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Dye-Loaded Polymersome-Based Lateral Flow Assay: Rational Design of a COVID-19 Testing Platform by Repurposing SARS-CoV-2 Antibody Cocktail and Antigens Obtained from Positive Human Samples

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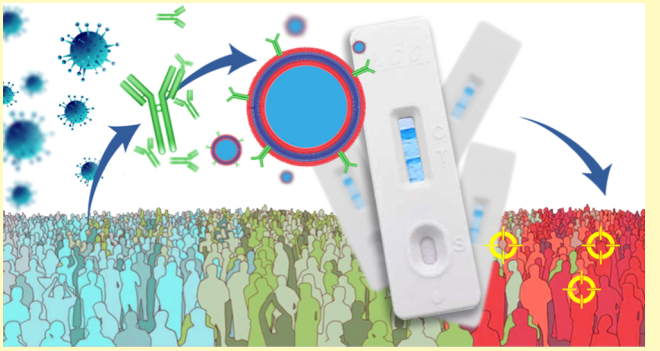
ABSTRACT: The global pandemic of COVID-19 continues to be an important threat, especially with the fast transmission rate observed after the discovery of novel mutations. In this perspective, prompt diagnosis requires massive economical and human resources to mitigate the disease. The current study proposes a rational design of a colorimetric lateral flow immunoassay (LFA) based on the repurposing of human samples to produce COVID-19-specific antigens and antibodies in combination with a novel dye-loaded polymersome for naked-eye detection. A group of 121 human samples (61 serums and 60 nasal swabs) were obtained and analyzed by RT-PCR and ELISA. Pooled samples were used to purify antibodies using affinity chromatography, while antigens were purified *via* magnetic nanoparticles-based affinity. The purified proteins were confirmed for their specificity to COVID-19 *via* commercial LFA, ELISA, and electrochemical tests in addition to sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. Polymersomes were prepared using methoxy polyethylene glycol-*b*-polycaprolactone (mPEG-*b*-PCL) diblock copolymers and loaded with a Coomassie Blue dye. The polymersomes were then functionalized with the purified antibodies and applied for the preparation of two types of LFA (antigen test and antibody test). Overall, the proposed diagnostic tests demonstrated 93 and 92.2% sensitivity for antigen and antibody tests, respectively. The repeatability (92−94%) and reproducibility (96−98%) of the tests highlight the potential of the proposed LFA. The LFA test was also analyzed for stability, and after 4 weeks, 91−97% correct diagnosis was observed. The current LFA platform is a valuable assay that has great economical and analytical potential for widespread applications.



KEYWORDS: *COVID-19, in vitro diagnostics, point-of-care (POC) platform, lateral flow assay, dye-loaded polymersome, sample repurposing*

Since the first outbreak of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the development and implementation of specific diagnostic assays were promptly ensured by various laboratories worldwide. Initially, the detection of viral RNA *via* molecular assays has been the gold standard for early and precise detection using polymerase chain reaction (PCR).[1] However, PCR faces stringent requirements for its application which consequently pushed pharmaceutical companies to look for more effective methods to bridge the gap.[2] Immunological-based testing seems like a dependable alternative for diagnostics such as the use of lateral flow assays (LFAs). LFA is an immunoassay-based platform that has a great impact in low-income countries. It has many applications in disease diagnostics including infectious diseases.[3] LFAs demonstrate a great advantage as a point-of-care (POC) platform due to fast turnaround and lower prices.[4] Their real importance resides in their assistance to health providers to make a quick diagnosis and proceed for faster treatment measures that can have an important impact during critical situations.[5] LFAs are generally based on visual observation that makes use of colorful molecules such as gold nanoparticles which are considered stable for LFA-based

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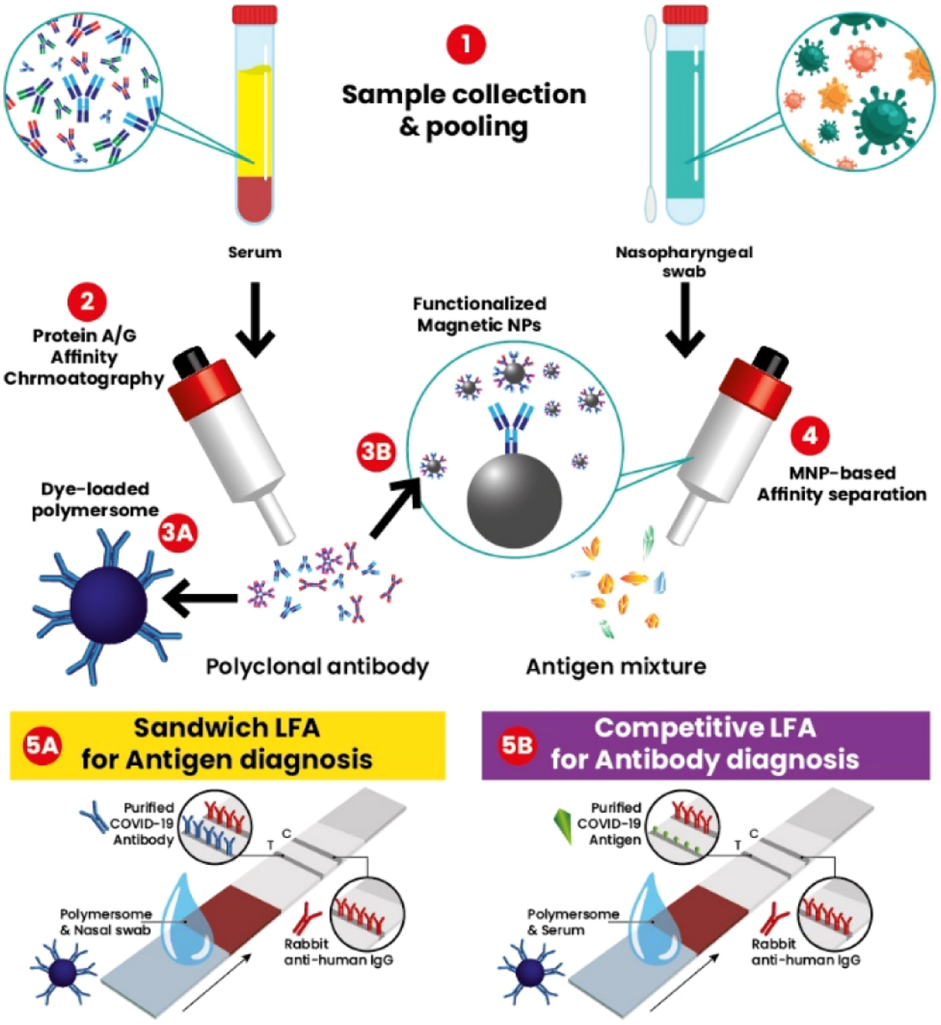
Scheme 1. Illustration of the Paper-Based LFA for COVID-19 Diagnosis through the Repurposing of Human Positive Serums and Nasal Swabs for Antibody–Antigen Purification[a]



[a]Numbers represent the order with which the approach was taken.

diagnosis.[6] Although gold has many advantages due to the easy synthesis and modification procedures,[7] there are still some disadvantages related to unsteadiness and aggregation, intrinsic plasmonics, and color bleaching.[7,8] Hence, alternative materials continue to be highly sought after. Polymer materials have also found a place in this category, especially polymersomes that can be prepared from the self-assembly of block polymers and create vesicles that can encapsulate a variety of molecules.[9] Additionally, polymersomes have great flexibility which facilitates functionalization and attachment of specific proteins and antibodies.[10]

Matching antibodies and specific antigen pairs is an important criterion for the success of immunological testing. The selection of high-affinity antibody–antigen pairs is a slow, laborious, and expensive process (especially for monoclonal and recombinant antibodies).[11] Antigens (*e.g.*, SARS-CoV-2 spike protein) can have mutations that lead to misrecognition. Consequently, targeting multiple antigens could enhance the efficiency of immunological tests.

