

A Closer Look into FDA-EUA Approved Diagnostic Techniques of Covid-19

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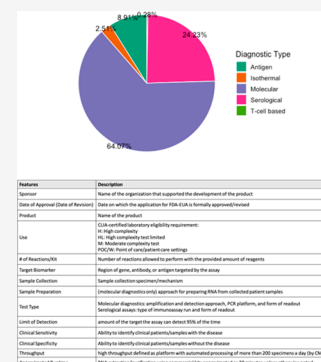
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ABSTRACT: The 2019 coronavirus disease (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 virus, caused a worldwide pandemic in 2020 and is the most urgent health issue worldwide. In this review, we highlight the details of Food and Drug Administration-Emergency Use Authorizations approved diagnostics kits, focusing on the similarities and differences. It is essential to understand the currently available options and the advantages and disadvantages each provides to select the appropriate products that maximize the testing efficiency. We believe this work will provide a holistic evaluation of the current COVID-19 diagnostic resources, including variations across the countries, and guide developing novel diagnostic techniques to improve and optimize the current testing options.



KEYWORDS: COVID-19, molecular diagnostics, serological diagnostics, FDA-EUA, multicountry analysis

INTRODUCTION

In December of 2019, 44 cases of pneumonia of unknown etiology in Wuhan, Hubei Province, China, were reported to the World Health Organization (WHO). This virus, which quickly spread to all parts of China and the neighboring countries in Asia and Europe in less than a month, was identified to be a novel coronavirus (severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)), an entirely different species from the known SARS coronavirus.¹ As the virus continued to spread across the globe to more than 200 countries, WHO declared the 2019 coronavirus disease (COVID-19) a global pandemic on March 11, 2020, the second time in the 21st century, following the Swine flu in 2009.² More than a year has passed since the WHO declaration, but the pandemic is still ongoing. As of August 2021, the total confirmed cases worldwide reached 207 million, along with the cumulative death toll of 435 000 000 surpassing the number of deaths from the Swine flu pandemic.³ However, these figures are greatly underestimated, according to United States (U.S.) media reports, and the damage is expected to be much greater considering the cases of deaths before being examined.⁴

In the past years, mankind has already experienced multiple outbreaks of coronavirus: Severe Acute Respiratory Syndrome in 2003, which was caused by SARS-CoV1, and Middle Eastern Respiratory Syndrome (MERS) in 2015, which was caused by MERS-CoV.⁵ However, these epidemics were quickly contained, without relatively large damage across the world, because the early development of vaccines and treatments successfully blocked the spread of the disease. In

the case of COVID-19, the virus is being transmitted person-to-person at the fastest rate of any other epidemics in the past, but the vaccines are administered in a slower pace than expected and are still short in supply in many countries. With the lack of these control measures, many countries are focusing on the prevention of the disease through an effective identification and isolation of the infected patients who are contagious and can transmit the diseases, using the widely available COVID-19 diagnostic testing tools.

The COVID-19 pandemic hit the United States the hardest, with more than 36 million cases confirmed, the largest number in the world (18% of the total confirmed cases worldwide), and the number is still rising.⁶ With the exponential increase in new cases, medical supplies such as masks and hand sanitizers as well as diagnostic resources, such as swabs, reaction reagents, and RNA isolation kits, are experiencing supply shortages. A previous study showed that more than 40% of the infected patients remain asymptomatic, demonstrating the increasing need for rapid, widespread testing to reduce the risk of unintentional transmission.⁷ Consequently, to facilitate the growing demands, the Food and Drug Administration (FDA) partnered with the Centers for Disease Control and Prevention

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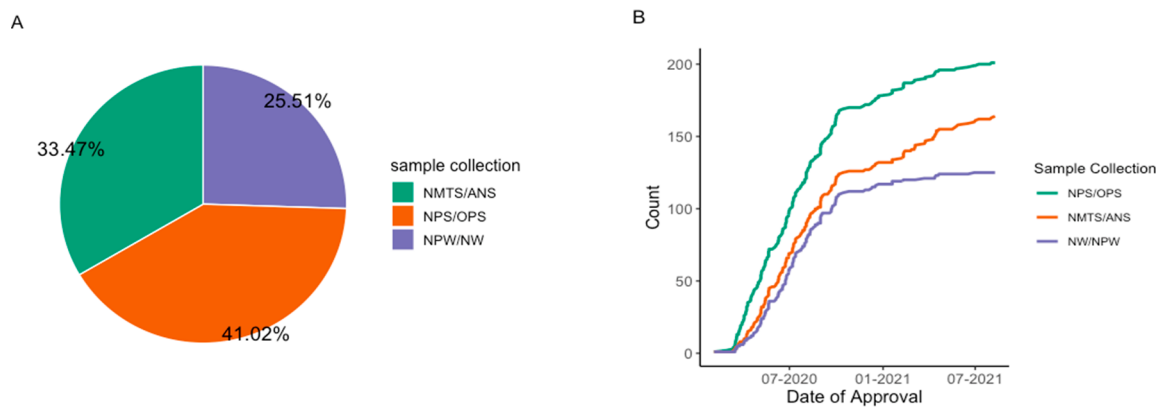


Figure 1. Sample collection mechanism of FDA-EUA approved molecular diagnostic devices. (A) Composition of the sample specimen type is summarized. All of the devices accept upper respiratory specimens, mainly from the nasopharynx and oropharynx. (B) Time trend of the sample collection type over time shows that the growing number of products offer more specimen options as the pandemic progresses.

(CDC) under Health and Human Services (HHS) to issue Emergency Use Authorizations (EUA), a bill that allows the FDA to authorize unapproved drugs and medical devices in response to a chemical, biological, radiological, and nuclear (CBRN) crisis. Accordingly, the FDA has approved various medical devices, including COVID-19 diagnostic testing kits, and, as of August 2021, up to 358 diagnostic products obtained the EUA approval, excluding specimen collection devices.⁸ Many countries are responding to the crisis in similar ways, announcing public health emergencies and rapidly approving diagnostic kits for use while regulating the export to secure medical supplies.

In response to the emergency approval issued by many countries, a growing number of COVID-19 diagnostic tests become available to the public, boosting the testing capacity in each country. Innovative designs and technologies are introduced to improve the efficiency of the diagnostic workflow and to provide more options for healthcare providers to choose from. Despite the rapidly evolving diagnostic landscape, a detailed side-by-side comparison of commercially available testing products is still lacking. Hence, it is important to provide a comprehensive review of the approved tools to provide a better understanding of the available options and further insight into future works. This article aims to provide a complete framework of the FDA-EUA diagnostic landscape of COVID-19. We will first describe the principles of the COVID-19 detection methods and introduce the FDA-EUA approved commercially available diagnostic products. Any differences in the testing streamline will be highlighted, and finally, they will be compared to the diagnostic kits with approvals from different continents, Europe, and Asia, to evaluate the FDA-EUA diagnostic kits in a broader context. A complete list of FDA-EUA approved devices and corresponding features can be seen in Table S1.

MOLECULAR DIAGNOSTICS

Overview. Since COVID-19 cannot be diagnosed based on the clinical symptoms alone, it is necessary to detect causative agents (SARS-CoV-2) in a patient's sample. Common viral testing methods include a virus culture, antigen, and antibody detection, but a nucleic acid detection remains the gold standard given a relatively short runtime and high specificity. As of August 2021, more than 230 molecular diagnostic products have been approved for emergency use and are

actively being used to diagnose COVID-19 in the United States.⁸

COVID-19 molecular diagnostic tests aim to detect the presence of SARS-CoV-2 gene in patient samples. SARS-CoV-2 is a spherical virus, 80–100 nm in diameter, and it contains single-stranded positive-sense RNA, with a genome of 30 kb in size.⁹ Since the genome of the virus is RNA, it is necessary to reverse-transcribe RNA into DNA before the amplification. The pan-coronavirus reverse transcription transcription-polymerase chain reaction (RT-PCR) assay, the first diagnostic method of COVID-19, compares the sample genetic sequence to that of six existing types of coronavirus (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV), but it takes more than 24 h to complete, and the primers used are often less sensitive.¹⁰ The discovery of the SARS-CoV-2 genomic sequence led to the development of the targeted testing method, real-time RT-PCR (rRT-PCR), which most devices adopt as their principal diagnostic technique.

