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Review

SERS Based Lateral Flow Immunoassay for Point-of-Care Detection of SARS-CoV-2 in Clinical Samples

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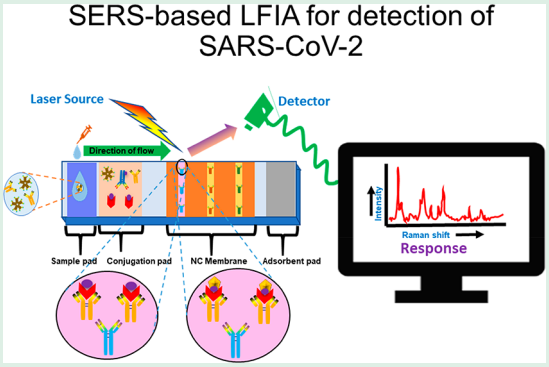
ABSTRACT: The current scenario, an ongoing pandemic of COVID-19, places a dreadful burden on the healthcare system worldwide. Subsequently, there is a need for a rapid, user-friendly, and inexpensive on-site monitoring system for diagnosis. The early and rapid diagnosis of SARS-CoV-2 plays an important role in combating the outbreak. Although conventional methods such as PCR, RT-PCR, and ELISA, etc., offer a gold-standard solution to manage the pandemic, they cannot be implemented as a point-of-care (POC) testing arrangement. Moreover, surface-enhanced Raman spectroscopy (SERS) having a high enhancement factor provides quantitative results with high specificity, sensitivity, and multiplex detection ability but lacks in POC setup. In contrast, POC devices such as lateral flow immunoassay (LFIA) offer rapid, simple-to-use, cost-effective, reliable platform. However, LFIA has limitations in quantitative and sensitive analyses of SARS-CoV-2 detection. To resolve these concerns, herein we discuss a unique modality that is an integration of SERS with LFIA for quantitative analyses of SARS-CoV-2. The miniaturization ability of SERS-based devices makes them promising in biosensor application and has the potential to make a better alternative of conventional diagnostic methods. This review also demonstrates the commercially available and FDA/ICMR approved LFIA kits for on-site diagnosis of SARS-CoV-2.



KEYWORDS: *surface-enhanced Raman spectroscopy, lateral flow immunoassay, SARS-CoV-2, COVID-19, POC devices*

1. INTRODUCTION

In past decades, three highly pathogenic human coronaviruses including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 have emerged and triggered global pandemics that resulted in high morbidity and mortality globally.[1,2] SARS-associated coronavirus, SARS-CoV, was the first outbreak in China, 2002 and spread swiftly to over 30 countries, resulting in more than 8000 cases of SARS-CoV.[3] The outbreak of zoonotic coronavirus in Saudi Arabia in 2012 known as MERS-CoV transferred to humans from dromedary camels. Over 27 countries have reported cases of MERS-CoV, leading to 858 total demises worldwide.[4] In December 2019, the first case of severe acute respiratory syndrome coronavirus (SARS-CoV-2) having pneumonia-like symptoms was identified in China. Since then, the COVID-19 epidemic has speedily spread throughout the world and has undergone serious health concerns and socio-economic burdens.[5] The WHO announced COVID-19 as a pandemic on Mar. 11, 2020.[6] Globally, as of Jan. 16, 2021, there have been over 92 million confirmed cases of COVID-19 out of which approximately 1.9 million demises have been reported and the numbers keep growing.[7]

The vital cause of the rapid spread of SARS-CoV-2 is the highly infectious nature of SARS-CoV-2 and transmission of the virus through physical contact. The most common symptoms reported in COVID-19 patients are fever, cough, running nose, difficulty in breathing, muscular pain, and diarrhea.[8] The incubation period of SARS-CoV-2 before the onset of symptoms is 2−7 days. This stage is mainly asymptomatic and infectious because the infected person shows non-specific symptoms. It can easily be transmitted from an infected person to a non-infected person.[9] Approximately 45% of the reported cases are asymptomatic. The main challenge in preventing and suppressing the viral spread is to track and monitor the COVID-19 patients (symptomatic and asymptomatic) who are staying in close contact with healthy individuals.[10] The infected person with particularly SARS-CoV-2 virus and other upper tract respiratory infections suffer

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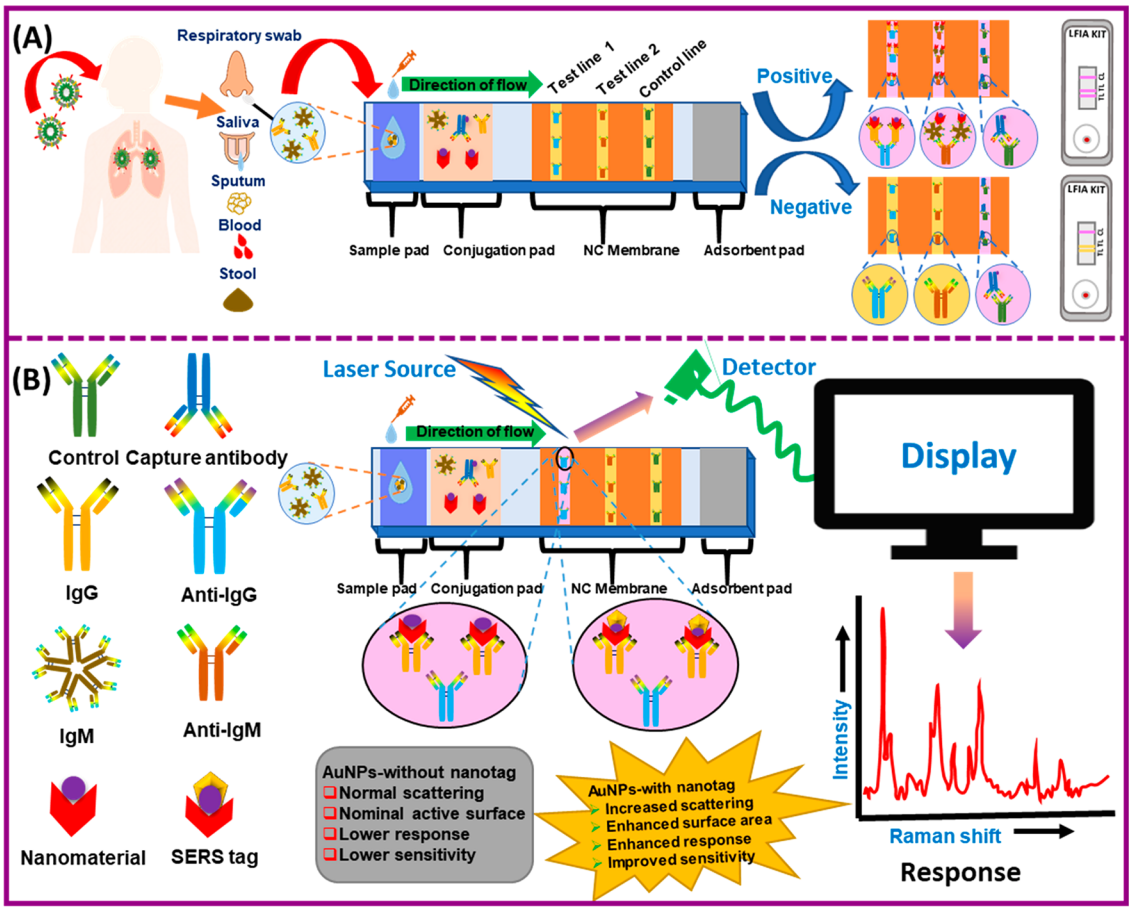

Figure 1. Schematic representation of the fabrication and working principle of (A) conventional LFIA and (B) SERS-based LFIA for detection of SARS-CoV-2.

a high number of complexities that lead to morbidities mainly in people who have prior health problems.[11] The increasing numbers of cases are the major challenge for the healthcare sector, and they are also imposing a socio-economic burden globally.

The massive growing threat of SARS-CoV-2 could be related to the scarcity of effective point-of-care testing (POCT) assessments for speedy and accurate diagnosis of SARS-CoV-2 infection. There is an urgent need for a fast and accurate laboratory method for the diagnosis of SARS-CoV-2 in an early stage. Since the outbreak of COVID-19, there have been endless efforts to develop effective diagnostic techniques for sensitive and specific identification of SARS-CoV-2.[12] The major challenges in diagnostics are sample collections, false positive and negative results, required sophisticated instruments, well-settled laboratories, skilled personnel, and expensive and time-consuming procedures.

The outbreak of SARS-CoV-2 came as an opportunity for research and development in various healthcare, science, and technology sectors all around the globe. The aim is to come up with accurate, rapid, and reliable detection techniques, which would be distributed on a global scale to control the pandemic. Because one sector of research is dedicated toward the curing and vaccination part, the other research community is coming up with better and better ways to help detect the virus since it has symptomatic as well as asymptomatic transmission which makes it hard to detect. PCR and RT-PCR tests are commonly used as a diagnostic technique that involves the detection of viral genetic material. They are gold-standard tests, since the demand is high, the processing time would delay the tests in a larger population of the developing nations. Another diagnostic technique that shows better results is ELISA test kits, but the time consumption and high cost of the test is a major drawback for the majority of the economically disadvantaged population.

Some advances have been made for detection through strip-based tests which will be cheap and effective to a large number of people. One of the most popularly used techniques is lateral flow immunoassay (LFIA), which can be used either alone or in a combination with other techniques to get better and more reliable results. One of advanced characterization techniques is surface-enhanced Raman spectroscopy (SERS), which is an advanced form of Raman spectroscopy. SERS makes use of unique properties of nanostructures to give enhanced and optimized results for extremely low levels of detection.

The use of SERS in combination with LFIA could become the right combination for reliable and accurate testing, with the merits of a quick and large number of tests. The representation of the fabrication and working principle of conventional LFIA is given in Figure 1A, and SERS-based LFIA is shown in Figure 1B, along with the SERS technique integrated with LFIA, which results in the enhancement of sensitivity and low detection limit of biomolecules. For instance, Lim et al. reported the label-free SERS-based biosensor of gold nanoparticles for the detection of the influenza virus.[13,14] Similarly, Kukushkin et al. detected the influenza virus through the

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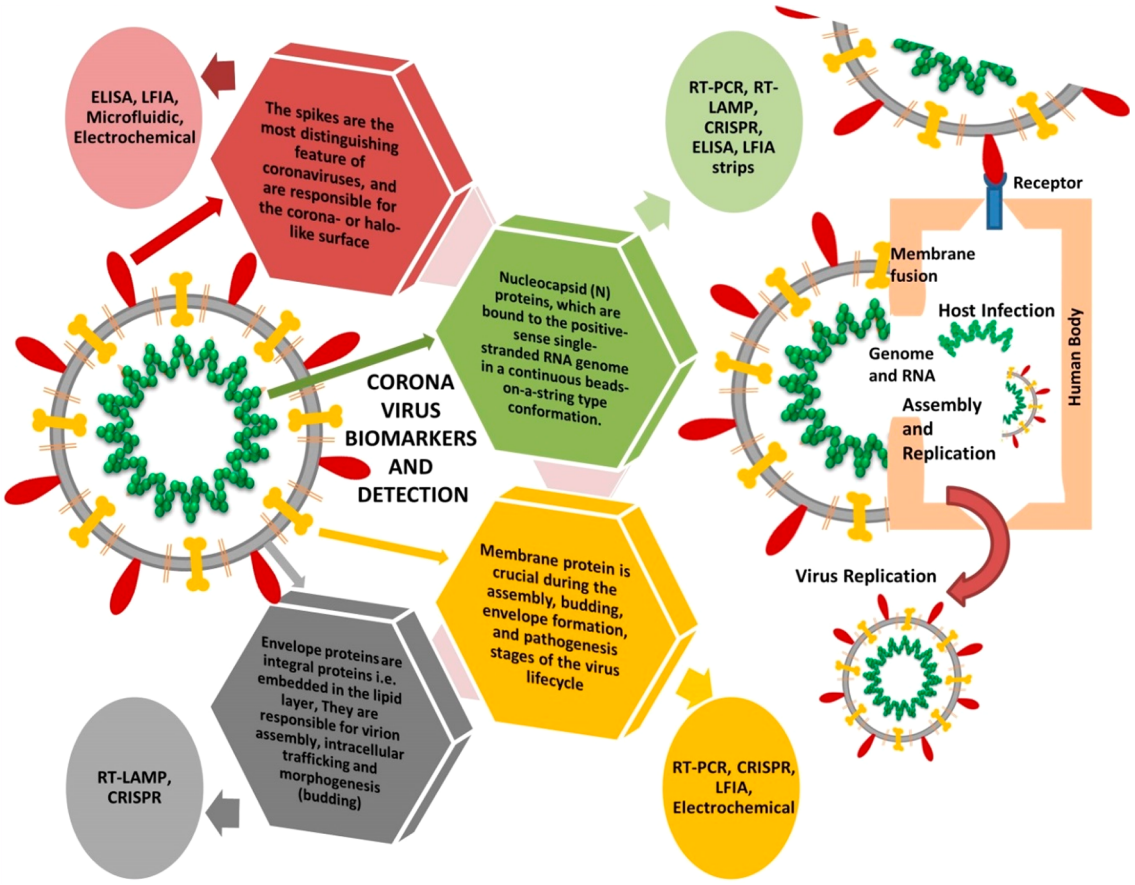

Figure 2. Representation of the proteins of SARS-CoV-2, their binding mechanism, and diagnostic methods.