The current study addressed a rational design of a dye-loaded polymersome colorimetric paper-based LFA platform for the detection of SARS-CoV-2 antigens and antibodies using anti-COVID-19 antibody–antigen pairs purified from human samples (Schemes 1 and S1). This work aims to propose a platform that can be both economical compared to other commercial counterparts and present a good alternative to quickly produce diagnostic platforms especially in low-income countries where access is limited.

EXPERIMENTAL SECTION

Chemicals and Reagents. An immunoaffinity spin column (protein A) was purchased from Thermo Fisher. Anti-SARS-CoV-2 ELISA IgG and IgA were obtained from EUROIMMUN (Luebeck, Germany). Iron oxide (II, III) magnetic nanoparticles (MNPs) (30 nm avg, amine-functionalized; 747327) were provided from Sigma-Aldrich (Germany). LFA COVID-19 IgG/IgM Rapid Test kit was obtained from Unscience Biotechnology Co., Wuhan, China. Nitrocellulose membrane cards were purchased from Millipore (Cheshire, CT). Rabbit anti-IgG was obtained from Raybiotech (GA, USA). All chemicals used in the current work are of high analytical grade and were purchased from Sigma-Aldrich (Germany) unless mentioned otherwise.

Human Samples and Selection. A total of 121 human samples (serums; n = 61 and nasal swabs; n = 60) collected by trained healthcare workers from patients whose personal information and medical records were anonymized and codified at Ege University Hospital (Izmir, Turkey) from November 2020 to March 2021. Molecular RT-PCR was performed by the hospital for COVID-19

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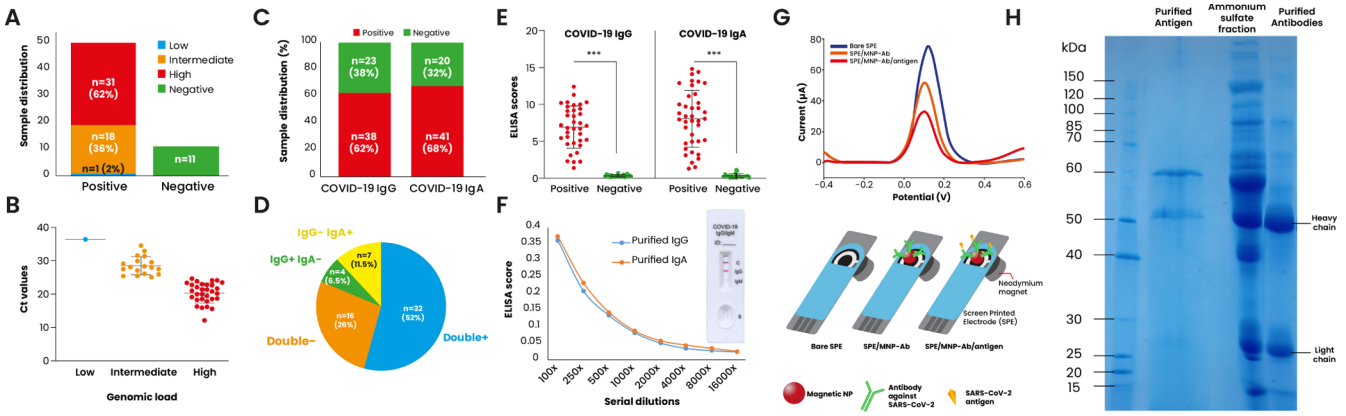

Figure 1. Analysis of human samples *via* RT-PCR and ELISA for COVID-19 antigen/antibody purification. (A) Nasal swab samples distribution after RT-PCR analysis showing negative (n = 11) and positive (n = 50) samples. (B) Categorization of the COVID-19 positive samples according to Ct values. (C−E) Human serum sample distribution after analysis for COVID-19 antibodies using commercial ELISA tests. (F) Antibody titer of the purified antibodies using commercial COVID-19 antibody ELISA test (the LFA represents a parallel test for COVID-19 IgG/IgM detection). (G) Electrochemical analysis of the purified antigens using MNPs functionalized with the purified antibodies tested over a SPE. (H) SDS-PAGE analysis of both purified antibodies and antigens.

diagnosis, after which, an ELISA was performed to determine specific Covid-19 antibody levels. The samples were assembled as negative and positive groups and stored at −20 °C until subsequent use. The study on human samples was approved by the Ege University, Clinical Research Ethics Committee (20-8T/28).

SARS-CoV-2 Specific Antigen and Antibody Purification. Human serum samples with high anti-COVID-19 IgG/IgA levels were used for the antibody purification using an immunoaffinity spin column (protein A column). The eluted antibody fraction was stored at −20 °C in the aliquots until used. The specificity of the purified antibodies for COVID-19 was determined using commercial anti-COVID-19 IgG and IgA ELISA and LFA kits.

Antigen purification was performed by an affinity-based interaction using antibody-functionalized MNPs (MNP-Ab) and swab samples with high viral loads. Samples were stored at −20 °C until further use. The purified antigen fraction was confirmed by a screen-printed electrode (SPE)-based electrochemical analysis using differential pulse voltammetry. The detection procedure has been described in detail in an earlier report[12] and in the Supporting Information. The purified antibodies and antigens were analyzed by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).[13]

Polymersome Preparation and Characterization. The detailed synthesis process and characterization of the dye-loaded polymersome is described in the Supporting Information section. Briefly, methoxy poly(ethylene glycol-*block*-caprolactone) copolymers (mPEG−PCL) were prepared according to a previously reported protocol.[14] The hydroxyl group of the mPEG−PCL was modified to create a carboxylic acid-end group (mPEG−PCL−COOH)[15] followed by an amine modification to obtain the final amine-modified mPEG−PCL (mPEG−PCL−NH_2) copolymers.[16] The amine modification is a necessary step for the subsequent oriented functionalization with the antibody. The dye encapsulation and polymersome formation were processed by following the previously reported co-assembly method[17] and Coomassie Brilliant Blue G-250 dye (CBBG) as the coloring agent.

The conjugation of the previously purified antibodies over the surface of the dye-loaded polymersome was performed using a standard EDC/NHS chemistry protocol. The final product (immunopolymersome) was kept at +4 °C for further use.