The general protocol for rRT-PCR diagnostics includes three steps: sample collection, sample extraction and purification, and sample amplification and detection. Patient samples from the upper and lower respiratory tracts are preferred, according to the CDC, but other types of samples, such as saliva, feces, blood, and urine, can be used to detect viral RNA. The collected samples are stored in a viral transport medium (VTM) and are transported to a certified laboratory for testing. From each sample, RNA is extracted and purified using silica column- or magnetic beads-based extraction kits and is converted into a single-stranded complementary-DNA (cDNA) through the reverse transcription process. Then, primers targeting specific regions of the SARS-CoV-2 virus are added to amplify the nucleic acids to an observable level, which is detected through a fluorescent signal readout. Multiple control measures for sample collection, extraction, and amplification are also included in each reaction tube. Most test reagents are designed to detect two or three target genes, and the results are based on the comprehensive analysis of individual gene responses. If only one of two or three genes is positive or unexpected results are obtained from the controls, the test should be repeated using different specimen types or new reagents.¹¹

The FDA-EUA approved molecular diagnostic kits provide a variety of alternative approaches to the general protocol, modifying the steps and implementing new technologies to maximize the performance of the testing. They alter different

features, such as run time, throughput volume, cost, or sensitivity, to fit the testing purposes. Consequently, some are better at processing high-throughput volumes, while others are more suitable for a rapid diagnosis outside of a laboratory setting at a point of care. An in-depth analysis of each modification is necessary to understand the current diagnostic landscape and to select the appropriate products that match the user's needs.

Sample Collection. Upper Respiratory Specimens. Patient samples from the upper respiratory tract include nasopharynx (NPS), oropharynx swabs (OPS), nasopharynx wash or aspirate (NPW/NW), nasal midturbinate swabs (NMTS), and anterior nasal swabs (ANS).^{8,11} The swab samples are collected by inserting a flocked swab into the nasal or oral cavity, often in a direction parallel to the palate, gently rubbing or rotating the swab at least four or five times against the pharynx to absorb the mucous secretion, and placing it into a sterile tube containing VTM for storage. To collect nasopharynx wash or aspirate, a clinician inserts a tube into the NP to pass the saline solution through the nasal passage and collect the washings.^{12,13} The CDC recommends upper specimens collected with flocked swabs, specifically NP swab specimens, because they involve less invasive steps and are less dense compared to the aspirates, which makes the sample processing easier in the automated workflow system. Previous research showed that there was no significant difference in the virus detection between the NP swab samples and aspirates, putting the swab samples as the most preferred choice above all.¹²

Reflecting the CDC guideline, the majority of the EUA-approved diagnostic devices requires NP or OP swabs, as shown in Figure 1A. As the pandemic progresses, however, a growing number of kits accept additional upper respiratory specimens, mainly nasal midturbinate swabs or anterior nasal swabs, but some take nasopharynx or nasal wash/aspirate specimens as well. This trend is reflected in Figure 1B, where the number of products accepting these types of specimens rapidly increases a few months after the pandemic, starting in May 2020. The midturbinate and anterior nasal swabs are getting more approvals because they have the potential to be self-administered. Currently, NP and OP swabs must be collected by a trained healthcare provider wearing level D or higher personal protective equipment (PPE).¹⁴ These requirements slow down the testing processes while putting the medical staff at risk of exposure. However, nasal swabs can be self-collected at a healthcare location, with guidance from a healthcare provider, or at home, using the home collection kits. Self-collection methods are less invasive and more comfortable, as they use much smaller swabs than that of the traditional NP or OP specimens,¹⁵ and clinicians do not have to get close to the patients, making the risk of transmission even lower. Hence, the implementation of the simple and easy self-administered collection techniques can greatly improve the testing efficiency while protecting healthcare workers and saving protective equipment. As of August 2021, there are 51 EUA-approved kits that use self-collected specimens at home, with or without the guidance provided by the clinicians. All of these self-collected specimens demonstrated comparable analytical and clinical performances; the limit of detection ranged from 0.012 to 40 copies/ μL , and clinical testing on contrived positive samples and negative patient samples showed greater than 90% positive and negative percent agreements, respectively.

Lower Respiratory Specimens. Three different types of specimens, namely, sputum, tracheal aspirates, and bronchoalveolar lavage (BAL), can be collected from the lower respiratory tract. Sputum samples are obtained when patients expectorate a deep cough into a sterilized container, but they should not be induced because aerosol particles produced from the cough can increase the risk of transmission.^{12,16} The tracheal aspirates and BAL specimens are collected by inserting a tube into the trachea or the lung; the saline solution is flushed into these locations and then removed for further analysis. In a recent study by Wang et al., researchers examined 1070 different types of samples and compared cycle threshold values to determine the viral loads in each type. They showed that both the upper and lower respiratory tract samples contained comparable amounts of virus, with the exception of nasal swabs that contained the highest viral loads.¹⁷ However, given the invasive nature of the technique, lower respiratory specimens are recommended to take only when clinically needed. In particular, these samples are collected when the tests performed using the upper respiratory tract specimens are negative but other clinical signs indicate a positive infection. Since the lower respiratory specimens are mainly used for the secondary confirmation testing, none of the EUA-approved products accepts the lower respiratory specimens alone, and only 11 accept all three types. However, many take at least one or two types, mainly BAL and sputum samples.

Sample Extraction and Purification. The SARS-CoV-2 RNA extraction and purification steps are important for molecular diagnostics because contaminated RNA samples interfere with the enzymatic reactions during RT-PCR, resulting in an insufficient amplification of the target genes. In general, the collected specimens are degraded using lysis buffer and detergents, and RNA is extracted from the lysate. The extracted RNA is purified using organic solvents or solid-phase materials, such as silica-filled columns and magnetic beads, and is eluted in the buffer, ready to be processed further.

The FDA-EUA approved diagnostic products share a list of commercially available extraction kits that are compatible with their test designs, which is summarized in Figure 2. The recommendation spans over a wide variety of choices, but Qiagen's QIAamp products have been most available at the initial state and hence shown in most EUAs. Other common kits include MagMAX Viral/Pathogen Nucleic Acid Isolation Kit from Thermo Fisher Scientific, Chemagic assay from PerkinElmer, and MagNA Pure DNA Viral NA extraction kit

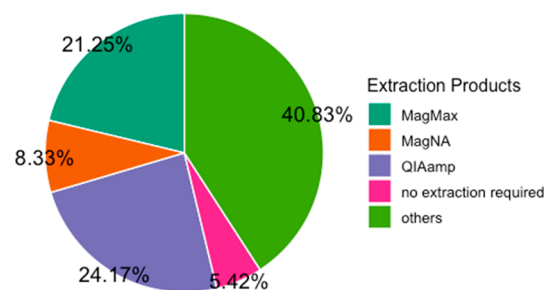


Figure 2. Summary of the most used bead-based extraction commercial products. High percentage of “others” suggests that the majority of the products use unique extraction reagents or often are integrated with the amplification process. A small percentage of the approved products eliminated the need for extraction, further simplifying the testing streamline.