SERS-based aptasensor. SERS sensor reaches a 10^6 to 10^9 times higher signal intensity, which results in the enhancement of the sensitivity of the sensor. This biosensor has an LOD of 10^4 virus particles per clinical sample.[15] In another work, Xiao et al. reported the SERS integrated LFIA strip using gold and silver nanomaterials for the detection of avian influenza virus (H7N9) within 20 min. The LFIA strips provide the qualitative information, but after its integration with the SERS techniques, it delivers the quantitative information of the analyte in clinical samples which can be portable as well.[16]

This review will be focusing on the SERS-based lateral flow immunoassay for the detection of antigen and/or antibodies biomarkers against SARS-CoV-2 in clinical specimens such as whole blood, plasma, serum, and saliva. Along with this, the overview on coronavirus and its associated biomarkers will also be discussed in brief. Further, the advantages, challenges, and future opportunities for applications of SERS-based lateral flow immunoassay in viral pathogen detection will be described.

2. CORONAVIRUS AND ASSOCIATED BIOMARKERS

Coronaviruses belong to the large family of viruses; generally, four subdivisions are included in this coronavirinae subclass, that is α-coronavirus, β-coronavirus, γ-coronavirus, and δ-coronavirus, out of which SARS-CoV-2 comes under the genus β-coronavirus of subclass coronavirinae in the family Coronaviridae of the Nidovirales order.[17] Until now, seven coronaviruses species have been reported which can be transferable through human contact, and out of these, HCoV-229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43 cause less harm. However, the other three β-coronaviruses, SARS-CoV, MERS-CoV, and SARS-CoV-2, cause serious harm and show deadly pathogenicity.[18,19]

Most researchers found that the genome sequence of the SARS-CoV-2 virus is approximately alike that of the previously emerged SARS-CoV-like genome and looks diverse from SARS-CoV and MERS-CoV viruses.[19] Usually, the rate of transmission of the virus is evaluated on the basis of the reproduction number (R0) variable. R0 is considered a crucial threshold parameter for measuring viral transferability.[20] The range of basic reproductive number (R0) between 2.2 and 5.7 is proposed for SARS-CoV-2 virus transmission. This parameter is a bit higher than the estimated value for the SARS-CoV virus; i.e., R0 ranges between 2.0 and 5.0, indicating that the transmissibility of SARS-CoV-2 is faster than the SARS-CoV virus.[20]

SARS-CoV-2 virus has a single-stranded positive sense RNA genome having the length of approximately 30,000 nucleotides and is a relatively large virus of virion diameter 70−140 nm with 9−12 nm identifiable spikes.[19,21] SARS-CoV-2 genome has four structural proteins, i.e., spike (S), envelope (E), membrane (M), and nucleocapsid (N).[22] In addition, the SARS-CoV-2 genome has five main open reading frames (ORFs), organized in the way of the 5′ untranslated (UTR)-replicase complex.[23] Tang et al. reported that 149 mutation sites are present in SARS-CoV-2. These are further evolved in two types such as L- and S-types which are responsible for 70% and 30% of the viral population, respectively, and make them highly contagious.[24] Recently, new strains of SARS-CoV-2, B.1.1.7, B.1.351, and P.1 variants, have been found in the United Kingdom (U.K.), South Africa, and Brazil respectively,

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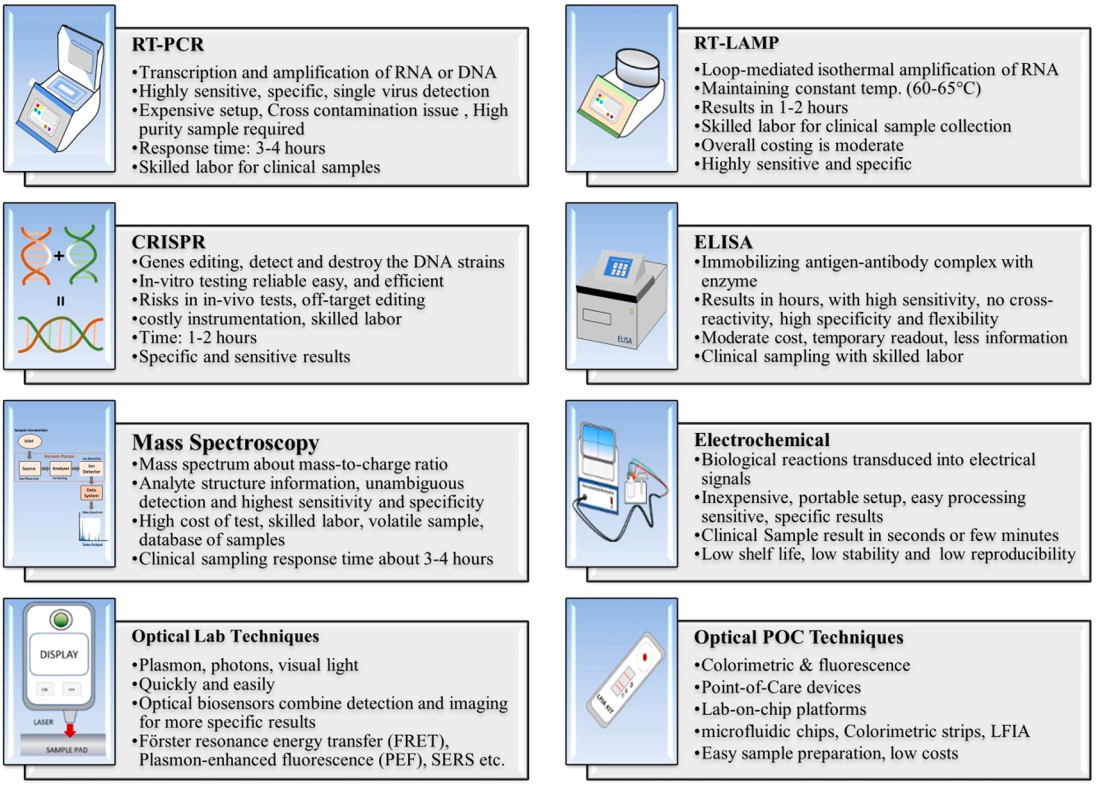

Figure 3. Conceptualized overview on current diagnostics methods, their working, advantages, and limitations.

wherein B.1.1.7, and B.1.1.351 variants have been found to have high transmission rate and spreading in other countries (India, Denmark, and Ireland), being almost 70% more contagious than the previous one.[25,26] Currently, in the U.K., over 60% of COVID-19 cases are reported due to infection of a new variant.[27] Commercial molecular assays for SARS-CoV-2 target the ORF region, as well as ES and/or N protein. Therefore, the accuracy of such assays would be theoretically affected by the new mutations in the SARS-CoV-2.[28] There is a dropout in S-gene-based assay that laboratories need to monitor and implement other genomic targets (e.g., E or RdRP genes), based on assays not previously incorporated in current diagnosis methods.[29]

The SARS-CoV-2 binds to receptors on the cell surface via a receptor-binding domain (RBD) present in their S1 subunit and enters cells by binding spike proteins to ACE2 receptor.[30] The M-protein has been found in two adaptations that are compact and elongated and hence support in assembling the virus; the E-protein interacts with the protein of host cells and performs the channelization of viroporin.[31] The different proteins of SARS-CoV-2 and their binding mechanism are shown in Figure 2.

Biomarkers are defined as the measurable biological indicators of the presence, severity, or type of disease. Biomarkers play a vital role in predicting the present, historical, and future status of infections and help in early diagnosis.[32] The prognosis of SARS-CoV-2 can be made by detection of certain biomarkers related to viral infection of SARS-CoV-2. The direct sensing of associated markers can help in the early detection of SARS-CoV-2.

The various inflammatory biomarkers associated with the SARS-CoV-2 infection are C-reactive proteins (CRP), interleukin-6, procalcitonin, and ferritin. CRP is produced in the liver in response to inflammation. A higher level of CRP indicates the viral infection. In a study, the CRP level in COVID-19 patients was found 100-fold higher than normal and it can increase about 1000 times in several other cases. In another study, the IL-6 values were also found 3-fold higher than normal. Ferritin is a widely researched protein; its value was found as high as 1000 ng/mL which was many folds higher than the normal individuals. The detection of these inflammatory biomarkers can help in the diagnosis of the virus in those patients who have been diagnosed with false negative results by traditional diagnostic approaches.[33,34]

On the other hand, antibodies are produced in the body to safeguard against harmful foreign substances. The various serological antibodies are widely used as an efficient biomarker. Upon exposure to SARS-CoV-2 viral infection, first the immunoglobulin (IgM) is produced to defend against the viral infection and later on the immunoglobulin IgG is generated which acts as humoral immunity and keeps the memory of this encounter for the long term. The immunoglobulin IgM and IgG can be detected for SARS-CoV-2 within a week before the onset of symptoms. The presence of a number of immunoglobulins predicts the future complexity and seriousness of SARS-CoV-2 infection.[35]

3. CURRENT DIAGNOSTICS APPROACHES FOR DETECTION OF SARS-COV-2

Several diagnostics methods have been developed for the diagnosis of viral infections depending upon the category of virus and their characteristics and also on the collected sample from infected patients. Currently, two diagnostic methods are available for the diagnosis of SARS-CoV-2 based upon the detection of the viral RNA and antibodies produced upon the disclosure of the viral infection.[36] At present, the polymerase

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ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

chain reaction (PCR) is used for the detection and amplification of deoxyribonucleic acid (DNA), having high sensitivity and specificity.[37] The conceptual simplicity of this method has made a widely used detection technique in molecular biology, and it can detect as little as a single fragment of DNA. The coronavirus genome contains RNA instead of DNA.[38] RT-PCR technique is most commonly used for the detection of viral RNA genome due to its high sensitivity and specificity. In RT-PCR, it isolates the RNA transcripts from biological samples and polymerases into complementary DNA (cDNA). Although, this method directly detects the nucleic acid, i.e., viral RNA, as well it accelerates and multiplies the genomic material. It can even utilize a small amount of viral RNA present in collected biological specimens.[39] Nonetheless, in many cases, the sensitivity of RT-PCR has been poor because of some concerns in performing the assay, such as changeable and low viral loads depending upon the duration of infection, sample classification (nasal swab vs oral swab, upper respiratory tract vs lower respiratory tract), collection of the sample, conservation, and transportation of specimens, etc.[40] In addition, they require a highly pure sample; otherwise, it produces a false negative result. In the case of antigen testing, the majority of patients who tested positive for COVID-19 by RT-PCR later tested negative using the rapid serological assay alone; this emphasized the potential for misdiagnosis of COVID-19 using the assay in isolation.[41] Moreover, the greater time consumption, low availability of trained personnel, and high ratio of false negative and false positive results make them a limitation in diagnostic applications.

