, P. Li, D. Mi, L. Ren, L. Guo, R. Guo, T. Chen, J. Hu, Nat. Commun. 2020, 11, 1.
[26] S. W. Wong, J. Data Sci. 2018, 10, 111.
[27] S. Wu, C. Tian, P. Liu, D. Guo, W. Zheng, X. Huang, Y. Zhang, L. Liu, J. Med. Virol. 2021, 93, 2132.
[28] T. Zhang, Q. Wu, Z. Zhang,Curr. Biol. 2020, 30, 1346.
[29] K. Li, B. Huang, M. Wu, A. Zhong, L. Li, Y. Cai, Z. Wang, L. Wu, M. Zhu, J. Li, Z. Wang, W. Wu, W. Li, B. Bosco, Z. Gan, Q. Qiao, J. Wu, Q. Wang, S. Wang, X. Xia, Nat. Commun. 2020, 11, 6044.
[30] D. Liu, C. Ju, C. Han, R. Shi, X. Chen, D. Duan, J. Yan, X. Yan, Biosens. Bioelectron. 2020, 173, 112817.
[31] D. Paul, A. Gupta, S. Roose, E. Gupta, J. Virol. Methods 2021, 298, 114299.
[32] R. Radha, S. K. Shahzadi, M. H. Al-Sayah, Molecules 2021, 26, 4812.
[33] M. Droybsh, A. Ramanaviciene, R. Viter, A. Ramanavicius, Micromachines 2021, 12, 390.
[34] A. Yakoh, U. Pimpitak, S. Rengpipat, N. Hirankarn, O. Chaisapakul, S. Chaiyo, Biosens. Bioelectron. 2021, 176, 112912.
[35] J. Kudr, P. Michalek, L. Ilieva, V. Adam, O. Zitka, Trends Anal. Chem. 2021, 136, 116192.
[36] Z. Zhang, R. Pandey, J. Li, J. Gu, D. White, H. D. Stacey, J. C. Ang, C. J. Steinberg, A. Capretta, C. D. Filipe, Angew. Chem., Int. Ed. 2021, 60, 24266.
[37] N. Cennamo, G. D’Agostino, C. Perri, F. Arcadio, G. Chiaretti, E. M. Parisio, G. Camarlinghi, C. Vettori, F. Di Marzo, R. Cennamo, Curr. Opin. Pharmacol. 2021, 60, 24266.
[38] J. S. Del Rio, O. Y. Henry, P. Jolly, D. E. Ingber, Nat. Nanotechnol. 2019, 14, 1143.
[39] B. Mojoska, S. Larsen, D. A. Olsen, J. S. Madsen, I. Brandslund, F. A. Alatraktchi, Sensors 2021, 21, 390.
Yun Zhang is a chair professor at the School of Pharmaceutical Science and Technology, Tianjin University, China. He received his Ph.D. at the University of Pennsylvania, School of Medicine in 2006. His research interests include metal trafficking, metalloenzymes, radical enzymes, and their catalytic mechanisms. His group combines chemically guided bioinformatics and biochemistry to explore new enzymes and new metabolic pathways in microbes. Other projects in his lab include synthetic biology, immuno-based human disease diagnosis, and new drug development.