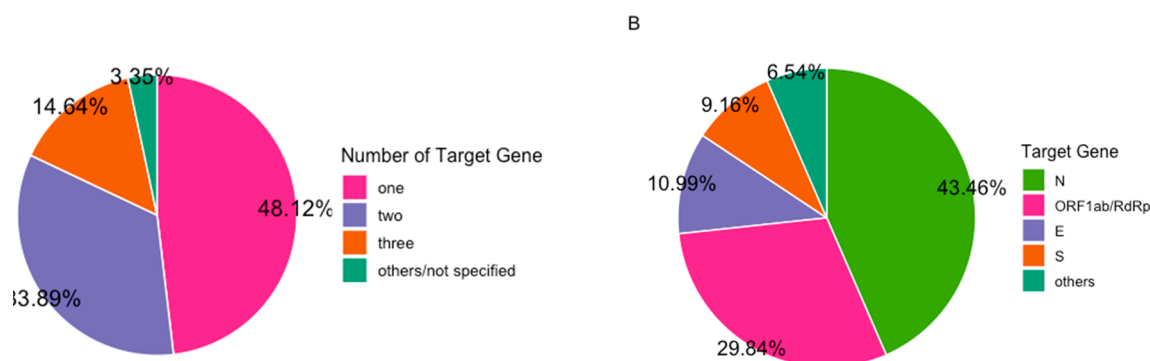


Figure 3. FDA-EUA approved-COVID-19 molecular diagnostics target gene. (A) Most identify one target gene, disregarding different regions of the same target gene, or two, first for the screening and second for the confirmation purpose. However, a comparison of their clinical performance shows that the number of target genes does not influence their diagnostic capability. (B) A total of eight different regions is amplified by the FDA-EUA approved diagnostic devices, but the N gene remains the most common target in the U.S.

from Roche. The average runtime for these commercial kits is 30 min, but there have been several efforts to further simplify these processes, either by a direct addition of samples to RT-PCR or through an automation of the entire processes.

Direct-to-Test Addition. A direct addition of the collected samples into RT-PCR removes the extraction and purification processes. This approach greatly shortens the test time and reduces the overall reliance on commercial reagents, which is critical in a time of pandemic, when countries are experiencing supply chain shortages for diagnostics. Several preliminary studies have validated the compatibility of the unprocessed specimens with the RT-PCR and concluded that a direct-to-test addition is capable of detecting the SARS-CoV-2 RNA, but only with a selective number of swab storage buffers. Researchers showed that RNA from swab samples stored in VTM or in water and Copan Universal Transport Media (UTM) at 4 °C had a comparable amplification during an RT-PCR reaction to that from the processed samples, while samples stored in Hank's medium or a saline solution did not.^{13,18,19} Other studies have noted that swab samples in VTM directly added to RT-PCR reached the detection threshold a few cycles later than the purified RNA from the same samples.^{13,20,21}

There are currently 13 EUA-approved diagnostic devices that do not require extraction steps. Diasorin Molecular's Simplexa COVID-19 Direct accepts swab samples in different buffer systems, including VTM and UTM, while three of the kits require specific buffer systems for the sample storage. In particular, Mesa Biotech's Accula SARS-Cov-2 Test employs a lysis buffer to pretreat the samples before the samples are added into the PCR chamber, further optimizing the test sensitivity. As seen in previous studies, these kits require a greater number of cycles before reaching the amplification threshold, and the iAMP COVID-19 Detection Kit from Atila Biosystems has the highest recommended cycle threshold cutoff value (<50) out of all EUA-approved devices. All 13 established powerful analytical and clinical performances, with an average limit of detection ranging from 0.125 copies/ μ L to 12 copies/ μ L, positive and negative percent agreements above 90%.

Automation. Current diagnostic devices perform a varying range of steps in an automated platform, including nucleic acid extraction and purification, transfer of samples into appropriate reaction wells, and target amplification and detection. Automated systems can process multiple specimens at the

same time, facilitating mass extraction and large-scale sampling, and a standardization of the procedure and reduction of user interaction increase the consistency and reproducibility of the results. The majority of EUA-approved kits include automated extraction platforms, most being combined with the PCR machine for an integrated analysis. Some of the common extraction platforms include the KingFisher Flex Magnetic Particle Processor from ThermoFisher Scientific, bioMérieux NucliSENS easyMAG system, or Chemagic MSM Instrument from PerkinElmer that integrate with liquid handling platforms, providing automation from start to finish.

Sample Amplification and Detection. Real-Time RT-PCR. The purpose of real-time RT-PCR is to amplify and detect a small amount of SARS-CoV-2 RNA present in the collected samples. The cycle consists of four steps: reverse transcription, denaturation, annealing, and elongation. A master mix containing dNTP (four nucleotides), primers, reverse transcriptase, and DNA polymerase is added to the reaction chamber with the patient sample. The primers bind to the specific sequence of the target viral gene and indicate the starting position of DNA polymerase that replicates the template DNA reversely transcribed from SARS-CoV-2 RNA. Another set of primers targeting a human RNase P is included as an internal control for sample collection, RNA extraction, and amplification. Each step in the cycle is performed at different temperatures, and millions of copies are created after 30–40 repeats. The whole process is monitored in real time, using a special probe that emits fluorescence in proportion to the amount of synthesized DNA present in the mixture.²² Some of the most commonly used probes include intercalators, such as SYBR Green, and hydrolysis probes, such as the TaqMan probe. The intercalating dyes can insert between the bases of cDNA, producing a nonspecific amplification to any DNA products, while TaqMan probes release the fluorophore only after breaking off from the specific gene of interest.²³ The fluorescence intensity emitted from the probes is analyzed to obtain the cycle threshold (Ct) value, and if the value is below the recommended cutoff value for two or more target genes, the sample is diagnosed as positive.

RT-PCR technology is the current standard for the majority of the approved devices, gaining 239 approvals from the FDA as of August 2020 (66.8% of the total approved kits, excluding the collection devices). The turnaround time for the test results of the 239 devices varies from 13 min to 2 d, but most take ~2–3 h to complete. A broad range of variations was

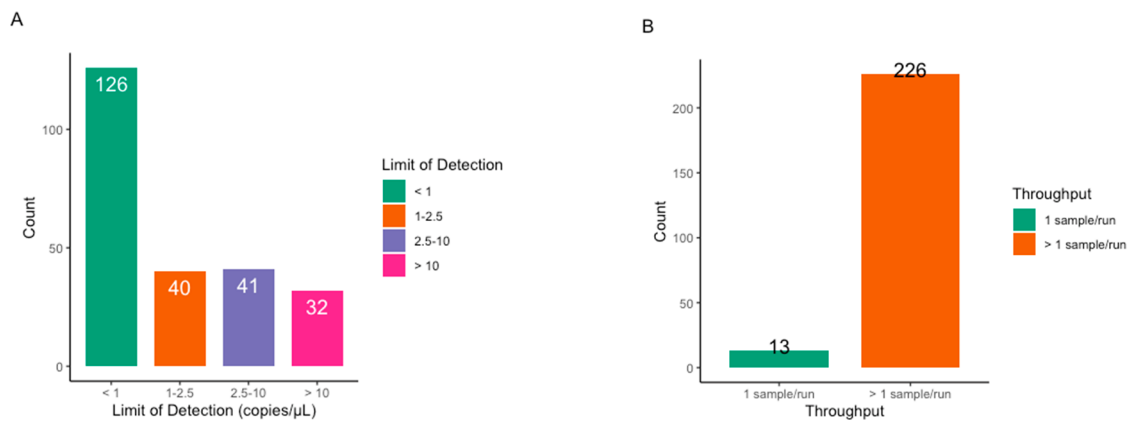


Figure 4. Limit of detection and throughput of FDA-EUA approved diagnostic devices. (A) The analytical performance of the products is analyzed by classifying the limit of detection into four different categories, as shown. The highest limit of detection value provided is chosen for this comparison purpose. Most demonstrate a highly accurate diagnostic capability, down to less than one copy per microliter. (B) The throughput of the products is analyzed by looking at the number of samples processed per run. The low throughput is in exchange for shorter runtime and the point-of-care screening.

noted in multiple aspects, including target genes, fluorescent probes, and multiplex ability.