On the other hand, a technique similar to RT-PCR known as reverse transcription loop-mediated isothermal amplification (RT-LAMP) has also been utilized for the detection of SARS-CoV-2.[42] RT-LAMP assay is used for detection of specifically the viral RNA of SARS-CoV-2 instead of DNA.[43] It amplifies RNA rather than DNA. This assay is similar to the traditional PCR, but, exceptionally, amplification of nucleic acids is done at a particular temperature rather than the thermal cycler required in PCR as compulsory equipment.[39] For instance, Lamb et al. reported development of a quick screening LAMP test for detection of SARS-CoV-2 infection within 30 min with visualization of the color.[43] RT-LAMP has no cross-reactivity with similar infectious viruses such as SARS-CoV, MERS-CoV, HCoV, and influenza viruses (type B, H1N1pdm, H3N2, H5N1, H5N6, H5N8, and H7N9) as well.[44] In addition, this assay is easy to perform, is cost-effective, gives quick results, and can be performed in laboratories for initial detection of SARS-CoV-2. Over the advantages, they have some drawbacks such as false positive results, high risk of contamination during handling of assay, and requirement of maintaining constant temperature (60−65 °C), which may limit their application.[45]

Colorimetry is another popular diagnostic platform for the detection of the SARS-CoV-2 virus. Since it is easy to interpret due to the development of color on the strip which is observed through naked eye. In addition, it is easy-to-use, less-expensive, requires minimum technical expertise, and less instrumentation facilities.[46] Over the advantages of a colorimetric assay, the sample contamination, false positive, false negative results, and the lower intensity of color on a strip of the test zone limits the applicability in biosensor applications.

Other complementary or alternative techniques for screening of SARS-CoV-2 are the serological-based techniques including enzyme-linked immunosorbent assay (ELISA), immunofluorescent assay (IFA), and neutralization assays.[47] The serological tests are effectively used for the diagnosis of asymptomatic patients as well as for patients with negative RT-PCR results.[48] ELISA is a sensitive diagnostic method for detecting the presence of antibodies produced against the SARS-CoV-2 antigen. It is specifically designed for the identification of IgM and IgG antibodies. The principle of ELISA is based on the interaction of antibody−antigen binding mechanism followed by enzyme reaction which produces the color. On the basis of these binding interactions, there are several formats such as direct, indirect, and sandwich, etc., which are performed by this assay. The advantageous role of this assay is that there is no requirement for sophisticated facilities and results are seen with naked eyes. Moreover, the major drawback of ELISA includes the lengthy processes, expensive kits, and complicated washing procedures.[49−51]

The clustered regularly-interspaced short palindromic repeats (CRISPR) is a powerful technology that has been used to engineer (cut, delete, and repeat) the genome. It has been modified from the prokaryotic adaptive immune system and is known to be a CRISPR (-Cas) system. Currently, it has provided a faster and cheaper diagnostic platform for virus detection in an ongoing pandemic situation. The CRISPR-Cas system focuses upon degrading the viral genome into the cell and its resulting viral mRNAs. Hence, CRISPR is used to identify the SARS-CoV-2 molecular properties and its therapeutic choice.[52] These systems have Cas proteins such as Cas12, Cas13, and so on, known for their collateral, nonspecific activities in succeeding recognition and cleavage of a specific target. In addition to this, collateral activities are responsible for nuclear detection methods with high specificity and sensitivity.[53] To provide the rapid and accurate result for COVID-19, different laboratories explored the capability of CRISPR-associated techniques for the detection of SARS-CoV-2.[54] Some examples of CRISPR-associated approaches such as SHERLOCK (specific, high-sensitivity enzymatic reporter unlocking) and DETECTR (DNA endonuclease-targeted CRISPR trans reporter) methods have gained much popularity for detection of SARS-CoV-2 viral genome in the recent scenario.[53] Despite many advantages such as specificity and flexibility, these existing CRISPR-based diagnostic platforms have some limitations such as the prerequisite of upfront extraction of nucleic acid, a voluminous amount of sample, and several physical steps involved in performing the test.[55]

One of the rarely commercialized techniques, mass spectroscopy (MS) is one of the promising techniques for the detection of viral infections. Recently, Nikolaev et al. reported the detection of the N-protein of SARS-CoV-2 in nasopharynx epithelial swabs through the mass spectroscopy technique. They deactivated the viral protein through heating and further added isopropanol for tryptic digestion. They have done the mass analysis which results in the fragmentation peak of viral protein, which confirms the presence of N-protein.[56] Similarly, Ihling et al. and Cardozo et al. reported the mass spectroscopy-based analysis of SARS-CoV-2 proteins in clinical samples.[57,58] MS analysis allows multiplex detection and ease of processing. But the complexity of data interpretation, a requirement of an expert technician, a well-equipped laboratory, costly setup, and the time consumption of about 3−4 h limit their diagnostics application. To eradicate the technological drawbacks such as labor-intensiveness, time-consumption, and complexity in sample preparation, etc., of conventional methods such as

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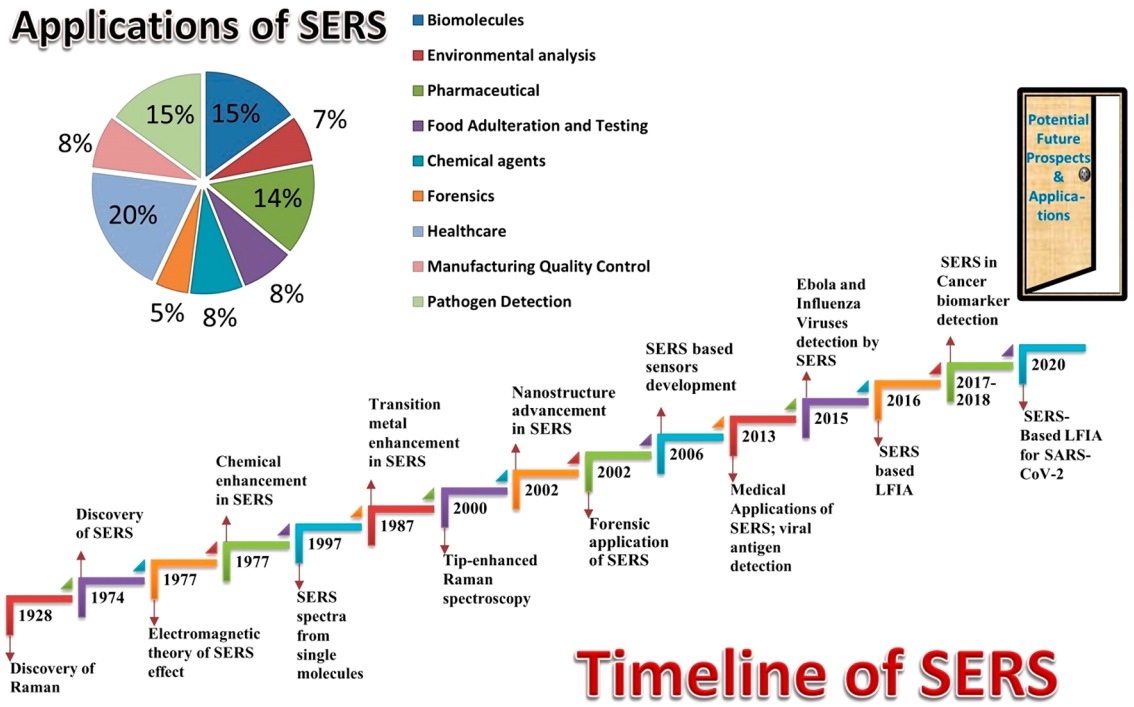

Figure 4. Historical overview and numerous applications of SERS for diagnostics platform.

RT-PCR, RT-LAMP, and ELISA, other substitute modalities can be developed.[59]

After the emerging of a new variant of SARS-CoV-2, several challenges are faced as they affected the accuracy of diagnostic tests and significantly reduced the sensitivity, which leads to the false negative result in several molecular tests.[60]

In this scenario, affordable, sensitive, specific, user-friendly, rapid, and robust, equipment-free, and deliverable platforms are required. Hence, POCT is relatively easy to perform and is a small, self-sufficient test for the detection of SARS-CoV-2 viral infection. Apart from these, they do not require trained personnel and other healthcare facilities and can be installed for on-site triage. Moreover, they do not require tedious sample preparation and the clock workload for performing the test.[61] Mujawar et al. in 2020 discussed COVID-19 management with miniaturized nanoenabled sensors embedded with artificial intelligence (AI) and Internet of Things (IoT) for POC diagnostics. They reported the potential advantages of AI that helps in fighting against pandemics at the global level.[62]

Therefore, POCT is the need of the hour platform to curb the swiftly growing pandemic across the globe. Consequently, the POC diagnostics device can fulfill a requirement for the detection of SARS-CoV-2 as well as in the surveillance of asymptomatic individuals. Since there is an urgent need for mass testing at a high ratio which could efficiently help to prevent the virus from spreading in a large chunk of the population from infected people. Recent POCT platforms such as LFIA and paper-based devices have the advantages to provide simpler, rapid, and economical platforms for the diagnosis of SARS-CoV-2. But they struggle with their challenges such as insufficient sensitivity, overall reliability, and selectivity, etc.[63,64]

Besides, biosensing technique is the emerging advanced sensing technique to propose the development of an effective diagnostic tool in a miniaturized shape, which is economical and easy to operate without high-level skills. The working of the biosensor propagates in such a manner that target analytes bind to biorecognition elements; a transducer amplifies the signal and generates the quantitative or semiquantitative information which records on a device. The classification of biosensors, based upon the class of transducer used, are named as optical, electrochemical, and mass-based biosensors.[65] All perspectives focus on the affinity of three main targets, i.e., nucleic acids, antibodies, and proteins.

Electrochemical biosensors gained much attention among analytical techniques due to their several advantages. They are superior in sensing biomolecules, because they can detect biomarkers with high specificity, sensitivity, and reliable accuracy. In addition, the ease-of-fabrication, miniaturization ability, budget-friendliness, simplicity, and user-friendly nature are attracting research toward them. They also have been used as POC testing at home and clinics.[66] A concise report on AI-based electrochemical sensing of SARS-CoV-2 at POC by Kaushik et al. describes a nanoenabled biosensor that aids in managing the pandemic. Their work shows the use of bioinformatics, machine learning, data exchange, and use of information with AI at the site of the epidemic to diagnose the disease.[67]

Moreover, the short shelf life, lower stability, and lower conductivity of nanomaterials fabricated on sensor surfaces make them a limitation in biosensor application. Over the past few years, plasmonic-based techniques have come out as an important contender in the growth of next level disease diagnosis platforms. It would significantly reduce the burden of contagious diseases in the developing sphere. They can overlap the gap between analytical chemistry and optics and provide inexpensive, real-time, sensible, and label-free detection of biomarkers of infectious diseases.[68] Recent work of Ahmadivand et al. in 2021 has developed a highly sensitive miniaturized THz toroidal plasmonic metasensor to detect SARS-CoV-2 spike proteins at femtomolar concentrations. Their proposed non-invasive, non-contact, and rapid modality