The characterization of the polymersome components in each reaction step was analyzed *via* ^{1}H NMR (Varian Mercury-plus/AS-400) and FTIR (Spectrum Two FT-IR Spectrometer—PerkinElmer). In addition, the dynamic size of the prepared polymersomes was analyzed *via* dynamic light scattering (DLS) (Zetasizer NanoZS, Malvern Instruments). Visual observations of the polymersomes were performed under scanning electron microscopy (SEM, Thermo Scientific Apreo S). The dye encapsulation efficiency was calculated by measuring the entrapped CBBG concentration and expressed in percentage.

LFA Design and Sample Analysis. The application of the purified antibody−antigen pairs for COVID-19 diagnosis consisted of assembling a paper-based LFA model and dye-loaded polymersomes for the creation of two LFA types; antigen test using sandwich-type LFA (1) and antibody test utilizing a competitive type LFA (2) (Scheme 1).

Immunoassay strips consisted of control (C) and test (T) lines drawn over a nitrocellulose membrane according to the method described by Shukla *et al.*[18] A detailed protocol for the LFA preparation is given in the Supporting Information. The prepared membrane pieces were kept in an airtight plastic bag at 4 °C until further experiments.

The assay format consisted of the immunopolymersome solution, serum and nasal swab samples, and nitrocellulose strips. In short, the assay was initiated by mixing 20 μL of polymersome solution with 10 μL of samples. The prepared mixture was then dropped over the sample pad and allowed for at least 5 min for migration.

Analytical Characteristics. *Repeatability and Reproducibility.* Same human samples were analyzed 10 times to determine the repeatability of the immunostrip, whereas reproducibility was examined over different immune strips prepared at different times (n = 5).

Stability of the Lateral Flow Test Strips. Samples were applied onto the strips for stability analysis using a batch of LFA strips stored at 25 °C with silica gel packs to absorb humidity. Samples were tested weekly over a period of 4 weeks.

Statistics. The results are presented as mean and standard deviation. The statistical differences were analyzed using a simple Student's t-test (Graphpad Prism 8.0). The p values < 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Since the worldwide outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), many diagnostic approaches have been used. The advantages and drawbacks of each technique should be considered by the healthcare physicians to properly manage and contain the spread of the virus. As such, the preparation of a specific targeting diagnosis test needs a proper understanding of the viral protein structures.[19] The most recognized protein is the spike glycoprotein (S) found on the surface, which facilitates the attachment to host cells. The nucleocapsid protein (N) allows

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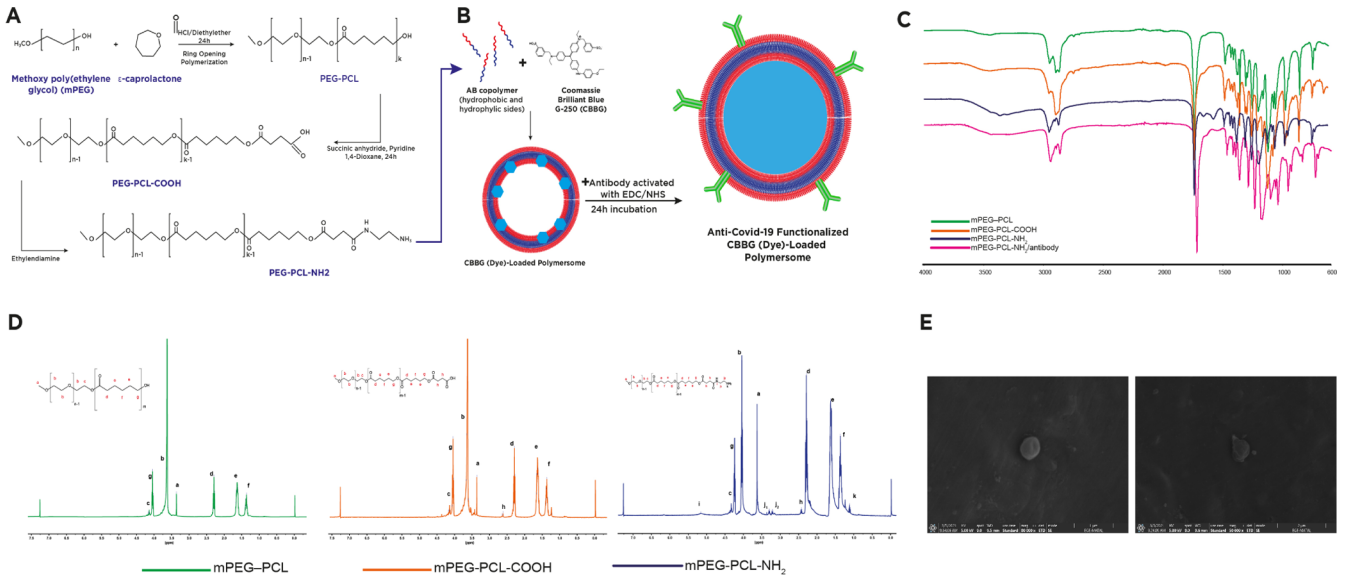

Figure 2. Synthesis and characterization of the dye-loaded polymersome. (A) Synthesis steps of the AB type copolymers with $-NH_2$ reactive groups. (B) Vesicle formation, dye encapsulation, and antibody attachment over the polymersome. (C,D) FT-IR and ^1H NMR characterization of the polymersome components. (E) SEM observation of the dye-loaded polymersome (left, 80,000× magnification) and antibody-functionalized dye-loaded polymersome (right, 50,000× magnification).

for the binding of the RNA and forms a nucleocapsid. Membrane proteins (M) are structural components that link membranes to capsids. The other important component of SARS-CoV-2 is the small envelope protein (E) which is implicated in the burgeoning of the virus.[2] Various countries are using one or a combination of these targets in their diagnostic applications.

The RT-PCR continues to be the standard strategy for COVID-19 detection. However, there has been less demand for this method due to the high cost and instability of transported RNA that can give false-negative results.[20] POC immunoassays are more appealing, given their sensitivity and fast turnaround. The key factor in immuno-based assays resides in the selection of adequate antibody−antigen pairs.[11,21]

The current study proposes a paper-based colorimetric LFA for the detection of COVID-19 antigens and antibodies using a blue dye-loaded polymersome. The main originality of the proposed platform resides in the rational design of a human serum and nasal swab repurposing system for diagnosis applications. This was achieved by purification of COVID-19 specific antigens and antibodies from swabs and serums, respectively (Scheme 1). In addition, the repurposing of human antibody or antigen mix containing different immunoglobulins and antigens is hypothesized to enhance the diagnosis capacity by recognizing various targets instead of a specific protein. In the case of antigen detection, the T-line in the LFA design comprised an "antibody cocktail" which can be considered as an "all-in-one" concept instead of single targeting using pure or monoclonal Ab. This is very important to provide more recognizing sites for virus capture.

