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ABSTRACT

The SARS-CoV-2 pandemic has had a significant impact worldwide. Currently, the most common detection methods for the virus are polymerase chain reaction (PCR) and lateral flow tests. PCR takes more than an hour to obtain the results and lateral flow tests have difficulty with detecting the virus at low concentrations. In this study, 60 clinical human saliva samples, which included 30 positive and 30 negative samples confirmed with RT-PCR, were screened for COVID-19 using disposable glucose biosensor strips and a reusable printed circuit board. The disposable strips were gold plated and functionalized to immobilize antibodies on the gold film. After functionalization, the strips were connected to the gate electrode of a metal-oxide-semiconductor field-effect transistor on the printed circuit board to amplify the test signals. A synchronous double-pulsed bias voltage was applied to the drain of the transistor and strips. The resulting change in drain waveforms was converted to digital readings. The RT-PCR-confirmed saliva samples were tested again using quantitative PCR (RT-qPCR) to determine cycling threshold (Ct) values. Ct values up to 45 refer to the number of amplification cycles needed to detect the presence of the virus. These PCR results were compared with digital readings from the sensor to better evaluate the sensor technology. The results indicate that the samples with a range of Ct values from 17.8 to 35 can be differentiated, which highlights the increased sensitivity of this sensor technology. This research exhibits the potential of this biosensor technology to be further developed into a cost-effective, point-of-care, and portable rapid detection method for SARS-CoV-2.

I. INTRODUCTION

The ongoing global pandemic, caused by the SARS-CoV-2 virus, has impacted world economies and has had a major impact on our daily lives. The constant development of new virus variants requires profound changes in our approach to mitigate the effects of the virus. Prior to the emergence of several variants, the incidence of COVID-19 cases seemed to be decreasing after the start of the vaccination campaign. However, just when this seemed like a turning point in the pandemic, an upturn in infections occurred due to the more virulent strain of SARS-CoV-2.[1] The Centers for Disease Control and Prevention also pointed out that this variant has increased breakthrough cases.[1] By the end of December 2021, there have been over 300×10^6 confirmed cases, with 5×10^6 deaths according to World Health Organization (WHO).[2] To stop this

pandemic, rapid, accurate, and cost-effective point-of-care testing methods are needed to help identify positive cases quickly even when these positive cases may have low virus concentrations.

Currently, polymerase chain reaction (PCR) and lateral flow tests are widely used to detect positive cases. PCR is a very accurate method that can verify positive samples that have low virus concentrations.[3,4] However, this test takes several hours to obtain the results, which increases the chance of continuing to spread the virus by social contact. Lateral flow tests, currently the most common point-of-care commercial products, on the other hand, demonstrate shorter detection times but are not accurate enough to identify positive specimens when testing samples with cycling threshold values (Ct values) higher than 28.[5]

Besides PCR and lateral flow tests, electrochemical detection methods have also been used to also detect SARS-CoV-2 cases because of their low limit of detection, cost-effectiveness, and short cycle time.[6,7] A rolling circle amplification (RCA)-based electrochemical biosensor was reported in February 2021.[8] Despite being able to differentiate positive samples from negative samples, the results obtained by using RCA-based electrochemical biosensors did not form a trendline corresponding to the Ct values or virus concentrations within each sample.[8] Another team from the University of Louisville applied electrochemical impedance to develop their rapid detection technology, but additional clinical samples are required to better quantify the performance of this device.[9] Seo *et al.* and Hwang *et al.* used field-effect transistor-based sensors to improve SARS-CoV-2 detection.[10,11] However, in both of their works, the graphene FET was used as a sensing device and exposed to the SARS-CoV-2 samples. After testing samples, the graphene FET will need to be discarded, which is not practical or cost-effective. Compared to their research, the FET in our sensor technology can be repeatedly used and other FETs, such as Si-metal-oxide-semiconductor field-effect transistor (MOSFET), GaAs HEMT, or GaN HEMT, can be used to replace the FET in our sensor technology.