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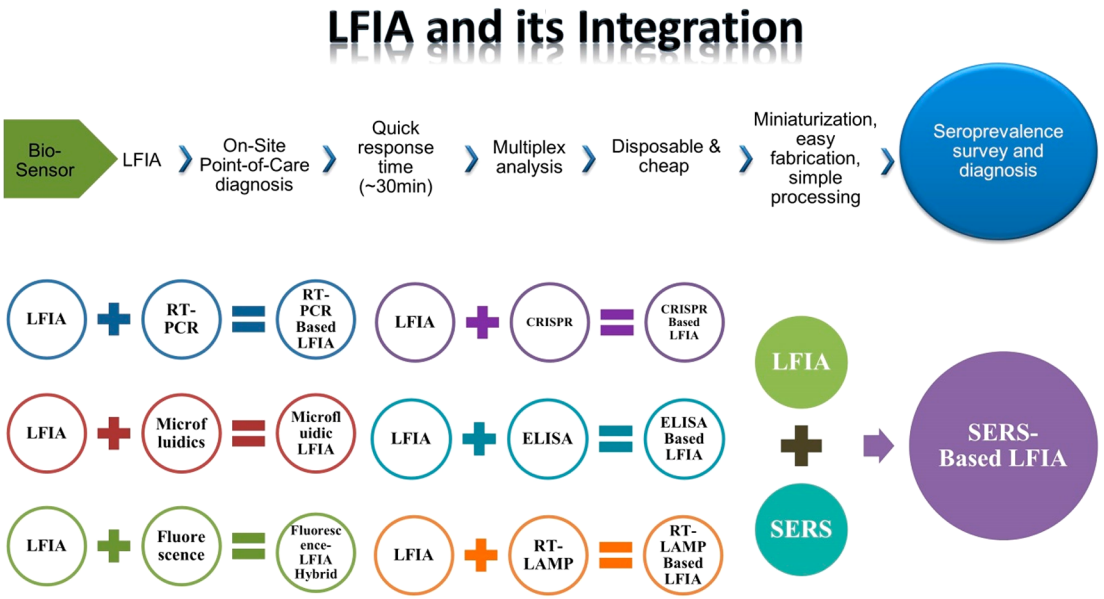

Figure 5. Representation of the potential of integration of LFIA with the other techniques.

research permits the analysis of SARS-CoV-2 spike protein at early stages with high accuracy.[69]

Several biosensing modalities such as surface-enhanced raman spectroscopy (SERS), surface plasmon resonance (SPR), localized surface plasmon resonance (LSPR), and colorimetric plasmonic assays have come out for early detection of biomarkers of infectious diseases. These depend on the principle of plasmonic-based optical detection platforms, i.e., absorption of light.[70] Their main advantages are high specificity, sensitivity, and ease of handling. At present, the working principle, advantages, and limitations of several diagnostics' platforms for the detection of SARS-CoV-2 are shown in Figure 3.

4. OVERVIEW OF SERS TECHNIQUE

Earlier use of technology was done individually for only one application. Later advancements led to multi-application-based techniques for the same detection setup. Even that had some limitations, which were later overcome by the integration of two or more techniques. With the recent discoveries and developments, some detection tools have optimized working. There is a huge scope of introduction of new methods with improved testing and enhanced results.

One such example is Raman spectroscopy. It enables the identification of molecular vibrations giving characteristic specific signals of each molecule. However, it has some limitations of its own such as low-density scattering signals are very weak and they get capped with other noise signals, which Fleischmann et al. discovered in 1974 and was later individually confirmed by Van Duyne and Creighton in 1977. Pyridine Raman spectra on a small roughened silver electrode had an enhanced spectrum which was later termed as surface-enhanced Raman scattering.[71−73] Nanoparticle size and surface make SERS is a distance dependent phenomenon. In 2015, Kumari et al. investigated this distance dependence of SERS as a function of nanoparticle size. Detection of biomolecules in an aqueous medium requires careful control over nanoparticle size. This directly affects the enhancement of Raman signal and filtering useful information.[74] SERS is a powerful technique which gains much attention in the research community due to its several advantages. Among emerging novel detection techniques, SERS has come out as a robust analytical tool for analysis of single-molecule level sensitivity and capacity of quantitative detection. They can be used for diagnosis purposes, when Raman spectroscopy is incorporated with intrinsic optical and chemical characteristics of plasmonic nanoparticles.[75,76] Classical plasmonic nanomaterials for SERS applications encompass colloidal nanoparticles, two-dimensional (2D) materials and arranged in a three-dimensional (3D) hierarchy.[77] The historical overview and wide applications of SERS in numerous fields are shown in Figure 4.

In the SERS sensing technique, an amplified inelastic light scatter from the molecules after being absorbed on the surface of metals.[78] The frequency modulation of excitation light and localized surface plasmonic resonance (LSPR) of the metallic nanomaterial could enhance the scattering signal by a thousand times. In addition to Raman spectroscopy, SERS has a highly sensitive and more specific detection and quantification platform.[79] The potential of SERS significantly increased after the discovery of its detection of microorganisms. The future clinical applications paved the way in healthcare for the rapid detection of pathogens, especially viruses.[80−82] SERS require a specific substrate but the sample preparation is simple, this helps in the safe detection of fragile and sensitive specimen. This was evident from the trace amount detection of viruses in very low concentration which is usually found underwater or in air.[81] SERS plays an important role in basic sciences research. It contributes toward clinical practices for disease prognosis, designing theragnostic agents, drug delivery monitoring, even diagnosis of diseases with real-time therapeutic response, which is possible due to the high enhancement factor of SERS.[79]

Metallic nanoparticles show enhanced scattering and can be used as SERS nanotags. It makes them a good candidate as labels, in which the signal of Raman dyes (RDs) with large Raman scattering cross-sections are enhanced by plasmonic nanoparticles of gold or silver.[82,83] "Hot spots" are the active sites on the substrate, where incident light gets greatly enhanced when exposed to a laser light due to the LSPR effect of SERS nanotags. The amplification of incident light

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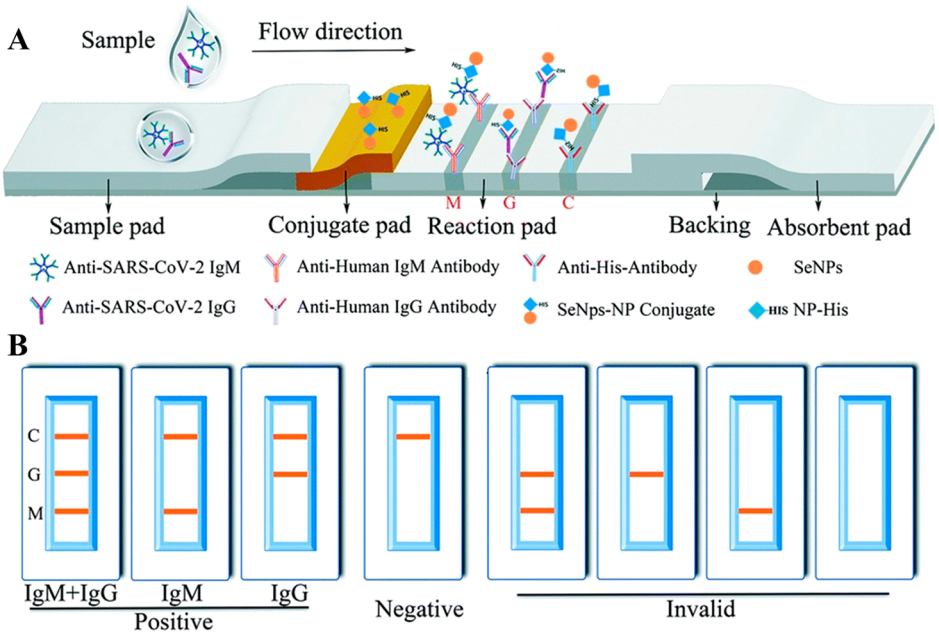

Figure 6. (A) Diagram and components of the anti-SARS-CoV-2 antibody immunoassay test strip and (B) visual assessment guidelines for interpreting the test strip results. Reproduced with permission from ref 135. Copyright 2020 American Chemical Society.

intensity is observed when the target molecules are placed near tiny nanogaps due to electromagnetic effects.[84,85] Even after considering several advantages of SERS, commercialization of SERS nanotags and surface is challenging due to the practicality of cost-effective fabrication, being highly sensitive, thermally and chemically stable, reusable, and reproducible SERS substrates.[86] Currently, SERS-based assay platforms have gained much awareness among medical sections because of their capacity of multiplex detection and comparative lower limit of detection than other techniques.[87]

Since 2007, making use of SERS for LFIA has emerged as highly specific and sensitive for viral pathogen detection methods. Precisely SERS tags can encapsulate with distinct Raman molecules that are used for conjugated detection antibodies for multiple target analytes and lead to multiplex detection on LFIA.[88] The current scenario of healthcare technology significantly influenced the diagnostic methods such as LFIA to get integrated with SERS to compensate for the drawbacks of conventional colloidal gold based LFIA.[89,90] The diverse applications of SERS-based LFIA include the detection of various target analytes, including proteins, nucleic acids, and infectious pathogens. Different elements, such as antibodies, aptamers, and DNA oligonucleotides, are also used as recognition receptors. The measurement principle in a SERS-based LFIA strip is the same as that in a conventional colorimetric LFIA strip, except for the use of Raman reporter-labeled SERS nanotags[91−104]

5. POC LFIA FOR DETECTION OF SARS-COV-2

The LFIA is an attractive detection tool that qualifies all required merits for colorimetric assays. A commonly used lateral flow device consists of four constituents that are sample pad, conjugate pad, detection membrane, and absorbent pad. It is fabricated in such a way that the continuous flow of samples through channels is done, where the flow is based on the mechanism of the capillary action.[105] The sample pad is made up of glass and/or cellulose, and the detection sample is applied to it. It is responsible for the transportation of samples to other components of the assay a smoothly and continuously way. A conjugate pad is placed where labeled biorecognition molecules are distributed. When the running sample is in contact with the material of the conjugate pad, it releases conjugate labels which are generally colloidal nanoparticles commonly AuNPs and AgNPs in the case of a traditional LFIA setup. These labeled conjugates affect the sensitivity of assays. The detection membrane is highly important in deciding the sensitivity of LFIA assay. Test lines and control lines are drawn on this membrane, and it supplies the support to better binding to capture probes such as antibodies, antigens, and so on. The adsorbent pad is connected to the detection membrane at the end of the assay, which works like the sink, also maintains the flow rate of the sample, and prevents the backward flow of sample. Moreover, the adsorption capacity of the holding sample plays a crucial role in the results of assays.[106] Traditional LFIA provides qualitative or semi-quantitative results on the basis of color visualization that can be observed by the naked eye. When color is visualized on both test line and control line, they indicate a positive result. However, if color is seen only on the control line, this indicates the negative result for the target analyte present in the sample.[107] In addition, LFIA does not require well-equipped laboratories and trained personnel for performing the test and provides a faster and reliable result.

Furthermore, LFIA tests are capable of denoting the existence of pathogens in various clinical samples, such as whole blood, plasma, serum, and saliva, etc., and are used as an ideal diagnostic assay for POC tests in limited resource surroundings and emergency divisions. In the need of antibody testing of SARS-CoV-2, LFIA raised as a rapid, accurate POCT method that is capable of detecting specific antibodies such as IgM, IgG, and IgA in whole blood, serum, and plasma and also provide distinguished results with specific antibodies detection.[108] For instance, Li et al. developed a LFIA prepared by using gold nanoparticles which are used for simultaneous detection of both antibodies IgM and IgG in the clinical sample within a few minutes.[109] Undoubtedly, LFIA has a