Antibody and Antigen Purification from Human COVID-19 Samples. Acquired samples were analyzed by RT-PCR and ELISA to determine the positive and negative samples. From the 61 swab samples, 50 were found positive and 11 were negative. Considering the threshold cycle (Ct) values, positive samples were further divided into low (n = 1), intermediate (n = 18), and high (n = 31) subgroups (Figure 1A,B). Ct refers to the number of cycles needed to amplify a viral RNA to reach a detectable level. These values are closely related to the virus load in the samples with high virus quantity needing a lower cycle number to be detected. Researchers have grouped patients' RT-PCR results into high (Ct ≤ 25), intermediate (25 < Ct < 35), and low (Ct ≥ 35) genomic load cohorts.[22,23] Considering the Ct values, high load samples were selected for the purification of antigens. On the other hand, serums (n = 60) were tested using commercial COVID-19 IgG and IgA ELISA tests. For the purification of COVID-19 antibodies, samples showing the presence of IgG and IgA (n = 32) were selected (Figure 1C−E). Antibody production in infected patients becomes detectable 4−7 days after the COVID-19 onset.[11] Our data corroborate the earlier information given that computed tomography results obtained estimated that patients were admitted 7−21 days post-infection (data not shown). Serums were treated with a standard protocol for antibody purification and the purified antibody mix was controlled with ELISA and LFA antibody tests (Figure 1F).

COVID-19 antigens were purified using MNPs and controlled using an electrochemistry-based immunoanalysis over an SPE (Figure 1G). We have shown in an earlier work that SPE-MNP biosensors possess a high sensitivity for selective and specific detection of biomolecules.[12] Both purified antibodies and antigens were analyzed *via* SDS-PAGE. Although the purified antibodies contain IgG (monomer) and IgA (monomer or dimer), the reduction by SDS in the electrophoresis demonstrates two bands of ∼50 and ∼25 kDa corresponding to the heavy and light chains of immunoglobulins (Figure 1H). The purified antigens showed two bands at ∼50 and ∼70 kDa (Figure 1H). These bands were determined as COVID-19 S and N proteins *via* mass spectrometry (data not shown).

Synthesis and Characterization of Dye-Loaded and Functionalized Polymersomes. The block copolymer of mPEG−PCL was synthesized *via* a ring-opening polymerization using the hydroxyl end group of PEG as an initiator and hydrochloric acid as a catalyst (Figure 2A). The molecular

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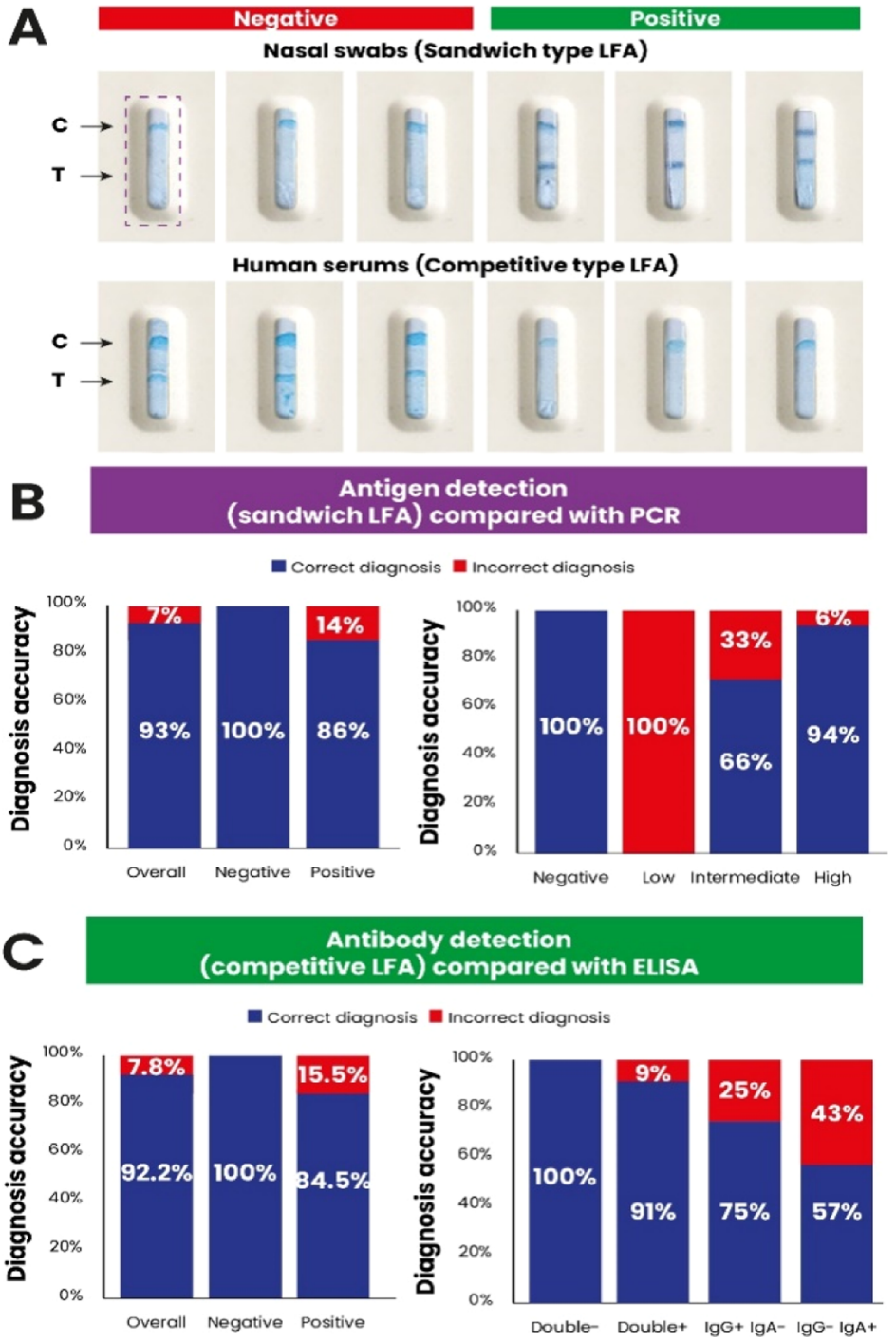

Figure 3. LFA application of the purified antigens and antibodies for COVID-19 diagnosis. (A) Visual observations of the proposed dye-loaded based LFA for the detection of COVID-19 antigens in the nasal swab sample (sandwich-type LFA, upper row) and antibodies in serum samples (competitive type, lower row). (B) Accuracy of the proposed LFA compared with RT-PCR data for antigen detection. (C) Accuracy of the prepared LFA compared with ELISA results for COVID-19 antibodies.

weight of the mPEG−PCL copolymer was calculated (M_w = 2510) by ^{1}H NMR from the integral ratio of the PEG methylene protons (3H, a) and PCL methylene protons (2H, f) (Figure 2D). A mPEG-PCL-COOH copolymer was also synthesized by reacting hydroxyl end group of mPEG−PCL with succinic anhydride. The appearance of a new proton signal at 2.65 ppm (the 2CH_2 proton of PCL (4H, h)) in ^{1}H NMR confirmed the structure (Figure 2A,D). FT-IR results showed that the intensity of the peak absorbance of the C═O functional group increased with O−H peak absorbance, which determined the structure of mPEG−PCL−COOH (Figure 2C). The structure of mPEG−PCL−NH_2 was identified by ^{1}H NMR and the amine proton signal at 1.10 ppm. Similar to ^{1}H NMR results, FT-IR results also determined the mPEG−PCL−NH_2 structure *via* peaks at 3357, 1643, 1560, and 1239 cm^{-1} (Figure 2C). The structure of polyclonal anti-COVID antibody conjugated polymersome was confirmed by FT-IR (Figure 2B,C). The comparison of mPEG−PCL−NH_2 with