The EUA-approved diagnostic devices detect varying numbers and types of SARS-CoV-2 genes, represented in Figure 3. Most of the devices identify one or two regions of the separate viral gene, but some screen for three. However, the number of target genes does not reflect their diagnostic performances; the kits testing for three different regions have a similar analytical sensitivity to those with two targets, with an average limit of detection of 4.06 copies/ μ L. Regarding the type of SARS-CoV-2 genes detected, there is a total of eight different regions amplified by the diagnostic devices, which include genes encoding for the nucleocapsid (N) protein, open reading frame 1a and/or b (Orf1ab), open reading frame 8 (Orf8), spike (S) protein, envelope (E) protein, membrane (M) protein, nonstructural poly protein a and/or b (pp1a/pp1ab), and nonstructural protein 2 (nsnp2). The N gene is the most common target of all, and 166 of the approved kits select various regions (N1, N2, N3, N4, N5) on the gene to detect the virus. Other common targets include E genes and RNA-dependent RNA polymerase (RdRp) genes (inside the Orf1ab polyprotein region). Many studies have evaluated different assays' analytical performances. In one study, the diagnosis targeting the S gene alone showed reduced sensitivity, which was improved by incorporating other viral-specific targets.^{24–26} Currently, out of 35 EUA-approved devices that detect the S gene, 33 use additional primers to amplify different regions of the viral genome. When clinical samples were tested, these devices obtained higher sensitivity (negative percent agreement of 99.3%, on average) relative to the ones that targeted S gene only (mean negative percent agreement of 97.7%). In another study by Kakhki et al., the Orf8 gene showed an improved specificity compared to the previously reported N, E, or RdRp genes; the probe located in the Orf8 region had fewer cross reactions with other types of coronaviruses than that located in the other two genes.²⁷ BioFire Defense's BioFire COVID-19 Test, the only device that detects the Orf8 region, provides 100% specificity when tested with 30 positive clinical specimens contrived with live SARS-CoV-2 virus. As the SARS-CoV-2 variants spread, the target gene becomes an important factor to consider. Genetic variations on the gene targeted by the diagnostic assays can

impact the performance, as the primers may fail to bind to and amplify the mutated target, producing false-negative results. Following the introduction of genetic variants in the United States, the FDA has initiated a postmarket analysis on the EUA-approved devices, monitoring the variants and their impacts on the test performances. They have concluded that the variants introduced do not interfere with the diagnostic test, given that most of the devices target multiple genes or multiple regions on one gene. However, they have identified three molecular kits that might be susceptible to the virus variants, which include the Accula SARS-CoV-2 Test, TaqPath COVID-19 Combo Kit, and Linea COVID-19 Assay Kit. The Accula SRAS-CoV-2 Test is affected by the genetic variant at position 28881, although the FDA concludes that the impact does not appear to be significant. For the last two kits, their ability to detect one of their targets, the S gene, is impacted by B.1.1.7 (alpha) variant, while their overall performance is not affected considering that they detect three target genes.^{28,29}

Many of the tests utilize TaqMan hydrolysis probes as their standard method for signal production, but they differ in their ability to detect individual targets. Some of the approved devices (e.g., Hologic, 2020; Abbott Molecular, 2020; QIAGEN GmbH, 2020; Atila BioSystems, 2020) show an undifferentiated amplification of their target regions, where the presence of either gene will produce fluorescent signals. Most of these devices detect different regions located on the orf1ab genes or use a combination of the RdRp and N genes. Since these genes are highly specific for COVID-19, as they are often used in the confirmation assays, the devices have adequate clinical and analytical validity, with the average limit of detection 2.8 down to 2.6 copies/ μ L, clinical specificity of 99.3%, and clinical sensitivity of 100%. Two kits from BioFire Diagnostics employ intercalating dyes to analyze the end-point melting curve after the amplification. The melting curve measures the change in the fluorescence as the double-stranded DNA, intercalated with the dyes, dissociates into a single strand at a high temperature. The melting temperature is calculated from the graph and compared with the expected value to identify the specific amplification of the target genes. The two products show reliable performances, with an analytical sensitivity of 0.33 copies/ μ L, on average, and clinical specificity and sensitivity above 98%. Given little differences in

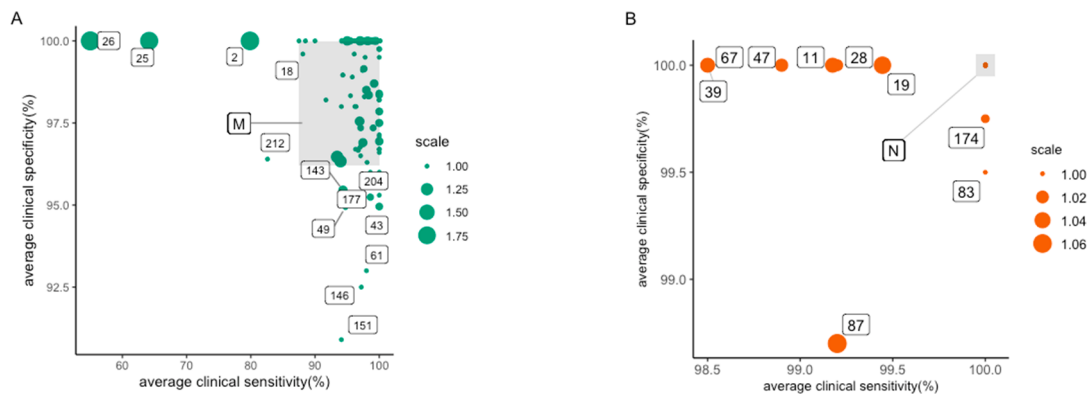


Figure 5. Clinical performance of FDA-EUA approved diagnostic devices. Each number represents the diagnostic product provided in Table S1. Range in clinical performance due to analytical instruments or target genes is accounted for by scaling each dot to a different relative size. (A) Letter “M” denotes a cluster of 195 companies with positive predictive value (PPV) above 87.5% and negative predictive value (NPV) above 97%. Greater variations in PPV compared to NPV show the importance of diagnosing those with the disease correctly in the time of pandemic where the available resources for diagnosis and treatment are limited. (B) Evaluation of diagnostic sensitivity and specificity by focusing on those with PPV and NPV greater than 98.5%. Letter “N” denotes a cluster of 108 companies with 100% PPV and NPV.

their diagnostic performances, both probe-based and dye-based RT-PCR provide an accurate detection of SARS-CoV-2 RNA.

Currently, the single-step multiplex technique is widely adopted by many EUA-approved devices to provide rapid, high-throughput diagnostics, as shown in Figure 4. This technique simultaneously detects multiple target regions in a single reaction well, and the amplification is completed in a single PCR reaction, reducing the runtime and the amount of samples and reagents required. The specimens are processed under the same amplification conditions, which lowers the risk of contamination and sources of experimental errors. In comparison with those using single-plex PCR (Trax Management Services, 2020), single-step multiplex-based diagnostic devices (e.g., InBios International, 2020; DiaCarta, 2020; OSANG Healthcare, 2020; Altona Diagnostics GmbH, 2020; SD Biosensor, 2020) show high speed and high sensitivity. They can process ~93 samples in less than 160 min, on average, while single-plex assays process 23 samples in 2–4 h. The ability to provide accurate high-throughput diagnostics is valuable in a time of the pandemic, when widespread scalable testing is needed to stop the spread. A multiplexing technique also allows for a high-throughput screening of various bacterial and viral infections besides COVID-19, as shown in five EUA-approved devices. These five devices detect 18 viral species and three bacterial species, on average, along with two or three separate regions on the SARS-CoV-2 genome. They provide results in less than 2 h, but the limit of detection for SARS-CoV-2 RNA varies by 2 orders of magnitude depending on the devices; Luminex Molecular Diagnostics’ NxTAG CoV Extended Panel Assay shows the lowest analytical sensitivity with 45 copies/ μL , and BioFire Diagnostics’ BioFire Respiratory Panel 2.1 shows the highest sensitivity of 0.16 copies/ μL . Considering that a consistent portion of COVID-19 patients presents with coinfections,^{30,31} especially among patients of a younger age, this multiplex technique provides a comprehensive report of patient’s conditions so that the clinicians can deliver appropriate treatments.