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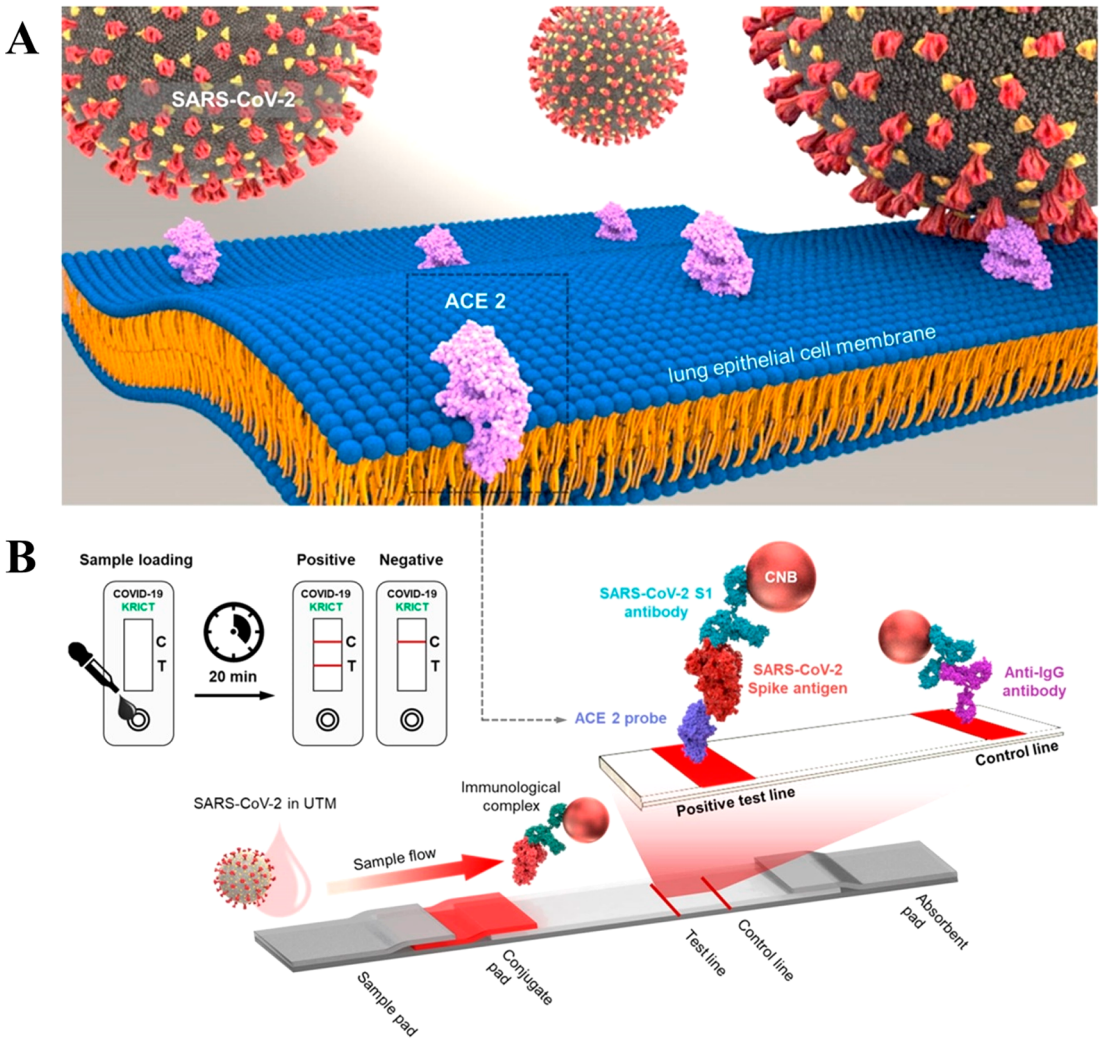

Figure 7. Cellular receptor (ACE2)-based LFIA. (A) Schematic of ACE2 receptor recognition by SARS-CoV-2. ACE2, a type 1 membrane protein expressed in the lung, heart, kidney, and intestine, is the cellular receptor for SARS-CoV-2. (B) Schematic of an ACE2-based LFIA consisting of a sample pad, conjugate pad, nitrocellulose membrane, and absorbent pad. The test line placed on the nitrocellulose membrane contains ACE2 for detection of the SARS-CoV-2 spike antigen. Anti-IgG antibody is used in the control line. Reproduced with permission from ref 140. Copyright 2020 Elsevier.

broader set of applications in both clinical and non-clinical setups. But, the limited sensitivity upon the higher concentration of analyte and poor reproducibility are the major drawbacks. Additionally, LFIA gives a rapid result, i.e., 10−30 min, and is simpler to use but it provides only qualitative facts and not so always with sufficient accuracy. Therefore, fast and delicate diagnostic methods are required to enlarge the capability of SARS-CoV-2 virus testing which are capable of high-throughput diagnosis and do not need high technical expertise or advanced tools.[110]

To overcome the aforementioned issues, LFIA has been integrated with other techniques such as SERS, CRISPR, RT-PCR, RT-LAMP, and ELISA, etc., which are capable of resolve these problems. The potential of integration of LFIA with the other techniques is shown in Figure 5.

Since the first reported case of COVID-19 last year, many publications and patents have been made in healthcare to develop the diagnostic kits for the detection of SARS-CoV-2. Some of the LFIA having significant advancement as biosensors for SARS-CoV-2 diagnosis is discussed in this section, in which some are either under R&D or commercialized.[111] For instance, Cavalera et al. used a multitarget LFIA and gold nanoparticles conjugate which gave better response and detection. The overall sensitivity for all antibodies combined was 94.6%. The response time after the sample was dropped on the LFIA strip was about 20 min. They used two test lines for the "total antibodies" detection of the SARS-CoV-2 antibodies.[112] In the publication of *Journal of Clinical Virology*, Nicol et al. did the comparative assessment of three commercially available immunoassays. They reported that the assays give better results for samples 14 days after symptoms onset. They did not observe any significant difference between the sensitivity of IgA ELISA and IgM LFIA which target two different proteins. Their study showed equivalent clinical performance for IgG of three immunoassays, and NG-Test is reliable and accurate for routine use in clinical laboratories.[113] In their research, Flower et al. did the evaluation of LFIA for seroprevalence survey which is widespread at the global scale. They took RT-PCR tested samples and did LFIA in the clinic and laboratory. Further, the results were compared with a sensitivity of 84.4% and specificity of 98.6% and moderate concordance. However,

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Table 1. List of LFIA-Based Diagnostic Devices for Detection of SARS-CoV-2 Biomarkers

specimen no.	technique	material	detection	sample	sensitivity	LOD	response time	ref
1	multitarget lateral flow immunoassay	gold nanoparticles	various antibodies	blood	94.6%		20 min	112
2	lateral flow immunoassay	commercial kit	IgG and IgM antibodies	serum or plasma	98.0%		15 min	113
3	lateral flow immunoassays	self-test kits	IgM and IgG	finger-prick capillary blood	serum (80.0%); whole blood (57.0%); finger prick (22.0%)			114
4	lateral flow immunoassay strip	colloidal gold nanoparticles	IgG antibody	inactivated human sera	69.1%		15−20 min	115
5	lateral flow assay	AuNPs	Glycoprotein Human IgG1	SARS-CoV-2 IgG	10.0 ng/mL	10^9 IgG/mL	5 min	116
6	LFIA	LFIA kits vs ELISA kits	IgM and IgG	plasma samples	95.0%			117
7	half-strip LFA	commercial reagent	Antibodies of SARS-CoV-2	commercial antigen samples	84.0%	0.65 ng/mL	20 min	118
8	LFIA	commercial kit	IgM and IgG	serum samples	95.0%			119
9	DR-LFA	rapid test kit INgezim COVID 19 CROM	Antibodies	blood, serum/plasma	91.2%		10 min	120
10	lateral flow immunoassay	europium-chelate-based fluorescent nanoparticles	FNP-labeled rabbit IgG	throat swab or sputum sample	95.0%	1000 TU/mL	less than an hour	121
11	lateral flow strip membrane assay		RdRp, ORF3a, and N genes	nasopharyngeal swabs and sputum	99.4%	10.0 copies/test	30 min	122
12	lateral flow assay	CLUNGENE SARS-CoV-2 colloid gold	IgG and IgM antibodies to SARS-CoV-2	convalescent donor plasma	87.3% IgG; 50.8% IgM		15 min	123
13	lateral flow immunoassay	lanthanide-doped nanoparticles	Anti-SARS-CoV-2 IgG	serum samples		100 μL (1:1000) dilution	10 min	124
14	lateral-flow assay	colloidal gold nanoparticle	IgM against SARS-CoV-2	serum samples	100%		15 min	125
15	lateral flow immunoassay	SiO_2@Au@QD nano beads	SARS-CoV-2-Specific IgM/IgG	human serum	100%	$1:10^6$	15 min	126
16	lateral flow POC diagnostic	multivalent gold nanoparticles bearing sialic acid	SARS-CoV-2 spike glycoprotein	throat, lower respiratory tract		5.0 μg/mL (5.0 nM)	30 min	127
17	lateral flow immunoassay	(colloidal gold) (Biolidics Ltd.) kit	IgG/IgM	venous blood draw and finger stick	92.0%		10 min	128
18	lateral flow assays	kits: BTNX Inc., ACON Laboratories, SD BIOSENSOR	Anti−SARS-CoV-2 IgG	serum, plasma, or whole blood	95.0%		10−15 min	129
19	lateral flow assay	Vazyme 2019-nCoV IgG/IgM Detection Kit	IgG	plasma/serum	95.9%		10 min	130
20	SERS-based lateral flow immunoassay	Ag shell on SiO_2 core SERS nanotag	Anti-SARS-CoV-2 IgM/IgG	serum and whole blood samples	>Au-LFA	1.0 pg/mL	25 min	131
21	lateral flow immunoassays	AllTest COVID-19 kit	IgG/IgM	serum	64.4%			132
22	lateral flow immunoassays	LabOn Time, Israel Avioq, China QuickZen, Belgium	IgG/IgM	whole blood, serum or plasma specimens	91.0−94.0%, after 14 days; overall, 70.0%		10 min	133
23	POC-lateral flow immunoassays	ALLTEST, Dynamiker, ASK COVID-19 and Wondfo SARS-CoV-2 Antibody Test	IgM/IgG	whole blood, serum, or plasma samples	within 14 days, 50.0%; between 15 and 21 days, 95.7%		5−15 min	134
24	lateral flow immunoassay	selenium nanoparticle	IgM/IgG	human serum	93.33%	IgM, 20.0 ng/mL; IgG, 5.0 ng/mL	10 min	135
25	lateral flow combined IgG-IgM immunochromatographic assay	gold nanoparticles (GNPs)	IgM and/or IgG	clinical blood samples	85.29%		15 min	136
26	lateral flow immunoassays strips	Wondfo, Sienna, Prometheus	IgM/IgG	serum samples	45.2%, 64.4%, and 75.5%, respectively		20 min	137

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ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

Table 1. continued

specimen no.	technique	material	detection	sample	sensitivity	LOD	response time	ref
27	lateral flow immunoassay cards	Wondfo, SGTi-Flex, and Innovita	IgM/IgG	serum samples	76.0%, 74.0%, and 58.0%, respectively		15 min	138
28	lateral flow immunochromatographic assay	commercial	IgM/IgG	serum	74.0%		10 and 30 min	139
29	LFIA		S1 protein	nasal swab		1.86×10^5 copies/mL	20 min	140

the sensitivity of all of the LFIA kits was comparatively less than either PCR or ELISA, but they all had sufficient specificity to be considered for survey studies. They summarized the results with emphasis on the need to evaluate and develop new more optimized tests for the population.[114] Wen et al. developed a LFIA strip for rapid detection of IgG antibodies against SARS-CoV-2. A standard LFIA strip for SARS-CoV-2 antibody detection was modified with AuNPs bioconjugates, to follow standard test procedure. The test results were obtained within 15−20 min. The sensitivity of their strips came to be around 69.1%. Optimizing the strip parameters such as Au labeling, Ag-Ab coupling, *p* value, blocking solution, and membrane selection, etc. can improve the performance of the strip. They summarized by aiming toward focusing on improving signal enhancement strategies and quantification systems.[115] Peng et al. worked toward enhancing the sensitivity of the LFIA strip for its optimized use in detection of SARS-CoV-2. Theoretically they analyzed with the help of a simple laser optical analysis to quantify the test sample while enhancing the sensitivity with the help of a photon-counting approach. They used AuNPs for absorbing incident light and scattering of enhanced signals. Consequently, they reported the lowest LOD among LFIA test kits.[116]