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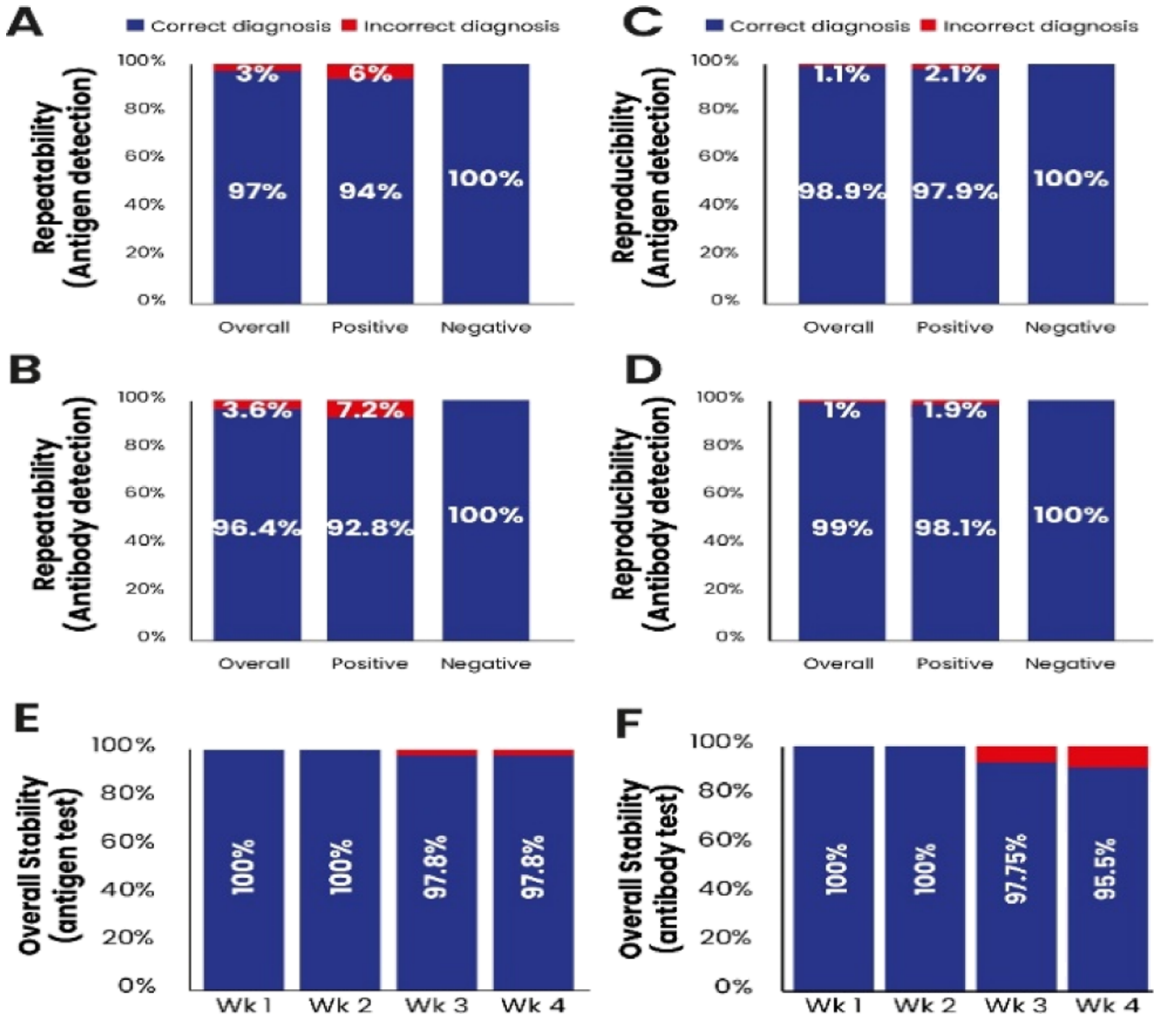

Figure 4. Repeatability, reproducibility, and stability of the proposed LFA diagnosis platform. (A,B) Repeatability analysis of the proposed LFAs for the detection of antibodies and antigens (n = 10). (C,D) Reproducibility of the platform using different immunostrips (n = 5). (E,F) Stability of the LFA platform during 4 weeks at room temperature conditions. Wk = week.

antibody-conjugated polymersomes showed some differences in the peak absorbance correlated to the antibody conjugation in the sample. The decrease in the intensity of the peak absorbance of the C═N stretching band as well as N−H bending in the antibody-conjugated polymersomes confirmed an antibody attachment on the polymersomes' surface.

The prepared polymersomes were further characterized by DLS and SEM analysis, showing a spherical structure (Figure 2E) and a dynamic size of 100 ± 13.23 nm. The encapsulation of the CBBG and functionalization with antibodies increased the size to 197.4 ± 22.25 nm (Table S1). The attachment of the antibodies on the surface of the polymersomes was further confirmed *via* zeta-potential analysis showing a decrease in surface charge. The inclusion of CBBG showed a high encapsulation efficiency reaching 93−95% (Table S1). The use of fluorescent and organic dyes has been widely applied in labeling molecules in complex environments. Nevertheless, the majority of dyes have hydrophobic characteristics, which greatly hinder their applications *in vitro* and *in vivo*.[24] The advancement in encapsulation technology allowed the development of various strategies to encapsulate hydrophobic dyes and easily use them for different biomedical applications. The concept of loading dyes in nanostructures is not recent. Many research studies have invested in the development of nanomaterials which can be considered as the state-of-art tools to replace the current generation of molecules (gold nanoparticles, fluorescent dye, *etc.*), especially in colorimetric and optical diagnostic applications.[24]

Applications of the Purified Antibody−Antigen and the Dye-Loaded Polymersome in Diagnostics. The next step of our investigation considered the use of the repurposed human samples (antigens and antibodies) to create a colorimetric paper-based biosensor. Two LFAs were proposed: (1) a sandwich-type LFA that used the purified antibodies over the nitrocellulose strips to detect the antigen presence in nasal swabs and (2) a competitive assay with the purified antigens anchored on the immunostrips to detect COVID-19 antibodies in suspected human serums (Figure 3A). Our obtained data were compared with the initial results obtained from RT-PCR and commercial ELISA testing and demonstrated an overall agreement of 93% for the antigen detection and 92.2% for antibody detection (Figure 3B,C). On the other hand, our diagnostic platform demonstrated a high level of sensitivity and showed 86 and 84.5% correct diagnosis for antigen and antibody detection, respectively (Figure 3B,C). The specificity and sensitivity of antigen detection have been demonstrated to be highly dependent on Ct values and decreases when viral load is low.[25,26] Indeed, the proposed LFA was further divided

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according to Ct values into high, intermediate, and low subgroups. Here, the high viral load group showed a 94% sensitivity and this sensitivity decreases with low Ct values (Figure 3B). However, given the limited number of samples with low viral load (n = 1), it is speculated that increasing the sample number would give a better evaluation of the sensitivity of the proposed LFA system. Antibody detection through the proposed LFA platform demonstrated a 91% sensitivity to double-positive samples (IgG+/IgA+) compared with 75 and 57% to single IgG+ and IgA+, respectively (Figure 3C). This can be attributed to IgG being the major component of immunoglobulins in serum samples.