As an alternative to the high-throughput testing, some EUA-approved devices are targeted for use at a point of care, offering a simple but rapid on-site diagnosis (Figure 4). The high-throughput RT-PCR method currently in use requires large, expensive analytical equipment and skilled professionals, which

limits the accessibility of the testing, especially during a pandemic like COVID-19. The constrained number of healthcare providers and laboratories, supply shortage of the reagents, and strict eligibility criteria for the testing are factors that further aggravate the problems. Therefore, there is an increasing need to develop rapid, reliable, and affordable point-of-care (POC) diagnostic devices that can be easily operated by the patients at home. Currently, there are 12 EUA-approved POC-PCR devices available for use. Cepheid’s Xpert Xpress SARS-CoV-2 test is the first product to receive EUA in a POC setting, and it performs real-time PCR on a simplified platform, GeneXpert Dx System, suitable for use in the physician’s office. The same company later gained approval for Xpert Xpress SARS-CoV-2/Flu/RSV and Xpert Omni SARS-CoV-2 that use the same platform. These devices take ~45 min to complete, and up to 2000 samples are processed per day. Mesa Biotech’s Accula SARS-CoV-2 reports a simplified procedure with a minimal number of steps; users inject the samples into the cartridge that contains all of the necessary reagents for the reaction and place it on the platform (Accula Dock or Silaris Dock). The test is completed in 30 min, and the results are displayed using a lateral flow assay, where the appearance of a blue band in the test line indicates a positive detection of the target gene. However, this device has a limited throughput of three samples per run or a maximum of 144 tests per day. The analytical sensitivity of the two tests differs by 3 orders of magnitude, with Cepheid’s products showing a higher sensitivity of 0.27 copies/ μL , on average, while Mesa Biotech’s Accula SARS-CoV-2 have a limit of detection of 40 copies/ μL . The disparity in the limit of detection could be due to the different number of genes detected. Cepheid’s kit targets two regions, the N and E genes, offering an additional confirmation measure, while Mesa Biotech’s kit targets only the N gene. However, both devices show reliable clinical performances, reaching a sensitivity and specificity above 95%.

The real-time RT-PCR is considered a gold standard of COVID-19 diagnosis because of its highly specific and sensitive detection even at low viral titer, in the early stage of infection. However, it still has many limitations, including the use of a complex thermocycler, need for trained personnel, long run times (of several hours), and high cost per test.³² In order to overcome these limitations, multiple technologies

have emerged as alternative diagnostic methods, including an isothermal amplification.

Isothermal Amplification. An isothermal amplification reaction does not require changes in the temperature that are typically required by the RT-PCR method, eliminating the need for the expensive thermocycler device. The most commonly used method is the loop-mediated isothermal amplification (LAMP), which uses 4–6 primers to target ~6–8 distinct regions of the DNA strand at ~60–65 °C, the optimal temperature for Bst polymerase. Multiple pairs of forward and backward primers of the LAMP reaction are complementary to each other, allowing self-hybridized loops to form at each end of the strand, producing an overall dumbbell-like structure. This particular structure provides multiple sites for the initiation of DNA synthesis, resulting in the production of 108 times more copies of the target region within an hour. Therefore, the LAMP allows a fast but highly sensitive and specific detection of SARS-CoV-2 RNAs at isothermal conditions, making it optimal for applications in resource-limited settings.^{11,12,25}

Currently, nine FDA-EUA approved diagnostic devices utilize RT-LAMP techniques. These devices have a significantly reduced runtime of ~38 min, on average, while maintaining a high specificity, with the minimum being 91.7% and a sensitivity of 98% in clinical evaluations. The limit of detection varies greatly depending on the devices, ranging from 0.125 to 20 copies/ μ L. Finally, the throughput volume reflects the potential uses of each device, either for widespread screening, processing 90 samples per run, or for portable rapid diagnostics, taking one sample per run.

The clinical performances of the EUA-approved molecular diagnostic devices are compared in Figure SA. The broad range of clinical sensitivity and specificity reflect the variations in the testing streamline, while approximately half of the devices show a highly accurate diagnostic capability with both clinical sensitivity and specificity above 98.5%, as shown in Figure SB.

In the Broader Context: Multi-Country Analysis. As of August 2021, the United States has recorded over 36 million COVID-19 confirmed cases, by far the highest in the world. The number of diagnostic tests performed also surpassed 544 million, remaining the largest in the world, followed by India.⁶ However, these figures, which are used to support superior diagnostic performances of the United States, fail to accurately reflect the reality, because they do not take into account the size of the population. Considering the number of diagnostic tests performed per million people, the United States does not rank among the top; it ranks in 31st place, with 1 665 032 cases per million.⁶ This ranking is relatively low compared to its high performance in the total number of tests conducted. Additionally, the number of tests per new confirmed case can be investigated to obtain a better overview of the diagnostic performances in the United States. The U.S. needs 13.9 tests to obtain a new confirmed case, which is similar to that of India, where the cases are growing at a fast rate with the new variant. In comparison, 37 tests are required for the United Kingdom and 52.6 for South Korea.³³ A lower number of tests per new confirmed case indicates that the testing is limited to the high-risk groups, implying a reduced availability of the mass public testing and an increased risk of transmission from potentially positive patients left untreated. Therefore, the United States overwhelms other countries in the quantity of the testing, but many loopholes exist with respect to the quality of the diagnostic tests. To further investigate

whether the current gaps in the quality come from the diagnostic devices available for use, EUA-approved devices are compared with those approved from other countries to assess their diagnostic performances.

The diagnostic devices approved from various countries around the world utilize similar mechanisms to detect SARS-CoV-2 viral RNA in patient samples. A total of 16 countries worldwide have announced hundreds of the RNA viral genome sequence, where an amplification of regions specific to SARS-CoV-2 will identify the virus in the patient samples.³⁴ Since most of the diagnostic kits use commercially available platforms for extraction and amplification, no significant difference is introduced by the equipment that they use. Therefore, the major differences in diagnostic performances between the kits originate from the PCR reagents and different rRT-PCR protocols that they adopt. In the absence of the international standard protocols for COVID-19 detection, each country has established its own guidelines for the diagnostic devices to refer to, which introduced variations across the countries in the assay designs, including the regions of the gene that the test kits target.

The WHO introduced reference rRT-PCR protocols developed by six countries, including Germany, Japan, Thailand, and France, and the United States shared its standard protocol on the U.S. CDC Web site. In summary, five protocols (China CDC, U.S. CDC, Japan National Institute of Infectious Diseases, Hong Kong University, Thailand National Institute of Health) detect different regions from the N gene, two protocols (France Institute Pasteur, Germany Charité) detect the RdRp gene, and two (France Institute Pasteur, Germany Charité) detect the E gene. A detailed comparison of the WHO reference rRT-PCR protocols is provided in Table 1. While many countries select the N gene as their

Table 1. WHO-Recommended rRT-PCR Protocol^a

sponsor	target gene
China CDC, China	N, ORF1ab
National Institute of Infectious Diseases, Japan	ORF1ab,S
HKU, Hong Kong SAR (China)	N (screening), ORF1b (confirmatory)
National Institute of Health, Thailand	N
Institut Pasteur, Paris, France	E (confirmatory), RdRp (IP2/IP4)
Charité, Germany	E (screening), RdRp (confirmatory)

^aDifferent countries developed their own initial guidelines that detect different gene regions of SARS-CoV-2 virus.

amplification target, some, including South Korea, choose the E and RdRp genes as their standard, following WHO's initial guideline. They use the E gene, common in sarbecovirus, as their first line of screening, and the RdRp gene, unique for SARS-CoV-2, for the confirmation assay. In the initial stage of the outbreak, seven diagnostic devices have gained emergency approval for use in South Korea, and all of them amplify E and RdRp genes at a minimum. Table 2 summarizes a list of devices that obtained initial approval in Korea. Given that the reference standard used to make diagnostic devices differs by country, there has been much controversy in determining the optimal target gene to use.