Whitman et al. evaluated the serological tests to check their performance in detecting SARS-CoV-2. They used commercially available 10 LFIA test kits (namely, Biomedomics, Bioperfectus, DecomBio, DeepBlue, Innovita, Premier, Sure, UCP, VivaChek, and Wondfo) and two ELISA test kits (namely, Epitope and In-house) for comparative performance testing. They got better results after 20 days of symptoms onset and variation in IgM results varied the test sensitivity. The specificity of LFIA kits was more after considering weak bands as negative, but it decreased the detection of antibodies (sensitivity) in RT-PCR positive cases.[117] Grant et al. developed a half-strip LFIA for the nucleocapsid antigen detection of SARS-CoV-2 and measured the response by two commercially available optical LFIA readers. The limits of detection of N-protein were 0.65 and 3.03 ng/mL for Genemedi and Genscript assays, respectively. The antibodies used for testing in this half-strip assay were originally developed for SARS-CoV. Moreover, the lack of specificity of assay due to cross-reactivity with SARS-CoV is not considered. The clinical sensitivity for SARS-CoV-2 in real samples has not been studied. It has been made for antigen detection as a possible diagnostic tool. Moreover, it opens new ways for further development on a clinical scale.[118] Lyu et al. used commercially available POC test kits for SARS-CoV-2 in pre-COVID-19 serums in Japan (400 healthy subjects in 2012−2015). LFIA and ELISA showed 1.5% and 1.75% for IgG positives, respectively. COVI040 LFIA kit was used for IgM or IgG detection, where weak signals were considered positive. RAI010R ELISA kit used the N-protein of SARS-CoV-2 as an antigen. The test was conducted for 40 pooled samples from 400 clinical samples. The sensitivity of the assay was 99.75%. The possibility of LFIA integration with other diagnostic methods could give better results. There is a need for careful interpretation of results and to adopt far better techniques.[119]

Hoste et al. used two serological methods, NovaLisa ELISA kit, namely, "DR-ELISA", and 2019-nCoV LFA, namely, "DR-LFA", for detection of antibodies of SARS-CoV-2. A total of 1056 human serum samples were tested for this study. The potential cross-reactivity of antibodies induced by infection

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ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

with seasonal coronaviruses was examined by both assays. No cross-reactivity was observed. The sensitivity of the DR-LFIA was reported as 91.2%, and that of DR-ELISA was 98.20%. LFIA took 10 min to give a single result, whereas ELISA took 75 min to analyze 92 samples. Total antibodies detection is beneficial because, in COVID-19, both IgM and IgG appear simultaneously.[120] Wang et al. reported an amplification-free nucleic acid immunoassay on a LFIA, named as "Hybrid Capture Fluorescence immunoassay HC-FLA". They developed a fluorescent nanoparticle monoclonal antibody that binds with DNA-RNA hybrid and helps to detect the ORF1ab, E- and N-regions of the SARS-CoV-2 genome. The test achieved a sensitivity of 100.0% and specificity of 99.0% for the trial sample of 670 individuals. These results were counter checked by RT-PCR results from the hospital. The consistency of results suggests the potential of such POC LFIA strips for rapid diagnosis of COVID-19 patients.[121] The novel lateral flow strip membrane (LFSM) assay was developed by Yu et al., which could be used for the simultaneous detection of RdRp, ORF3a, and N-genes at 25 °C after the RT-PCR. It has a detection limit of 10.0 copies per test for each gene. The result is out in 15 min. Previously detected by commercial assay, 162 clinical samples were reanalyzed to check the performance of the LFSM assay. The results were in concordance with commercial assays having high sensitivity. The developed LFSM assay is concluded to have the potential of complementary use with the RT-PCR or other methods for the diagnosis.[122] Another study using commercial CLUNGENE LFA test for SARS-CoV-2 virus analyzed the samples from COVID-19 convalescent plasma donors. Rasgnesola et al. presented their study which can reconfirm the positive individuals that have recovered from COVID-19. This would help in contact tracing and help the patient and community to take necessary safety steps. The LFIA test had a high degree of sensitivity (IgG had 87.3% and IgM had 50.8%). They have retested the samples to check reproducibility, from which some results were inconsistent due to several other reasons.[123]

Another development of LFIA with nanomaterial was reported by Chen et al., where they used lanthanide-doped polystyrene nanoparticles (LNPs) to detect SARS-CoV-2 IgG in human serum. The LNPs were functionalized with protein molecules for specific target binding. The use of nanoparticles influenced significantly the response of fluorescence results. The clinical testing was done on 51 normal serum samples and 7 post RT-PCR samples. The assay was reproducible and gave the results in 10 min. Thus, this rapid and sensitive test is essential to control the outbreak and diagnose the bulk population for early detection and prevention.[124] One of the very first research studies was done for the detection of IgM antibodies against the SARS-CoV-2 virus by Huang et al.; they developed a lateral flow assay using colloidal gold nanoparticles (AuNP-LF) to enhance the binding and detection of the virus from human serum. They used COVID-19 patient samples and normal humans then cross-checked their results with RT-PCR. The response time for the test was 15 min, and a sensitivity of 100% was achieved. Even the cross-reactivity of the test was validated. Hence, the feasibility of the test is recommended for use in a large number of clinical testings.[125]

Very recently, Wang et al. reported lateral flow immunosensor for the diagnosis of IgG and IgM against SARS-CoV-2 in a microliter of serum specimen within 15 min. Herein, they utilized the SiO_2@AuNPs@QD nanobeads functionalized with S-protein to target IgG and IgM. The proposed biosensor displays dual-mode signals as colorimetric and fluorescence. Moreover, this biosensor has 100 times higher sensitivity than the gold nanoparticles-based immunoassay strip. They utilized the quantum dots in biosensor fabrication since they possess excellent optical properties due to their quantum confinement effect. In addition, it has long-term light stability and a wide excitation range. Further, they validate the biosensor through the examination of 57 patient samples (16 positive and 41 negative) and found satisfactory performance with almost 100% specificity.[126]

Recently, Baker et al. developed a paper-based POC LFIA for the detection of the spike protein of SARS-CoV-2 within 30 min. Herein, they utilized the glycopolymer functionalized gold nanoparticles-based biosensor platform, where they studied that the functional chain of *N*-acetylneuraminic acid efficiently targets capture of the spike protein of SARS-CoV-2. They also tested that there is no such type of cross-reactivity over spike protein of SARS-CoV in a clinical sample. The LOD of this LFIA was 5.0 μg/mL.[127] Black et al. utilizing a commercial LFIA named "Biolidics 2019-nCoV IgG/IgM detection kit" for the diagnosis of IgG/IgM antibodies against SARS-CoV-2 in serum/plasma/whole blood sample within 10 min. They evaluate that the sensitivity of the test strip is 92.0% while specificity is 92.0% and 100% for IgM and IgG, respectively. They validate the test through the examination of 62 patients (both positive and negative) which were earlier confirmed by RT-PCR and also compared to gold-standard ELISA test. There was no significant alteration of the result of the Biolidics kit as compared to ELISA at day-0 and day-7. But the sensitivity of Biolidics at initial diagnosis of antibodies at day-0 was 29.0% and increases to 97.0% at day-7. So, the large variation of sensitivity at day-0 to day-7 may limit their application.[128] Similarly, McAulay et al. validated the four commercial LFIA test kits (BTNX kit 1, BTNX kit 2, ACON, and SD) from BTNX Inc., ACON Laboratories, and SD Biosensor Companies. They tested 352 patients of both positive and negative samples to clinically validate detection of the IgG antibodies against SARS-CoV-2 (anti-SARS-CoV-2 IgG) in plasma, serum, whole blood samples. Moreover, some of these test kits are also offered to detect IgM in the same strip. They dropped the 10.0−15.0 μL of the clinical sample followed by the 1−2 drops of buffer solution to dilute the sample which helps to flow the sample on the strip. After 10−15 min the result is shown for IgG/IgM detection. They evaluated that the sensitivity of BTNX 1 and BTNX 2 was 16.0% and 13.0% in the first week and increases to 95.0% and 91.0% after the second week, respectively. Moreover, the sensitivity for ACON and SD was found to be 5.0% and 92.0%, respectively. In addition, they evaluated that the specificities of biosensors are 98.0% for BTNX 1, 98.0% for ACON, and 100% for BTNX 2 and SD test kits. Moreover, few false-positive reports were also examined through the whole determination of IgG.[129] Similarly, Xie et al. validate the commercial LFIA kit Vazyme 2019-nCoV IgG/IgM kit (Nanjing Vazyme Medical Technology Co. Ltd.) from 100 serum/plasma samples. The test kits have efficiently detected the IgG in samples, which have high sensitivity and specificities of 95.9% and 96.1%, respectively. The company applied for the emergency approval for detection of antibodies (IgG/IgM) against SARS-CoV-2. But they failed to get approval due to poor diagnosis performance of the kit for IgM detection.[130]

Liu et al. reported that they used SERS-based LFIA for the simultaneous detection of anti-SARS-CoV-2 IgM/IgG. The

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ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

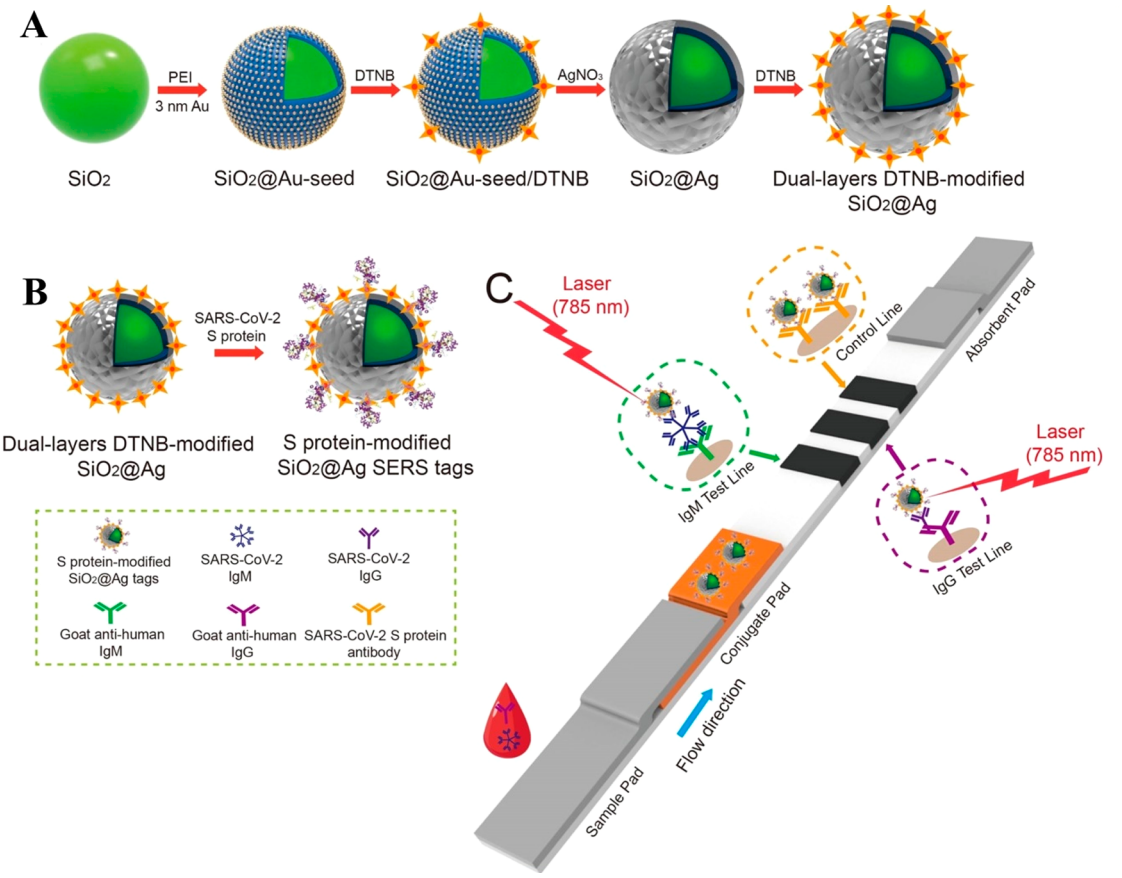

Figure 8. (A) Schematic diagram of the preparation of the dual-layers DTNB-modified SiO_2@Ag NPs. (B) Preparation of SARS-CoV-2 S protein-modified SiO_2@Ag SERS tags. (C) Operating principle of the high-sensitivity and simultaneous analysis of anti-SARS-CoV-2 IgM/IgG via the SERS-LFIA strip. Reproduced with permission from ref 131. Copyright 2020 Elsevier.