Antibodies can be detected in patients around a week after infection. This period is different with each antibody species. Therefore, antibody recognition in samples offers an indication of the time of infection onset. This is important to provide adequate treatments.[11]

The proposed dye-loaded COVID-19 LFA platform using repurposed antigens and antibodies from human samples aimed to an overall detection. We hypothesized that enhancing the number of detectable regions (by using a mix of antigens or a polyclonal antibody mix) can enhance the detection ability of the diagnostic platform. Various studies published in the recent years suggested multiplexing the diagnostic tools for antibodies and antigens[27] and fewer have analyzed IgA in COVID-19 cases.[28]

There have been several paper-based diagnostic approaches developed for the colorimetric and fluorimetric detection of COVID-19. Gold nanoparticles (AuNPs) are considered as the most employed molecule in colorimetric assays (Table S2). Indeed, some teams developed paper-based optical antigen assays for the diagnosis of COVID-19, where they made use of the fluorescence of various nanoparticles to produce signals.[29,30] The agreement with the PCR was around 73−99% which is close to the results found in the current study. The use of fluorescence in these studies necessitate the usage of specific detectors which can compromise portability.[30] As such, the use of colorimetric indicators provides an advantageous position in POC development. The use of AuNPs for the detection of antibodies has also been studied extensively during the pandemic. Li *et al.* developed a portable colorimetric LFA assay for the detection of IgG/IgM antibodies.[31] Their data demonstrated an 80−90% specificity. Another study by Cavalera *et al.* proposed an LFA system for the detection of various antibodies using AuNPs conjugated with nucleocapsid proteins.[32] Their results showed a 95.7−97.8% sensitivity. Comparing the various studies with the current dye-loaded polymersome-based LFA system shows that the performances are quite close, whereas our LFA system boasts the fastest detection time (5 min) (Table S2). Other paper-based systems using target and signal amplifications such as gene replication and fluorescence enhancement can reach a sensitivity of 99% (Table S2). These methods need longer sample processing and preparation in addition to loss of portability in some cases.

Repeatability, Reproducibility, and Stability of LFA Platform. The repeatability of the current COVID-19 biosensor was analyzed through repeated testing of the same samples. The sample testing showed a 97 and 96.4% repeatability for both antigen and antibody diagnosis (Figure 4A,B). Additionally, the reproducibility was tested over different batches of immunostrips, which was 98.9 and 99% (Figure 4C,D). Importance of repeatability and reproducibility is crucial in nanobiosensor development. It is recommended that a coefficient of variation lower than 10% is needed to validate and increase the reliability of developed biosensors.[33] The stability of the LFAs was also followed during 4 weeks after preparation. Data showed high stability of the prepared platform with a 100% rate for both positive and negative samples during the first 2 weeks after preparation. Starting from the third week, a slight decrease of 3−4% was observed (Figures 4E,F and S1). The stocking conditions of the current immunostrips consisted of stable temperature and humidity. It is suspected that exposure to air affected the proteins and the use of sealed packaging conditions could elongate the stability of the platform longer.

Mitigating the spread of a pandemic necessitates fast response in developing diagnostic tools that are fast, easy to use, and low in price. The improvements seen in COVID-19 diagnostics have been very encouraging, especially in POC development. Presently, molecular-based diagnostics (*i.e.,* PCR) remains the standard approach for COVID-19 detection. However, it is heavily burdened by the need for equipped facilities and trained staff that are highly lacking in remote regions and low-income countries.[34] As such, thinking of alternatives to lower the cost and make easy-to-prepare diagnostic tools, we proposed the repurposing of human samples to obtain COVID-19-specific antigens and antibodies instead of purchasing antibodies that might be costly.

Our approach considered simple affinity techniques for the purification process and the utilization of these proteins in LFA development demonstrated a high potential for a bigger patient cohort screening. Indeed, the technique suffers from some limitations regarding the sensitivity in comparison with RT-PCR where genomic content is amplified.[35] There is also the fact that the current sample group contains only one sample for the low virus load which cannot be a representative of the efficiency of the proposed system. As such, many options to enhance the current platform are possible: (1) enhancing the sample number and distribution to encompass the various levels of virus content (high, intermediate, and low). (2) Employing a second step of antibody purification to separate the COVID-19-specific antibodies from the rest will be very advantageous for enhancing sensitivity and selectivity. (3) Using quantitative tools for the immunostrip analysis such as smartphone-assisted image analysis can also help standardize the measurement and lower arbitrary conclusions. (4) The utilization of fluorescent dyes might enhance the detection of low viral load-containing samples; however, fluorescent analysis equipment would be needed. It should be understood that asymptomatic individuals comprise 20−80% of total cases and immunological diagnosis represents an advantage in large-scale screening.[36] Additionally, the concept of using a cocktail of antibodies recognizing different epitopes has been shown to produce enhanced recognition capacity compared to specific monoclonal antibodies.[37,38]

Lastly, the potential of dye-loaded polymersomes as a material for colorimetric assays remains in its first steps compared to the well-established tests that use gold nanoparticles. It would be interesting to perform a comparative study between the two materials in order to provide a better understanding of the advantages provided by the polymersomes.

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CONCLUSIONS

We have established a paper-based LFA nanodiagnostic platform using dye-loaded polymersomes and repurposed human samples for the purification of antigens and antibodies. Our data showed the successful use of LFA as a practical adaptable POC tool for the other possible pandemic cases. The continuation of this study is planned as clinical study. Moreover, the approach is meant to provide an alternative and supplement of diagnostic kits, especially with the continuously increasing demand. In conclusion, with the urgent need for timely detection of infection, our proposed platform is a useful assay, nevertheless, caution is needed before implementation in the community.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.1c00854.

Detailed illustration of the development of the dye-loaded polymersome-based LFA system, characterization of the synthesized polymersome, paper-based biosensor developed for the diagnosis of SARS-CoV-2 genes, antigens, and antibodies using colorimetric and fluorometric detection methods, and stability analysis of the two proposed LFA systems according to various criteria (PDF)

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Author Contributions

F.G. and H.M. contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Charlton, C. L.; Kanji, J. N.; Johal, K.; Bailey, A.; Plitt, S. S.; MacDonald, C.; Kunst, A.; Buss, E.; Burnes, L. E.; Fonseca, K.; Berenger, B. M.; Schnabl, K.; Hu, J.; Stokes, W.; Zelyas, N.; Tipples, G. Evaluation of Six Commercial Mid- to High-Volume Antibody and Six Point-of-Care Lateral Flow Assays for Detection of SARS-CoV-2 Antibodies. *J. Clin. Microbiol.* **2020**, *58*, No. e01361.