Most controversies pivot around genetic mutation,³³ as countries are attempting to select the target genes that are less

Table 2. South Korea Initial Emergency-Approved Diagnostic Kits^a

sponsor	product	target gene
KogeneBiotech Co, Ltd.	PowerChek 2019-nCoV Real-time PCR Kit	E, RdRp
Seegene, Inc.	Allplex 2019-nCoV Assay	E, N, RdRp
SD Biosensor, Inc.	STANDARD M nCoV Real-Time Detection kit	E, ORF1ab (RdRp)
SolGentCo., Ltd.	DiaPlexQ Novel Coronavirus (2019-nCoV) Detection Kit	N, ORF1a
BioSewoom, Inc.	Real-Q 2019-nCoV Detection Kit	E, RdRp
BioCore Co., Ltd.	BioCore 2019-nCoV Real Time PCR Kit	N, RdRp
Wells Bio, Inc.	CareGENETM N-CoV RT-PCRkit	E gene, RdRP1, RdRP2 (confirmatory)

^aMost of them targeted the RdRp region for screening and the E gene for confirmation, following WHO's initial guideline.

likely to mutate.³⁴ Viruses typically have high mutation rates because they contain RNA as their nucleic acids, which is less stable than DNA. Since the detection of the SARS-CoV-2 gene uses primers that attach to specific regions on the viral genome, the accumulation of mutations can induce a sequence change and prevent the primers from binding. There has been a collective effort to monitor the emergence of new variants as well as the frequency and extent of the mutations, and the sequencing information of SARS-CoV-2 genes from each country is shared worldwide through the GISAID virus information-sharing network, operated through the WHO.

While the N gene is most frequently targeted by many countries, several studies reported problems with the protocols.^{35–38} In one study, researchers evaluated the primers released by the U.S. CDC, targeting the N2 and N3 (removed from the kit 3/15/20) regions of the viral RNA.³⁵ When the team prepared two primers using the sequence released from the U.S. CDC and tested using negative specimens, they obtained high false-positive results in the absence of the virus. They observed that the primers began to self-assemble and amplified random DNA in the sample, yielding positive results. Another study has noted a high mutational frequency of N genes with a total number of 871 nucleotide mutations and 569 protein mutations.³⁶ It is the third most mutated site, next to Orf1ab and S genes, and if the trend continues, the N gene might not be detected by the approved-diagnostic devices currently in use. However, despite these challenges, N gene-based protocols have been reported to have a higher sensitivity than that of RdRp, up to 43 times, in a few clinical review papers, putting it at an advantage of detecting low viral concentrations, which is especially useful in the early stage of an infection.³⁷ The RdRp gene provides an attractive alternative, considering that no mutation has been reported on this gene.³⁶ With the least potential for mutation, the RdRp-based protocol can be highly specific to SARS-CoV-2. Although some studies suggest that the RT-PCR method targeting the RdRp gene has insufficient sensitivity, a preliminary study performed using Seegene's Allplex 2019-nCoV Assay detected the sensitivity in the increasing order of N, RdRp, and E genes.³⁸ The varying order of sensitivity of the target genes obtained from previous studies suggests that other components of the protocol, such as buffer and reagent compositions, may play a more important role in determining the performance of the diagnostic device than the target genes.

As the pandemic continues to accelerate, countries are granting emergency approvals to diagnostic devices that deviate from their recommended protocols. In the United States, COVID-19 diagnostic devices that use various target genes, such as the E gene, Orf1 gene, Orf1ab gene, and S gene, are granted EUA approvals. Similarly, in Korea, in addition to E and RdRp genes, other genes, including N and Orf1a genes, are accepted for emergency use. Considering that these devices demonstrate comparative performances to each other in both specificity and sensitivity, it is difficult to evaluate the diagnostic performances with respect to the target genes, and other components in the protocol, such as test reagents composition, sample quality, and patient condition, might play a greater role in determining the quality of the testing. However, in order to stop the controversies involving target genes, there should be an effort made to establish a global standard protocol for an SARS-CoV-2 diagnosis.

Serologic Testing. Serologic testing is another option in the diagnosis of SARS-CoV-2. It detects the antibodies (IgM, IgG) developed in response to the infection as a supporting diagnostic method for molecular diagnostics. In comparison to the molecular testing, serologic testing provides a faster testing time (generally less than 60 min) with samples that are safer to collect—using blood, serum, or plasma samples, which pose a lower risk than respiratory specimens to the healthcare providers—and a higher throughput volume. As of August 2021, 87 kits are EUA-approved for serologic testing. However, its usage in the clinical setting is limited by its relatively low specificity and sensitivity, as the technique depends on the patient's immune response that varies greatly by person.

Immunity. In terms of the human immune response toward COVID-19, antibodies arise late in the course of the illness, where the median duration of COVID-19 IgM and IgA antibody detection is found to be 5 d and IgG detection ~14 d after the symptom onset.³⁹ The seroconversion rate varies by patient, and in very mild cases, patients may not even produce a detectable amount of antibodies, increasing the potential for false-negative results. Hence, the diagnosis of COVID-19 through a serologic testing should be delayed until the host immune response is sufficiently elicited. A previous study had demonstrated that, after 5.5 d of a symptom onset, however, the detection efficiency of IgM enzyme-linked immunosorbent assay (ELISA) was greater than that of RT-PCR and that the positive detection rate was significantly increased (98.6%) when the IgM ELISA assay was combined with the PCR compared to a single qPCR test (51.9%).³⁹ The details of the immune response, such as SARS-CoV-2 antibody longevity and the role of IgA, are yet to be found, but there is little doubt that serologic testing has great potential as a supportive diagnostic test.

Diagnostic Landscape of FDA EUA-Approved Serology Test. In order to analyze the performance of the serology tests, an extensive review of the kit inserts of the EUA-approved commercially available diagnostic devices was performed, which is summarized in Table S2.

The serologic diagnostic devices are relatively simple to perform, with three main steps: sample collection, sample extraction, and reaction with recombinant antigens. The specimen types include serum, plasma, and whole blood (venous/fingerstick), which are collected by the healthcare providers. The additional extraction step is needed for serum or plasma samples, which are obtained by centrifuging whole

blood samples and extracting the top layer above the buffer region. The test kits contain recombinant antigens of SARS-CoV-2 (spike glycoprotein (S) and nucleocapsid phosphoprotein (N)) that the antibodies, if present in the patient sample, can bind to form immune complexes. These complexes are detected by multiple methods, including lateral flow immunoassays, ELISA, or chemiluminescent immunoassays.

In general, the serologic testing provides a rapid diagnosis, with minimal healthcare provider-patient interactions when collecting the samples. Despite the low specificity and sensitivity by itself, it is capable of improving the diagnostic accuracy when used along with RT-PCR and is suitable for mass screening and surveillance efforts to correctly identify and stratify people in the community into different categories, including newly infected or asymptomatic patients. In addition, with vaccine development in the future, the need for serologic testing will surge, as it is an essential tool to monitor the vaccine efficiency and formation of herd immunity. For other types of coronaviruses, such as MERS CoV and SARS-CoV, it was discovered that the antibodies declined over time, possibly allowing for the reinfection to occur.⁴⁰ Hence, serologic testing can be used to investigate the antibody response to COVID-19 by testing for the antibody formation in response to the newly developed vaccines and the longevity of their response.

Type of Specimen Accepted. Currently, 84 EUA-approved serological testing devices accept serum and/or plasma specimens. A few kits (18 out of 87) accept whole blood samples as well, which further streamlines the testing process by eliminating the centrifugation step. An increasing number (2 out of 87) of diagnostic devices accept fingerstick blood as a dry blood spot (DBS), easing the self-collection process by the patients and reducing the waiting time to get the results. These kits demonstrate a comparable performance to those that accept serum specimens, with the clinical sensitivity of 99% and specificity of 99%. Consequently, the diagnostic value of using the fingerstick dried blood spot could be explored further to facilitate mass testing.

Types of Serological Testing. There are four major types of serologic testing: lateral flow assays (LFA), chemiluminescent assays (CLIA), enzyme-linked immunosorbent assays (ELISA), and neutralization assays.⁴¹ LFA generally takes the shortest time to obtain results, ranging from 10 to 30 min. They detect the presence of antibodies against a viral antigen in patient samples, and given its rapidity and portability, they are most appropriate for point-of-care testing. CLIA takes 1–2 h to obtain the results and can both detect and quantify the antiviral antibodies, while ELISA takes 2–5 h for similar outcomes. Neutralization assays take the longest, ranging from 3 to 5 d, but they can measure the competency of the antibodies, showing whether the sample antibodies are able to prevent susceptible cells from being infected by a standard dose of the virus.