use of a novel SERS tag consisted of a silver shell over a silicon dioxide core. They used 90.0 μL of the sample to operate SERS-LFIA strips, and the response time was 25 min. The clinical validation of their device was checked with 68 patient samples with 100% accuracy.[131] Very recently, Perez-Garcia et al. validated the AllTest COVID-19 IgG/IgM test kit (AllTest BioTech) for the detection of IgG and IgM antibodies in clinical samples after 14 days of symptom onset. They tested the 90 positive and 100 negative samples. The specificity of sensor was 100% and sensitivity of 64.4%. The sensitivity increases to 88.0% after 14 days onset on viral infection.[132] Similarly, Montesinos et al. validated the five commercial kits for the detection of IgM, IgG, and IgA specific to SARS-CoV-2 in 200 (128 positive and 72 negative) clinical samples, in which they compare the performance of two quantitative immunosensors (Maglumi2019-n-CoV IgG and IgM and Euroimmun Anti-SARS-CoV-2 IgG and IgA kit) and got results showing that the Maglumi assay is less sensitive (64.3%) than the Euroimmun assay (84.4%). Moreover, the specificities of both assays are quite similar. In addition, the three LFIA (LaboOn Time, Avioq, and QuickZen) have approximately equal sensitivity around 70.0%.[133] Similarly, Wu et al. validated the four LFIA (ALLTEST 2019-nCoV IgG/IgM Rapid Test, Dynamiker 2019-nCoV IgG/IgM Rapid Test, ASK COVID-19 IgG/IgM Rapid Test, and Wondfo SARS-CoV-2 Antibody Test) for the detection of IgM/IgG antibodies against SARS-CoV-2 within 15 min. These four immunoassays have 100% sensitivity and specificity for antibodies to detect SARS-CoV-2 after three week onset of symptoms.[134]

Wang et al. assembled a LFIA for the detection of SARS-CoV-2 IgM and IgG in serum and blood samples within 10 min. Herein, they prepared the selenium nanoparticles and then conjugated by nucleoprotein. They fabricated the conjugate pad of assay by N-protein functionalized selenium nanoparticles while the two test lines (M and G), and control line by antihuman IgM antibodies, antihuman IgG antibodies, and antihis antibodies, respectively. When a drop of clinical sample is dropped on the conjugate pad, it flows on the strip and color develops on M, G, and C lines, which indicate the presence of IgM and IgG in the sample. With simplicity, the result is easy to observe through the naked eye. The sensitivity of LFIA is 93.33% and specificity of 97.34%. The LODs of the test kit are 20.0 ng/mL and 5.0 ng/mL for IgM and IgG, respectively. In addition, the shelf life of the kit is 30 days when it is stored at 37 °C temperature. Further, they validate the kit by examination of 90 positive and 263 negative samples and result in the satisfactory performance. They also tested against rheumatoid factor, antinuclear antibodies, influenza A, and influenza B and found no cross-reactivity against such diseases (Figure 6 A,B).[135]

Zeng et al. reported an immunosensor chip for simultaneous detection of IgM and IgG in a single test kit in blood specimen and could detect antibodies within 15 min. Herein, they fabricated the LFIA kit by the separate immobilization of gold nanoparticles labeled S-protein and rabbit IgG antibodies on sample pad. However, the two-test line (M and G) and control

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ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

lines were fabricated by their respective anti-antibodies. When the red line appears on M line or G line with C line, they indicate the presence of IgM or IgG in a clinical sample. Moreover, the red color on both M and G line with C line indicates both antibodies in the sample. They further tested the 80 (positive and negative) patients samples and found that the sensitivity of the kit is 85.29%. However, specificity is 100%. They also revealed that the sensitivity of immunoassay was low when it detected either IgM or IgG, but it dramatically increases when both antibodies are present.[136] Recently, Guedez-Lopez et al. validated the three lateral flow immunoassays (Sienna, Wondfo, and Prometheus) for the detection of IgM and IgG against SARS-CoV-2 in serum specimens. The overall sensitivities of the three LFIAs are 64.4%, 45.2%, and 75.5%, and specificities are 75.0%, 81.8%, and 12.5% for Sienna, Wondfo, and Prometheus, respectively. Moreover, the sensitivities of these immunoassays are enhanced to 100%, 83.3%, and 100%, respectively, after the third week of onset on disease.[137] Similarly, Gutierrez et al. validated the ten immunoassays (three LFIA rapid tests, four ELISA, and three chemiluminescence assays) for the detection of antibodies (IgM, IgG, and IgA) specific to SARS-CoV-2. The three LFIA kits are Wondfo, SGTi-Flex and Innovita; the four ELISA kits are COVID-19 ELISA IgG, COVID-19 ELISA IgM+IgA (Vircell, Spain, S.L.), EUROIMMUN ELISA Anti SARS-CoV-2 IgG, and Anti SARS-CoV-2 IgA (Medizinische Labordiagnostika AG). The first two ELISA kits utilized the S-protein and N-protein while the last two only the S-protein to specifically detect antibodies. Moreover, three chemiluminescence kits are VIRCLIA IgG Monotest, VIRCLIA IgM+A Monotest (Vircell, Spain, S.L.), and SARS-CoV-2 IgG Architect (Abbott). The first two chemiluminescence kits utilized the S-protein and N-protein while the third only the N-protein to specifically detect antibodies.[138] Fabian et al. validated two commercial test kits, anti-SARS-CoV-2 IgA EUROIMMUN and ENZY-WELL SARS-CoV-2 IgA, for the detection of IgA specific to SARS-CoV-2, instead of IgG/IgM in 65 clinical samples (39 positive and 26 negative) which are tested to PCR.[139]

Very recently, Lee et al. reported the LFIA for the detection of S1 protein of SARS-CoV-2 in clinical sample within 20 min. ACE2 receptor potentially binds to the S1 protein of SARS-CoV-2 on a LFIA strip. They have high sensitivity up to 1.0 ng/reaction to the RBD and below 5.0 ng/reaction S1 protein of SARS-CoV-2. The LOD of the assay was 1.86×10^5 copies/mL in sample. They further tested the cross-reactivity of LFIA and found that there are no such properties for S1 protein of SARS-CoV and MERS-CoV. Cellular receptor (ACE2)-based LFIA is shown in Figure 7A,B.[140] Recently reported SERS and LFIA-based biosensor for detection of COVID-19 in clinical specimens are listed in Table 1.

6. SERS-BASED LFIA POC DEVICE

The LFIA in itself is a rapid and POC diagnostic tool that has been used for quick diagnosis. It has its own merits and demerits. To enhance the performance and overcome the demerits, several nanotechnology-based enhancements have been made for internal optimization. There are few other methods like the integration of LFIA kit with other diagnostic tools to improve performance; still, there are a few drawbacks for them. One of the recent advancements is the development and utilization of the SERS technique. The applications of SERS are numerous and very promising to be coupled with another basic technique to achieve qualitative as well as quantitative results. SERS-based LFIA makes use of properties of a nanomaterial as a SERS tag and advanced characterization technique being highly specific. Along with rapid detection assay which collectively gives an enhanced signal response, accuracy and high sensitivity in results. Further, SERS dramatically enhanced the intensity up to 10^6 times and even it could detect the single molecule.[141] One novel research is from the group of Liu et al. where they developed a SERS-based LFIA biosensor. They used dual-layer Raman molecule-loaded Ag-coated SiO_2 NPs as advanced SERS tags. The SERS tag was then conjugated with the spike protein of the SARS-CoV-2 virus for the simultaneous detection of IgG/IgM antibodies. SERS measurements were performed on 19 COVID-19 positive serum and 49 COVID-19 negative serum samples by using 785 nm excitation with a power of 10.0 mW for 1.0 s. The results were out in 25 min (optimized time), and LOD was found to be 1.0 ng/mL of S-protein antibody in the case of 240 nm SiO_2@Ag tag that gave the best results. Further for the clinical samples, the LOD was 1.0 pg/mL from the SERS results, which is 1000 times more than the visualization results. Considering such enhanced results, the SERS-LFIA technique is proposed for rapid screening and bulk diagnosis at ultralow detection levels when other commonly used methods are not able to detect the virus. The schematic of the preparation of the dual-layers DTNB-modified SiO_2@Ag NPs based LIFA is shown in Figure 8A−C.[131]

7. COMMERCIAL KITS FOR DETECTION OF SARS-COV-2

Since the outbreak of the COVID-19 pandemic, there has been a tremendous amount of research and development for the testing of the clinical samples either in laboratory or POC test kits. Initial testing had several issues such as false negative or false positive results due to inaccurate detection techniques and incomplete knowledge about the virus. This leads to the spread of the virus on a global scale that leads to the high demand for the testing required in developing as well as developed nations. In the foresight of the above, many of the commercial kits were given emergency approval. However, the licenses of few distributors have been canceled. The approving agencies are responsible for validating the test kits which have clinically passed the sample testing and the chances of false results are null. Some of the commercially available approved kits are listed in Table 2.[142−146]

8. CHALLENGES ASSOCIATED WITH SERS-BASED COVID-19 DIAGNOSTICS MANAGEMENT

The diagnostic techniques generally implied in the laboratory go toward the commercial market after considering their advantages with respect to other similar techniques available. About the SERS technique, it has overcome many drawbacks of previous methods and presented an advancement in technology, and as an output, a significantly optimized and improved result is achieved. First, the applicability of such integration of techniques which can make the current diagnosis error free and more sensitive and quicker is worth mentioning among the advantages. In this review, we have discussed the integration of SERS with LFIA, which makes the POC diagnosis and at the same time gives accurate results even at multiplexed detection. To mention some of the main advantages of SERS, it has increased sensitivity which means

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ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

Table 2. List of Commercially Available Diagnostic Devices for Detection of SARS-CoV-2 Biomarkers[a]