(2) Moulahoum, H.; Ghorbanizamani, F.; Zihnioglu, F.; Turhan, K.; Timur, S. How should diagnostic kits development adapt quickly in COVID 19-like pandemic models? Pros and cons of sensory platforms used in COVID-19 sensing. *Talanta* **2021**, *222*, 121534.

(3) Downs, C.; Milovancev, M.; Fu, E. Rational design and characterization of a lateral flow assay for canine C-reactive protein in wound exudate. *Talanta* **2020**, *220*, 121319.

(4) Pallett, S. J. C.; Rayment, M.; Patel, A.; Fitzgerald-Smith, S. A. M.; Denny, S. J.; Charani, E.; Mai, A. L.; Gilmour, K. C.; Hatcher, J.; Scott, C.; Randell, P.; Mughal, N.; Jones, R.; Moore, L. S. P.; Davies, G. W. Point-of-care serological assays for delayed SARS-CoV-2 case identification among health-care workers in the UK: a prospective multicentre cohort study. *Lancet Respir. Med.* **2020**, *8*, 885−894.

(5) Ahmed, M. U.; Saaem, I.; Wu, P. C.; Brown, A. S. Personalized diagnostics and biosensors: a review of the biology and technology needed for personalized medicine. *Crit. Rev. Biotechnol.* **2014**, *34*, 180−196.

(6) Nguyen, Q. H.; Kim, M. I. Nanomaterial-mediated paper-based biosensors for colorimetric pathogen detection. *Trends Anal. Chem.* **2020**, *132*, 116038.

(7) Liu, L.; Yang, D.; Liu, G. Signal amplification strategies for paper-based analytical devices. *Biosens. Bioelectron.* **2019**, *136*, 60−75.

(8) Deng, X.; Wang, C.; Gao, Y.; Li, J.; Wen, W.; Zhang, X.; Wang, S. Applying strand displacement amplification to quantum dots-based fluorescent lateral flow assay strips for HIV-DNA detection. *Biosens. Bioelectron.* **2018**, *105*, 211−217.

(9) Ghorbanizamani, F.; Moulahoum, H.; Sanli, S.; Bayir, E.; Zihnioglu, F.; Timur, S. pH-bioresponsive poly(epsilon-caprolactone)-based polymersome for effective drug delivery in cancer and protein glycoxidation prevention. *Arch. Biochem. Biophys.* **2020**, *695*, 108643.