Of 87 EUA-approved serological diagnostic devices, 24 use LFA, 32 are based on CLIA, 16 are from ELISA, and one uses a neutralization assay, as represented in Figure 6. In one study, researchers compared the diagnostic performances of LFA and ELISA, testing their ability to detect positive serum samples obtained more than 31 d after the symptom onset. The latter assay detected 100% of the samples, demonstrating a higher sensitivity than LFA that only identified 44% of the samples.⁴² The results suggest that ELISA assays may be more sensitive to lower concentrations of antibodies than LFA, although the performance is influenced by other factors as well, including

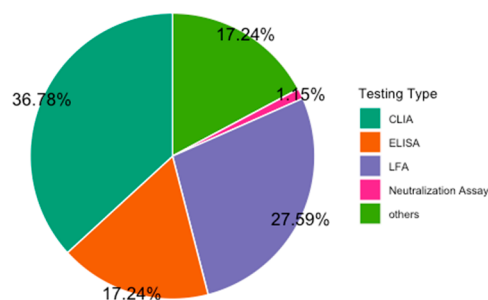


Figure 6. FDA-EUA approved serological testing type. Majority of the devices use CLIA and LFA due to their simpler procedure and shorter runtime, although neutralization assays give the most detailed results, including the competency of the antibodies.

the type of antibody (IgG/IgM) and antigen (N/S) utilized in the assay. Despite these findings, the majority of the EUA-approved devices employ LFA, preferring the shorter turnaround time and simpler procedure.

Neutralization assays are the golden standard for serologic testing, as they are the only type that can test the functionality of the antibodies and monitor the patients' immunity. However, these assays can only be performed at high-containment laboratories, limiting the accessibility of the assays, and, currently, cPass SARS-CoV-2 Neutralization Antibody Detection Kit by GenScript USA Inc. is the only serological device utilizing this assay technique.⁴⁰

Type of Antibodies Detected. The serological testing devices vary by the target antibody and detection antigen. In terms of the antibody tested, the EUA-approved serological devices can detect IgG only, IgM only, IgG and IgM, or total antibodies (IgG, IgM, and IgA), which is summarized in Figure 7A. The approved devices span equally across all four types, providing greater options and flexibility for the healthcare providers to choose from.

To detect any COVID-19 patients using serologic testing, the patients must be seropositive. Seroconversion is defined as the time required for the patient to develop antibodies following exposure, and the patient turns from seronegative to seropositive. It serves as an important factor determining the usefulness of serologic testing. A small cohort study investigated the seroconversion rate between IgG and IgM of SARS-Cov-2 and observed that all three types are evenly prevalent in their patient samples: synchronous seroconversion (9 out of 26 patients), earlier IgM seroconversion (7 out of 26 patients), and earlier IgM seroconversion (10 out of 26 patients).⁴³ Another study observed that assays detecting SARS-CoV-2 IgM and IgG antibodies individually did not add an overall diagnostic value compared to assays detecting the mixed antibodies,⁴⁴ and hence the CDC stated that there is no identified advantage in any of these tests.

As for the target antigen, the diagnostic kits mostly test for either antinucleocapsid (N) protein antibody or antispikes (S) protein antibody (or its subsets S1, S2, or Receptor Binding Domain) or both, as shown in Figure 7B. The spike and nucleocapsid antigens are of interest in serological testing for SARS-CoV-2, as the assays measuring antibodies against these antigens show a strong correlation between the antibody response and neutralizing antibody titer,⁴⁵ implying that these are the main viral antigens against which antibodies are produced.

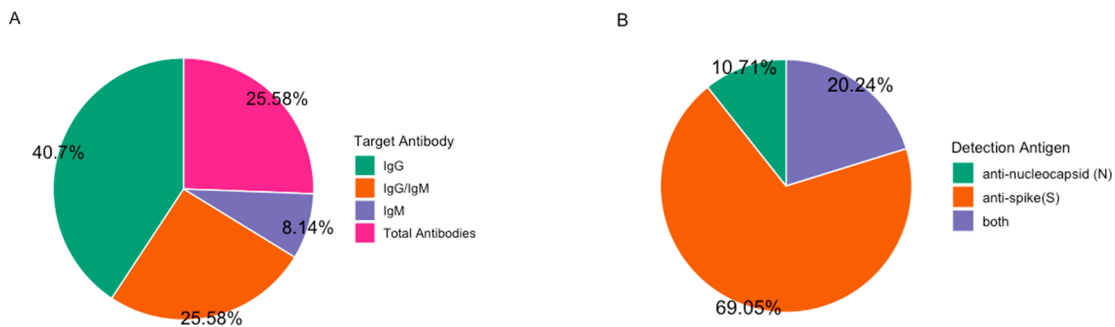


Figure 7. FDA-EUA approved serological testing target. (A) The types of target antibody that the serological kits detect are evenly distributed, giving more flexibility and options for the healthcare providers. (B) Most devices detect antispike (S) protein, which is known to give a better specificity than the antinucleocapsid (N) protein.

The target antigen is an important factor that determines the performance of the testing kits. According to the CDC, the N protein is more conserved, triggering more cross reactions across different coronavirus species and reducing the specificity. While many devices test for the full S protein, there are some devices that target different subsets of S proteins (S1, S2), such as DiaSorin's LIAISON SARS-CoV-2 S1/S2 IgG. Considering that smaller spike fragments are less likely to have a cross reactivity and, thus, a higher specificity,⁴⁰ assays testing for subsets of the S protein are more specific than those targeting the full-length S protein; similarly, assays testing for the S protein may have a higher specificity than those for the N protein. Mount Sinai Laboratory's COVID-19 ELISA IgG Antibody Test suggests an alternative approach to increase the device's diagnostic performance. This kit first screens for RBD to sort preliminary positives and retests them, targeting the full-length S protein to confirm the infection. With such an approach, the device obtains a negative percent agreement (compared with the RT-PCR results) of 100% (94%–100%).

Of all target antigens, several studies suggest that assays measuring the spike RBD antibodies are the most reliable methods for counting the number of COVID-19 cases when a large number of people is tested. In a study comparing assays measuring the total antibodies to RBD and IgG to N, total antibodies to RBD was positive in 93.1% of the patients (161 out of 173), with a median response time of 11 d, whereas IgG to N was positive in 64.7% of the patients (112 out of 173), with a median response time of 14 d.⁴⁰ Other than the delayed response time, the results suggested that anti-N antibodies wane faster than anti-S1 and anti-RBD antibodies,³⁸ making the assays that measure anti-N antibodies more affected by the patient's disease condition at the time of sample collection—possibly lowering the sensitivity. Nonetheless, it can serve as an advantage when tracking patient cases, as the presence of anti-N antibodies suggests that the infection occurred relatively recently. It was also observed that, for samples collected after 15 d of infection, assays measuring RBD antibodies detected seroconversion in 100% of the patients, whereas anti-N IgG was only detected in 79.8% of the patients.⁴⁰ A comparable study obtained similar results for samples collected 14 d after the infection, corroborating the previous findings.⁴⁵ Considering that these studies collected samples from later stages of infection, antibody-diagnostic devices have minimal implications for early diagnosis. However, they can potentially be applied to seroepidemiological studies and used in comple-

ment with the molecular diagnostics to provide a broader view of the COVID-19 pandemic.

Cellular Immunity Testing. While antibody testing has been the current diagnostic modality for evaluating the COVID-19 infection and exposure, it has several limitations, including a low amount or absence of antibodies for individuals with minimal or no symptoms, the rapid decline of antibodies over time, and cross reactivity to other infections.⁴⁶ Consequently, there has been a growing interest in T-cell-mediated immunity, leading to the FDA-EUA approval of the first T-cell test, Adaptive Biotechnologies' T-Detect COVID-19 test in March 2021.