Our previous study introduced an inexpensive, accurate, and rapid detection technology by using a printable circuit board where a synchronous double-pulsed bias voltage is applied on the drain of the MOSFET and disposable glucose strips functionalized with antibodies.[12] The antibody immobilization technique on solid-state devices has been used to attach the desired antibodies to the biosensor to capture target biomarkers.[13,14] Most importantly, this synchronous double-pulsed measurement can help reduce the sensing time and increase the sensitivity of sensors.[15] In this study, human saliva samples consisting of both positive and negative specimens were applied to the sensor system to further demonstrate our sensor technology. The goal of this research is to determine the ability of this sensor technology to detect the SARS-CoV-2 virus in saliva and assess the sensitivity compared with the current gold standard PCR test for detecting the virus.

II. MATERIAL AND METHOD

Disposable glucose test strips were used to perform human saliva sample testing after being gold plated and biofunctionalized. The strips were treated with a gold-plating solution to grow a 1 μm thick film on a carbon electrode for attaching SARS-CoV-2 antibodies. When functionalizing the test strips, 10 mM thioglycolic acid in ethanol was introduced into a microfluidic channel. The presence of the gold layer allowed the formation of Au–S bonding that provides a platform for subsequent attachment of functional layers specific for reacting with the antibodies. These disposable test strips were rinsed with acetonitrile and dried with nitrogen gas before applying 0.1 mM *N*,*N*′-dicyclohexylcarbodiimide and 0.1 mM *N*-hydroxysuccinimide in acetonitrile for 2 h. Once the reaction was completed, isopropyl alcohol and de-ionized water were used to wash off excess reactants in the microfluidic channel, which then was dried by filtered nitrogen gas. 1% Phosphate buffer solution (PBS) was prepared by diluting purchased 10% PBS in de-ionized water for antibody preparation. Then, two different antibodies, polyclonal spike antibody (ProSci Inc., Poway, CA) and MCA-5G8 monoclonal antibody (ProSci Inc., Poway, CA), were diluted to 20 μg/ml in 1% PBS and were injected into the microfluidic channel and stored at 4 °C for 18 h. Test strips were rinsed with 1% PBS and stored in a refrigerator at 4 °C for future use. Inactivated virus VR-1986HK (ATCC, Manassas, VA) was diluted in saliva to prepare virus standards with different concentrations, including 100 plaque-forming units per milliliter (PFU/ml), 250, 500, 1250, and 2500 PFU/ml, which were then stored in the refrigerator at 4 °C.

A silicon MOSFET (STMicroelectronics STP200N3LL) attached on a printed circuit board was used to amplify the electrical signal from the test strips. Synchronous pulse voltage signals were sent to drain and gate electrodes. The electrode on the test strips, which were functionalized with antibodies, was connected to the gate electrode of the MOSFET. A pair of synchronous pulses were delivered to both the auxiliary electrode on the test strips and the drain electrode of MOSFET. The duration of each pair of synchronous pulses was 1.1 ms. The analog drain waveform was obtained by using an Agilent infiniiVision DSO7054B oscilloscope at a fixed gate voltage of 1.5 V. For each sample measurement, ten pulse signals were received and averaged. The voltage reading at 750 μs was used as the analog reading. The waveform was integrated and converted to a four-digital reading by an Arduino microcontroller that was integrated on the circuit board (Fig. 1). The result of the digital readings was shown on a built-in LCD screen. The detailed information of the printed circuit board and this double-pulse method have been described in the previous work.[15,16]

Sixty human saliva samples, which included 30 positive and 30 negative samples determined by RT-qPCR, were received from the University of Florida Clinical Translational Science Institute (CTSI) Biorepository. These samples were de-identified and only COVID diagnosis was included. RT-qPCR test was conducted again using primers of N1 and N2 genes of SARS-CoV-2 to determine the cycle threshold value (Ct value up to 45) for each human saliva sample. When processing RT-qPCR, the Luna Probe One-Step RT-qPCR 4X Mix with UDG (New England BioLabs Inc., Ipswich, MA) and CDC 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR panel primers and probes were used. Before screening with disposable test strips, human samples were stored in a freezer at −20 °C. The reading for every sample was measured within a minute after the human sample was applied. The entire measurement was performed in the laboratory, where rigorous safety protocols were followed to ensure no risk of