(10) Ghorbanizamani, F.; Moulahoum, H.; Zihnioglu, F.; Timur, S. Nanohybrid carriers: the yin-yang equilibrium between natural and synthetic in biomedicine. *Biomater. Sci.* **2020**, *8*, 3237−3247.
(11) Orooji, Y.; Sohrabi, H.; Hemmat, N.; Oroojalian, F.; Baradaran, B.; Mokhtarzadeh, A.; Mohaghegh, M.; Karimi-Maleh, H. An Overview on SARS-CoV-2 (COVID-19) and Other Human Coronaviruses and Their Detection Capability via Amplification Assay, Chemical Sensing, Biosensing, Immunosensing, and Clinical Assays. *Nano-Micro Lett.* **2021**, *13*, 18.
(12) Sanli, S.; Ghorbani-Zamani, F.; Moulahoum, H.; Gumus, Z. P.; Coskunol, H.; Odaci Demirkol, D.; Timur, S. Application of Biofunctionalized Magnetic Nanoparticles Based-Sensing in Abused Drugs Diagnostics. *Anal. Chem.* **2020**, *92*, 1033−1040.
(13) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.
(14) Kim, M. S.; Seo, K. S.; Khang, G.; Cho, S. H.; Lee, H. B. Preparation of poly(ethylene glycol)-block-poly(caprolactone) copolymers and their applications as thermo-sensitive materials. *J. Biomed. Mater. Res., Part A* **2004**, *70*, 154−158.
(15) Kang, S. W.; Li, Y.; Park, J. H.; Lee, D. S. pH-triggered unimer/vesicle-transformable and biodegradable polymersomes based on PEG-b-PCL−grafted poly(β-amino ester) for anti-cancer drug delivery. *Polymer* **2013**, *54*, 102−110.
(16) Noguchi, A.; Takahashi, T.; Yamaguchi, T.; Kitamura, K.; Takakura, Y.; Hashida, M.; Sezaki, H. Preparation and properties of the immunoconjugate composed of anti-human colon cancer monoclonal antibody and mitomycin C-dextran conjugate. *Bioconjugate Chem.* **1992**, *3*, 132−137.
(17) Scarpa, E.; Bailey, J. L.; Janeczek, A. A.; Stumpf, P. S.; Johnston, A. H.; Oreffo, R. O. C.; Woo, Y. L.; Cheong, Y. C.; Evans, N. D.; Newman, T. A. Quantification of intracellular payload release from polymersome nanoparticles. *Sci. Rep.* **2016**, *6*, 29460.
(18) Shukla, S.; Leem, H.; Kim, M. Development of a liposome-based immunochromatographic strip assay for the detection of Salmonella. *Anal. Bioanal. Chem.* **2011**, *401*, 2581−2590.
(19) Chang, M. C.; Hur, J.; Park, D. Interpreting the COVID-19 Test Results: A Guide for Physiatrists. *Am. J. Phys. Med. Rehab.* **2020**, *99*, 583−585.
(20) Li, D.; Wang, D.; Dong, J.; Wang, N.; Huang, H.; Xu, H.; Xia, C. False-Negative Results of Real-Time Reverse-Transcriptase Polymerase Chain Reaction for Severe Acute Respiratory Syndrome Coronavirus 2: Role of Deep-Learning-Based CT Diagnosis and Insights from Two Cases. *Korean J. Radiol.* **2020**, *21*, 505−508.
(21) Lee, J.-H.; Choi, M.; Jung, Y.; Lee, S. K.; Lee, C.-S.; Kim, J.; Kim, J.; Kim, N. H.; Kim, B.-T.; Kim, H. G. A novel rapid detection for SARS-CoV-2 spike 1 antigens using human angiotensin converting enzyme 2 (ACE2). *Biosens. Bioelectron.* **2021**, *171*, 112715.
(22) Faíco-Filho, K. S.; Passarelli, V. C.; Bellei, N. Is Higher Viral Load in SARS-CoV-2 Associated with Death? *Am. J. Trop. Med. Hyg.* **2020**, *103*, 2019−2021.
(23) Zacharioudakis, I. M.; Prasad, P. J.; Zervou, F. N.; Basu, A.; Inglima, K.; Weisenberg, S. A.; Aguero-Rosenfeld, M. E. Association of SARS-CoV-2 Genomic Load with COVID-19 Patient Outcomes. *Ann. Am. Thorac. Soc.* **2021**, *18*, 900.
(24) Chang, S.; Wu, X.; Li, Y.; Niu, D.; Ma, Z.; Zhao, W.; Gu, J.; Dong, W.; Ding, F.; Zhu, W.; Shi, J. A hydrophobic dye-encapsulated nano-hybrid as an efficient fluorescent probe for living cell imaging. *Adv. Healthcare Mater.* **2012**, *1*, 475−479.
(25) Peña-Rodríguez, M.; Viera-Segura, O.; García-Chagollán, M.; Zepeda-Nuño, J. S.; Muñoz-Valle, J. F.; Mora-Mora, J.; Espinoza-De León, G.; Bustillo-Armendáriz, G.; García-Cedillo, F.; Vega-Magaña, N. Performance evaluation of a lateral flow assay for nasopharyngeal antigen detection for SARS-CoV-2 diagnosis. *J. Clin. Lab. Anal.* **2021**, *35*, No. e23745.
(26) Walsh, K. A.; Jordan, K.; Clyne, B.; Rohde, D.; Drummond, L.; Byrne, P.; Ahern, S.; Carty, P. G.; O'Brien, K. K.; O'Murchu, E.; O'Neill, M.; Smith, S. M.; Ryan, M.; Harrington, P. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. *J. Infect.* **2020**, *81*, 357−371.
(27) Rosado, J.; Pelleau, S.; Cockram, C.; Merkling, S. H.; Nekkab, N.; Demeret, C.; Meola, A.; Kerneis, S.; Terrier, B.; Fafi-Kremer, S.; de Seze, J.; Bruel, T.; Dejardin, F.; Petres, S.; Longley, R.; Fontanet, A.; Backovic, M.; Mueller, I.; White, M. T. Multiplex assays for the identification of serological signatures of SARS-CoV-2 infection: an antibody-based diagnostic and machine learning study. *Lancet Microbe* **2021**, *2*, e60−e69.
(28) Padoan, A.; Sciacovelli, L.; Basso, D.; Negrini, D.; Zuin, S.; Cosma, C.; Faggian, D.; Matricardi, P.; Plebani, M. IgA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: A longitudinal study. *Clin. Chim. Acta* **2020**, *507*, 164−166.
(29) Diao, B.; Wen, K.; Chen, J.; Liu, Y.; Yuan, Z.; Han, C.; Chen, J.; Pan, Y.; Chen, L.; Dan, Y.; Wang, J.; Chen, Y.; Deng, G.; Zhou, H.; Wu, Y. Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein. *medRxiv* **2020**, DOI: 10.1101/2020.03.07.20032524.
(30) Grant, B. D.; Anderson, C. E.; Williford, J. R.; Alonzo, L. F.; Glukhova, V. A.; Boyle, D. S.; Weigl, B. H.; Nichols, K. P. SARS-CoV-2 Coronavirus Nucleocapsid Antigen-Detecting Half-Strip Lateral Flow Assay Toward the Development of Point of Care Tests Using Commercially Available Reagents. *Anal. Chem.* **2020**, *92*, 11305−11309.
(31) Li, Z.; Yi, Y.; Luo, X.; Xiong, N.; Liu, Y.; Li, S.; Sun, R.; Wang, Y.; Hu, B.; Chen, W.; Zhang, Y.; Wang, J.; Huang, B.; Lin, Y.; Yang, J.; Cai, W.; Wang, X.; Cheng, J.; Chen, Z.; Sun, K.; Pan, W.; Zhan, Z.; Chen, L.; Ye, F. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *J. Med. Virol.* **2020**, *92*, 1518−1524.
(32) Cavalera, S.; Colitti, B.; Rosati, S.; Ferrara, G.; Bertolotti, L.; Nogarol, C.; Guiotto, C.; Cagnazzo, C.; Denina, M.; Fagioli, F.; Di Nardo, F.; Chiarello, M.; Baggiani, C.; Anfossi, L. A multi-target lateral flow immunoassay enabling the specific and sensitive detection of total antibodies to SARS COV-2. *Talanta* **2021**, *223*, 121737.
(33) Chen, L.-C.; Wang, E.; Tai, C.-S.; Chiu, Y.-C.; Li, C.-W.; Lin, Y.-R.; Lee, T.-H.; Huang, C.-W.; Chen, J.-C.; Chen, W. L. Improving the reproducibility, accuracy, and stability of an electrochemical biosensor platform for point-of-care use. *Biosens. Bioelectron.* **2020**, *155*, 112111.
(34) Wen, T.; Huang, C.; Shi, F.-J.; Zeng, X.-Y.; Lu, T.; Ding, S.-N.; Jiao, Y.-J. Development of a lateral flow immunoassay strip for rapid detection of IgG antibody against SARS-CoV-2 virus. *Analyst* **2020**, *145*, 5345−5352.
(35) Carrell, C.; Kava, A.; Nguyen, M.; Menger, R.; Munshi, Z.; Call, Z.; Nussbaum, M.; Henry, C. Beyond the lateral flow assay: A review of paper-based microfluidics. *Microelectron. Eng.* **2019**, *206*, 45−54.
(36) Li, R.; Pei, S.; Chen, B.; Song, Y.; Zhang, T.; Yang, W.; Shaman, J. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). *Science* **2020**, *368*, 489−493.
(37) Chen, R. E.; Zhang, X.; Case, J. B.; Winkler, E. S.; Liu, Y.; VanBlargan, L. A.; Liu, J.; Errico, J. M.; Xie, X.; Suryadevara, N.; Gilchuk, P.; Zost, S. J.; Tahan, S.; Droit, L.; Turner, J. S.; Kim, W.; Schmitz, A. J.; Thapa, M.; Wang, D.; Boon, A. C. M.; Presti, R. M.; O'Halloran, J. A.; Kim, A. H. J.; Deepak, P.; Pinto, D.; Fremont, D. H.; Crowe, J. E., Jr.; Corti, D.; Virgin, H. W.; Ellebedy, A. H.; Shi, P.-Y.; Diamond, M. S. Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. *Nat. Med.* **2021**, *27*, 717−726.
(38) Shrock, E.; Fujimura, E.; Kula, T.; Timms, R. T.; Lee, I.-H.; Leng, Y.; Robinson, M. L.; Sie, B. M.; Li, M. Z.; Chen, Y.; Logue, J.; Zuiani, A.; McCulloch, D.; Lelis, F. J. N.; Henson, S.; Monaco, D. R.; Travers, M.; Habibi, S.; Clarke, W. A.; Caturegli, P.; Laeyendecker, O.; Piechocka-Trocha, A.; Li, J. Z.; Khatri, A.; Chu, H. Y.; Villani, A.-C.; Kays, K.; Goldberg, M. B.; Hacohen, N.; Filbin, M. R.; Yu, X. G.; Walker, B. D.; Wesemann, D. R.; Larman, H. B.; Lederer, J. A.; Elledge, S. J.; Lederer, J. A.; Elledge, S. J. Viral epitope profiling of

https://doi.org/10.1021/acssensors.1c00854
ACS Sens. XXXX, XXX, XXX−XXX

COVID-19 patients reveals cross-reactivity and correlates of severity. *Science* **2020**, *370*, No. eabd4250.

https://doi.org/10.1021/acssensors.1c00854
ACS Sens. XXXX, XXX, XXX−XXX