Adaptive Biotechnologies' T-Detect COVID-19 test is a novel technology based on the next-generation sequencing of the T-cell receptor and immune repertoire profiling from whole blood samples collected by the healthcare providers. While the test fails to identify an active infection, it shows an improved performance on identifying a recent or prior infection compared to the serological testing, as studies have shown that a T-cell mediated immunity is maintained at least six months following the primary infection, even in the absence of seroconversion in some cases.^{47–49} When evaluated on the patient samples collected 15 d or more after the symptom onset, the T-Detect COVID-19 test correctly identified 94.5% of the patients, while the serological test had an 89% accuracy, on average.⁵⁰ Given its high performance on both positive and negative percent agreement and its cross reactivity, the T-Detect COVID-19 test has great potential in managing the long-term response to the pandemic, such as in clinical monitoring, epidemiological public surveillance, risk stratification, and assessment of protective immunity.

Antigen Testing. Antigen tests are also available for the diagnosis of SARS-CoV-2 and have received growing attention due to their low cost, rapid turnaround time, affordability, and ease of use. Consequently, upon the FDA-EUA approval of the first over-the-counter (OTC) at-home antigen testing in December 2020, the market of antigen testing is expanding, and as of August 2021, there are 32 available EUA-approved antigen testing devices, seven offering OTC home testing.⁵¹ Antigen tests involve a direct test for the viral antigen in the patient samples, most targeting the detection of the nucleocapsid (N) antigen, while there are two kits that test for the RBDs of SARS-CoV-2 spike protein antigen. They are less dependent on individual immune responses compared to the serological testing, and most return the results within 30 min. However, the use of antigen testing is considerably limited due to its low sensitivity, particularly in asymptomatic

or low-viral load patients. Multiple studies have evaluated the sensitivity and positive predictive values of various antigen kits in different settings, and most demonstrated a sensitivity of ~50%, the lowest being 41.2% on the asymptomatic population. The value increases with the specimens' viral load, reaching the highest when tested on the samples collected within 5 d of the symptom onset or those with Ct values below 20.^{52–54} A recent study of the performance of different types of testing over the course of COVID infection shows that the daily sensitivity of the antigen testing decreases rapidly after the first day of virus shedding, concluding that the short duration of antigen positivity may limit its clinical uses. However, the same study has suggested the use of serial testing multiple times per week, showing that the protocol sensitivity remains over 98% for testing performed in at least a three-day interval.⁵⁵ Another study has modeled the effectiveness of the diagnostic screening using viral load kinetics and concluded that it depends less on the testing sensitivity than the frequency and the turnaround time of the testing.⁵⁶ Hence, the reliability and viability of the antigen test can be maintained when it is limited to symptomatic individuals or populations with a high prevalence of SARS-CoV-2, when it adheres to serial testing, and when other confirmatory measures are used for negative results, as suggested by the CDC. Currently, antigen testing is also offered in nonclinical settings as a self-test. However, comprehensive data for its use by untrained individuals are lacking, while a recent publication has shown that the sensitivity of the parent/caregiver or self-administered antigen test, with or without the guided instruction, is ~15% lower than that collected by the healthcare provider.⁵⁷ Despite at-home antigen testing providing a convenient testing option for COVID-19, its clinical significance needs further evaluation, and the expansion of home-use testing should proceed with caution. More information is provided in Table S3.

In the Broader Context: Multi-Country Analysis. In a comparison of the use of serological testing across the globe, countries expressed a range of attitudes toward applying the serological testing for the diagnosis of SARS-CoV-2. Most countries tend to prefer molecular diagnostics over serological testing, as it offers a higher sensitivity and specificity despite a greater cost and time. In the case of the United States, serological testing occupies a supportive role in diagnostic testing second to the RT-PCR, as studies have shown that using them in a complementary manner can improve the diagnostic value.⁴⁰ Their preference for PCR-based diagnostics is also demonstrated by the approval process for the two different types of kits—for RT-PCR testing products, it is not a requirement to perform the clinical trial in the U.S. to obtain the emergency approval. However, for serological testing kits, they must be tested and approved by one of the U.S. medical institutions (National Cancer Institute (NCI) or National Institutes of Health (NIH)), making it more difficult for foreign kits to gain the EUA.⁵⁸

Interestingly, as of July 24, 2020, Korea's Ministry of Health and Welfare has not approved any serological testing, despite the existence of some Korean companies producing and exporting COVID-19 serological testing kits to the U.S.⁴⁵ This is due to the relatively low accuracy as mentioned and also due to the sufficient supply of PCR testing kits. With the large testing capacity that Korea already has, it was sufficient to manage the cases, and hence, there is no need to employ an alternative method to improve the throughput.⁵⁸ However, it is

worth noting that there are calls to employ serological testing to test the eligibility for plasma donation by cured patients for treatment purposes and to provide universal testing to detect asymptomatic carriers.

In contrast, China is an example of a country that relies on serological testing relatively heavily. Of the initial 20 government-approved COVID-19 testing kits, eight were serological testing kits, with five using lateral flow assays and three using chemiluminescent microparticle immunoassays.⁵⁹ It can be speculated that, since China was the epicenter of the SARS-CoV-2 pandemic, there may have been insufficient time to prepare a large amount of RT-PCR testing to perform a mass public screening and contact tracing. Moreover, even before the COVID-19 pandemic, China had a larger market in antibody testing (35%) compared to that of PCR,⁶⁰ which could explain why China has a relatively high percentage of serological testing kits. In looking at China's serological testing kits, there were no official documents that could be found reporting the clinical performance. However, upon the clinical evaluation of the Antibody Reagent Test Kits for the Novel Coronavirus (2019-nCoV) (GICA) produced by Guangzhou Wondfo Biotech Co., the sensitivity was reported to be 86.4%, and the specificity was 99.6%, with an overall accuracy rate of 91.2%, which does not satisfy the U.S. EUA standards of sensitivity 90% and specificity 95%.⁴⁴ Though there is insufficient information to conclude on the general clinical performance of China's testing kits, a lower sensitivity suggests that China's standards for approving SARS-CoV-2 diagnostic devices may be lower.

■ CONCLUSIONS

The COVID-19, caused by the SARS-CoV-2 virus, caused a worldwide pandemic in 2020 and is the most urgent health issue worldwide as of today. The virus is still circulating around the world, and a robust but rapid testing infrastructure of SARS-CoV-2 is essential to stop the spread of the disease. In this review, we have covered two categories of diagnostic methods that are currently in practice: molecular diagnostics and serological/antigen testing. Molecular diagnostics, using the real-time RT-PCR technique, is the gold standard of COVID-19 diagnosis, providing a rapid and reliable approach to detect the viral RNA in the patient samples. The four steps of the protocol are covered in depth, highlighting the current landscape of the EUA-approved diagnostic devices and alternatives that optimize the conventional diagnostic workflow. While serological testing provides a complementary role to molecular diagnostics, improving diagnostic accuracy, it has other potential applications in vaccine monitoring, epidemiological studies, and in donor screening for a plasma treatment. The FDA-EUA approved serological testing kits are analyzed, focusing on the similarities and differences between the kits.

Despite the development of COVID-19 vaccines, the number of confirmed cases is still fluctuating due to the introduction of new variants and vaccination hesitancy across the nation. The diagnostics will continue to play an important role in the future to help identify a new wave of outbreaks or changes in epidemiology, including the demographics of infected individuals or the severity and transmissibility of the virus, to understand and control the pandemic. Therefore, it is important to understand the currently available options, advantages, and disadvantages each provides to select the appropriate tests that maximize the testing efficiency. Future work is needed to establish the COVID-19 diagnostic standard

to reduce variations across the countries and to continue exploring novel diagnostic techniques to improve and optimize the current COVID-19 diagnostics. The ultimate goal is to improve testing rates and the general diagnostic accuracy.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsinfecdis.1c00268>.

List of FDA-EUA approved molecular diagnostic kits and its selected features, list of FDA-EUA approved serological diagnostic kits and its selected features, list of FDA-EUA approved antigen diagnostic kits and its selected features (XLSX)

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These authors contributed equally.

Notes

The authors declare no competing financial interest.

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