specimen no.	kit name	technique	name of company	country of origin	approval agency
	Antibody-Based Rapid Test Kits Approved by ICMR, India[142]				
1	Coronavirus (COVID-19) IgG/IgM Rapid Test	antibody based	Voxtur Bio Ltd., Surat (Gujarat)	India	ICMR
2	COVID-19 IgM/IgG Antibody Detection Card Test	antibody based	VANGUARD Diagnostics, Delhi	India	ICMR
3	Makesure COVID-19 Rapid test	antibody based	HLL Lifecare Limited, Gurugram (India)	India	ICMR
4	ACCUCARE IgM/IgG Lateral Flow Assay kit	antibody based	Lab Care Diagnostics India Pvt. Ltd., Mumbai (Maharashtra)	India	ICMR
5	Abchek COVID-19 IgM/IgG Antibody Rapid Test	antibody based	NuLifecare, Noida (UP)	India	ICMR
6	One Step Corona Virus (COVID-19) IgM/IgG Antibody Test	antibody based	Alpine Biomedicals, Ambala (Haryana)	India	ICMR
7	COVID 19 IgM/IgG Rapid Test Kit (ver 2.0)	antibody based	Medsource Ozone Biomedicals, Haryana	India	ICMR
8	Immuno Quick Rapid Test for Detection of Novel Coronavirus (COVID-19) IgM/IgG Antibodies	antibody based	Immuno Science India Pvt. Ltd., Pune	India	ICMR
9	BMT COVID-19 IgG/IgM Rapid Test Kit	antibody based	BMT Diagnostics (Rafael Diagnostic)	Israel	ICMR
10	One Step COVID-19 IgM/IgG Antibody	antibody based	SIDAK Life Care Pvt. Ltd., New Delhi	India	ICMR
11	Xamin COVID-19 Rapid Test Device	antibody based	Diagnocure, Solan (HP)	India	ICMR
12	ImmunoQuick COVID-19 IgG Rapid Test Device	antibody based	ImmunoScience India Pvt. Ltd.	Singapore	ICMR
13	PCL COVID-19 IgG/IgM Rapid Gold	antibody based	PCL, Inc., (Hemogenomics Pvt. Ltd.)	Seoul, Republic of Korea	ICMR
14	Rapid test - Corona Antibody (IgM/IgG)	antibody based	Oscar Medicare Pvt. Ltd., (Delhi)	India	ICMR
15	Edinburgh Genetics COVID-19 Colloidal Gold Immunoassay Testing kit IgG/IgM combined	antibody based	Edinburgh Genetics	UK	ICMR
16	COVID-19 Rapid Test	antibody based	BioPanda Reagents, (Inbios (Delhi)	UK, India	ICMR
17	TRUSTline COVID-19 Ab Rapid Test	antibody based	Athenese-Dx Private Limited, Chennai (Tamil Nadu)	India	ICMR
18	Corona virus (COVID-19) Rapid Test	antibody based	Beijing Zhangjian Antai Diagnostic Technology, (Aura Medical Devices Pvt. Ltd.)	China	ICMR
19	Rapid Test for detection of IgM and IgG antibodies	antibody based	Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd.)	India	ICMR
20	Corona IgG Ab detection Test (OSCOVID-IgG)	antibody based	Oscar Medicare Pvt Ltd., (Delhi)	India	ICMR
21	IgG Covimmuno Device	antibody based	Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd.)	India	ICMR
22	EdgeXpress Covid-19 Ab detection Test Kit (IgG/IgM)	antibody based	Edge Pharma Pvt. Ltd., Mumbai (Maharashtra)	India	ICMR
23	EzDxTM Covid-19 IgG/IgM Rapid Antibody Test	antibody based	Advy Chemicals Pvt. Ltd.	India	ICMR
24	Optra Shield COVID-19 (IgG) Rapid Test Kit	antibody based	Oscar Medicare Pvt. Ltd.	India	ICMR
	Antigen-Based Rapid Test Kits Approved by ICMR, India[143]				
25	STANDARD Q COVID-19 Ag	antigen based	SD Biosensor	South Korea/India	ICMR
26	COVID-19 Antigen Lateral Test Device	antigen based	LabCare Diagnostics Ltd., Valsad (Gujarat)	India	ICMR
27	BIOCARD Pro COVID-19 Rapid Ag test kit	antigen based	Trivitron Healthcare Pvt. Ltd., Chennai (TN)	India	ICMR
28	VSTRIP COVID-19 Antigen Rapid Test	antigen based	Panion & BF Biotech.	Taiwan	ICMR
29	PCL COVID-19 Rapid FIA	antigen based	PCL Inc.	South Korea	ICMR
30	Sure status COVID-19 Ag Test	antigen based	Premier Medical Corporation, Valsad (Gujarat)	India	ICMR
31	Angcard COVID-19 rapid Antigen Test kit	antigen based	Angstrom Biotech Pvt. Ltd., Alwar (Rajasthan)	India	ICMR
32	GenBody COVID-19 Ag rapid Test kit (POCT)	antigen based	GenBody Inc.	South Korea	ICMR
33	SENSIT Rapid COVID-19 Ag kit	antigen based	Ubio Biotechnology Systems Pvt. Ltd., Kochin (Kerala)	India	ICMR
34	COVID-19 Antigen Detection Test	antigen based	Meril Diagnostics, Vapi (Gujarat)	India	ICMR
35	Alpine COVID-19 Antigen Rapid Test kit	antigen based	Alpine Biomedicals Pvt. Ltd., Ambala (Haryana)	India	ICMR
36	Oscar CORONA Rapid Ag Test kit	antigen based	Oscar Medicare Pvt. Ltd., Delhi	India	ICMR
37	ImmunoQuick COVID19 Antigen Rapid Card test kit	antigen based	ImmunoScience India Pvt. Ltd., Pune (Maharashtra)	India	ICMR

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ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

Table 2. continued

specimen no.	kit name	technique	name of company	country of origin	approval agency
		Antigen-Based Rapid Test Kits Approved by ICMR, India[143]			
38	iNSTAXPLOR COVID19 Ag – Rapid Antigen Test	antigen based	STRUmed Solutions Pvt. Ltd., Chennai (Tamil Nadu)	India	ICMR
		Antibody-Based Lateral Flow Assay Rapid Test Kits Approved by FDA, USA[144]			
39	CareStart COVID-19 IgM/IgG	antibody-based LFA	Access Bio, Inc.	USA	FDA
40	Assure COVID-19 IgG/IgM Rapid Test Device	antibody-based LFA	Assure Tech. (Hangzhou Co., Ltd.)	China	FDA
41	WANTAI SARS-CoV-2 Ab Rapid Test	antibody-based LFA	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.	China	FDA
42	Tell Me Fast Novel Coronavirus (COVID-19) IgG/IgM Antibody Test	antibody-based LFA	Biocan Diagnostics Inc.	Canada	FDA
43	Biohit SARS-CoV-2 IgM/IgG Antibody Test Kit	antibody-based LFA	Biohit Healthcare (Hefei)	China	FDA
44	qSARS-CoV-2 IgG/IgM Rapid Test	antibody-based LFA	Cellex, Inc.	USA	FDA
45	RightSign COVID-19 IgG/IgM Rapid Test Cassette	antibody-based LFA	Hangzhou Biotest Biotech	China	FDA
46	LYHER Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit (colloidal gold)	antibody-based LFA	Hangzhou Laihe Biotech	China	FDA
47	COVID-19 IgG/IgM Rapid Test Cassette	antibody-based LFA	Healgen	USA	FDA
48	Innovita 2019-nCoV Ab Test (colloidal gold)	antibody-based LFA	Innovita (Tangshan) Biological Technology Co., Ltd.	USA	FDA
49	Orawell IgM/IgG Rapid Test	antibody-based LFA	Jiangsu Well Biotech	China	FDA
50	Rapid COVID-19 IgM/IgG Combo Test Kit	antibody-based LFA	Megna Health, Inc.	USA	FDA
51	Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit	antibody-based LFA	Nirmidas Biotech, Inc.	USA	FDA
52	Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test Cassette	antibody-based LFA	Salofa Oy	Finland	FDA
53	SGTi-flex COVID-19 IgG	antibody-based LFA	Sugentech, Inc.	Republic of Korea	FDA
54	TBG SARS-CoV-2 IgG/IgM Rapid Test Kit	antibody-based LFA	TBG Biotechnology Corp.	Taiwan	FDA
55	BIOTIME SARS-CoV-2 IgG/IgM Rapid Qualitative Test	antibody-based LFA	Xiamen Biotime Biotechnology Co., Ltd.	China	FDA
		Antibody-Based Lateral Flow Assay Rapid Test Kits Approved by U.S. FDA EUA[145]			
56	Anti-SARS-CoV-2	antibody based	Autobio Diagnostics Co., Ltd.	China	U.S. FDA EUA
57	ScheBo SARS-CoV-2 Quick	antibody based	ScheBo Biotech AG	Germany	U.S. FDA EUA
58	MAGLUMI 2019-nCoV IgG (CLIA)	antibody based	Snibe Co., Ltd. (Shenzhen New Industries Biomedical Engineering Co., Ltd.)	China	U.S. FDA EUA
		Antigen-Based Lateral Flow Assay Rapid Test Kits Approved by U.S. FDA EUA[146]			
59	BinaxNOW COVID-19 Ag Card	antigen based	Abbott Diagnostics Scarborough, Inc.	USA	U.S. FDA EUA
60	BD Veritor System for Rapid Detection of SARS-CoV-2	antigen based	Becton Dickinson & Co.	USA	U.S. FDA EUA
61	LumiraDx SARS-CoV-2 Ag Test	antigen based	LumiraDx UK Ltd.	U.K.	U.S. FDA EUA
62	SARS-CoV-2 Antigen Rapid Test Cassette (Swab)	antigen based	Spring Healthcare Services AG	Switzerland	U.S. FDA EUA

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ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

Table 2. continued

specimen no.	kit name	technique	name of company	country of origin	approval agency
Antigen-Based Lateral Flow Assay Rapid Test Kits Approved by U.S. FDA EUA[146]					
63	Sofia 2 Flu + SARS Antigen FIA, Sofia 2 SARS Antigen FIA	antigen based	Quidel	USA	U.S. FDA EUA

[a]ICMR, Indian Council of Medical Research Department of Health Research, Ministry of Health and Family Welfare, Government of India; FDA, Food and Drug Administration, U.S.A.; EUA, emergency use authorization.

it can detect single molecules. Selectivity is extremely high which makes the detection of specific target molecule in a large complex much easier on the basis of the signature of molecules. There are several applications where SERS can be used where no other technique was able to diagnose previously. Among the few are the cellular analyses, which can do a highly localized chemical-specific study of the sample with the help of nanosized substrate being SERS active.[147] SERS analysis can detect the actual molecule under study, being specific to the molecular level. This opens the way for in-situ identification, especially without the need for separation in the case of multiple species. The surface treatment of a substrate makes it SERS active. It can become beneficial for several other diagnostics applications.

However, at the same time, there are a few challenges when making use of the same technique in various applications. One such example is when the analysis of a protein is to be made; the surface treatment of the substrate to make it SERS active can cause conformation changes in the protein. This can be overcome by another merit of SERS being highly selective. It can still observe the reaction change with respect to the environment change, giving unique spectra for each of them.[148] With regard to the mentioned SERS-based LFIA technique, especially in the case of SARS-CoV-2, the specificity of the analyte after careful sample collection is of utmost importance since the technique is being used in healthcare. This highlights the need for the instrumental part to have the biocompatible components optimized for clinical usage. The use of rough surfaces at the nanoscale or metallic surfaces as SERS active surfaces is an advantage for the detection of other molecules. This gives rise to one of the inherent drawbacks of this technique; that is, it cannot be used for metals or alloys, since the sensitivity is very low and fluorescence of some materials can dim and cover the response of others. Moreover, nonspecific binding is one of the limitations which gives false results. This could be easily extended in terms of clinical samples and diagnosis of analytes at extremely lower concentrations. It makes it harder for each analyte to be detected specifically.[149] Significantly, this can be extended to the healthcare applications of SERS in the disease's regime for the pathogen, bacterial, and viruses' diagnostics. Moreover, this can be termed as a significant disadvantage of SERS in terms of the recent use of this technique in the medical field. Since the equipment needs to be modified with the compatibility of biological samples, currently physicists are more inclined toward use and operation.

Clinical communities need to adopt this technology for use in wide-ranging studies. The principle is the same independently and can govern the testing when coupled with other standard techniques like PCR instruments in clinical areas.[150] One of the main areas which need the attention of researchers and is responsible for signal response and enhancement is the development of substrate properties. There is a need to develop proper fabrication methods for the special purpose of SERS tag synthesis. Properties of well-dispersed nanoparticles show a weak but homogeneous enhancement which needs to experimentally be controlled. Optimized conditions would give spectral reproducibility and even enhance the response effects of nanoparticle aggregates which are already highly ordered with surface uniformity.[151]

Few other technical limitations of the SERS technique include the contact between the enhancing surface and the analyte. This causes the substrates to degrade with time,

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ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

resulting in a decrease in signal and hence making the substrate poorly reusable, and one main issue is having a reproducible SERS signal within a substrate.[152] Concerning the extremely small cross-sectional area of the samples, this leads to a longer acquisition period of analysis and a high concentration of the sample may lead to noise or irregular results.[153,154]

9. CONCLUSION AND FUTURE PERSPECTIVE

After analyzing the advantages and disadvantages of the SERS technique, one must have some future direction for the required modification and scope of the method to be made useful when integrated with other techniques. One of the highly focused clinical applications of LFIA with regard to its usefulness as a detection kit for SARS-CoV-2 and ease the diagnosis efficiently. The prospects of SERS-based LFIA or individually any technique lies in the possible use of the method. Furthermore, it strongly needs improvements in its integral setup or methodology for broader applications. One of the most required developments is making the laboratory scale SERS technique into portable SERS. This would be an opportunity to use it as POC device and as a rapid diagnostic tool for clinical application. Normally the SERS signals for most metals are found near the visible range or near-infrared wavelength. It gives a nudge to explore the ultraviolet frequency range and can be desirable for biological molecules and lead in applications such as disease diagnosis, food quality control, forensics, and others. Another area where potential improvement can be made is the testing time a SERS-based test would take, since currently the scan is time-consuming and requires molecule-by-molecule scanning for higher sensitivity and specificity. The efficient fast spectroscopies are the need of the hour for integrated techniques. One such example is the femtosecond stimulated Raman spectroscopy. The least explored area in SERS-based AFM extended development is TERS, which will make use of sub-diffraction-limited imaging for a wide variety of numerous applications.

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Notes

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