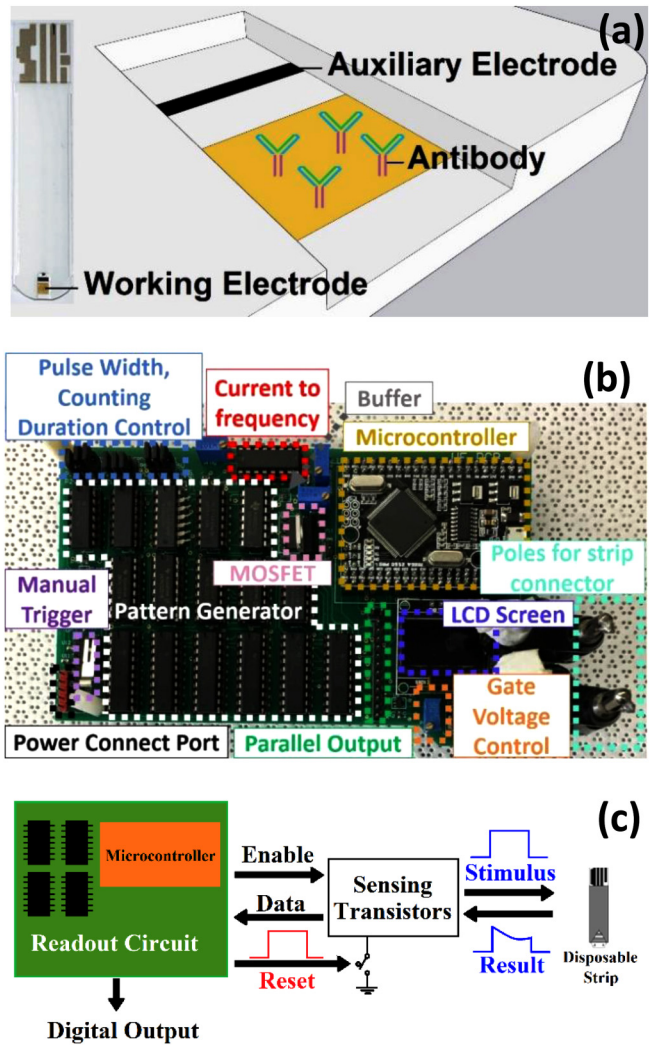

FIG. 1. Photographs of (a) the sensor strip, (b) the printed circuit board, and (c) block diagram illustrating the synchronous double-pulse method.

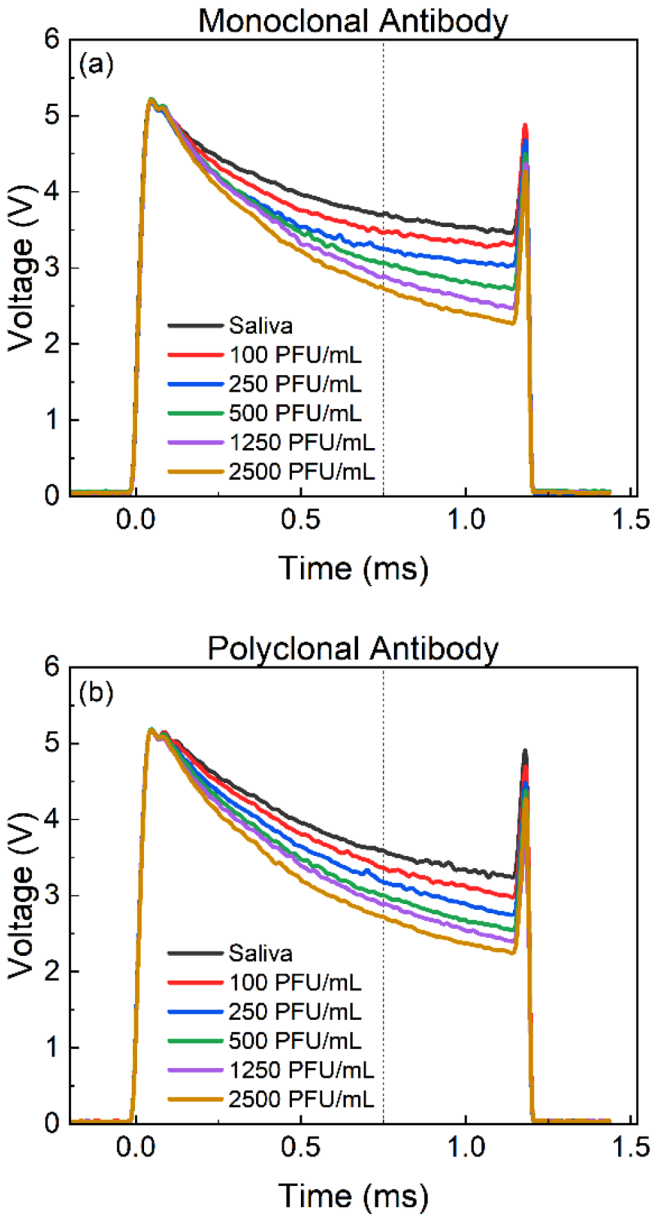

FIG. 2. MOSFET drain waveforms of (a) monoclonal antibody and (b) polyclonal antibody sensor strips obtained at 1.5 V for gate voltage for virus standards with different concentrations. The voltage at 0.75 ms (dashed line) was extracted from each waveform.

contagion, including wearing appropriate respirators, goggles, face shields, and disposable lab coats. While testing, each strip was used only once and bleached for an hour after the reading was obtained in order to denature the virus in samples in the microfluidic channel before disposing of the biohazardous waste.

III. RESULTS AND DISCUSSION

The correlation between spike protein concentration and digital readings, as well as voltage, was demonstrated in our previous study.[12] The time-dependent drain waveforms of different virus standards from pure saliva, which consists of 0 PFU/ml, up to 2500 PFU/ml virus standard, are shown in Figs. 2(a) and 2(b) for both monoclonal and polyclonal antibodies. These waveforms were obtained by measuring the potential change between the drain and the source. Both Figs. 2(a) and 2(b) indicate that increasing the inactivated virus concentration leads to a decrease in the drain voltage level for both monoclonal and polyclonal antibodies. The drain waveform of each virus standard was the average of ten waveforms from ten voltage pulse signals. All individual waveforms

were integrated, and the converted four-digital readings are compared in Fig. 3(a). The drain voltage of waveforms at 0.75 μs (dashed lines) was extracted and is analyzed in Fig. 3(b). Raising the concentration of inactivated virus boosts the amount of antigen-antibody formation, leading to the increase in conductance. This produces the reduction in drain voltage shown in Fig. 3(b) and the integrated digital readings in Fig. 3(a). A sensitivity of 368/decade was calculated for the monoclonal antibody from the average readings in Fig. 3(a) and in a similar fashion, a value of 354/decade was calculated for the polyclonal antibody. Figure 3(b) shows the change of drain voltage at 0.75 μs corresponding to virus standards with different concentrations. The sensitivities of 511 mV/decade for monoclonal antibody and 495 mV/decade for polyclonal antibody were then calculated from this data.

To analyze the correlation between digital readings and human samples that have different virus concentrations, RT-qPCR was applied to human saliva samples in order to collect the Ct values. Figures 4(a) and 4(b) show the digital reading of each sample corresponding to the N1 Ct value for monoclonal and polyclonal antibodies separately. Regression curves in Figs. 4(a) and 4(b) were obtained by analyzing digital readings within positive specimens. Both monoclonal and polyclonal antibody strips show similar sensitivity, and the trend that lower Ct value samples have lower digital readings fits the previous discussion where virus standards with higher concentrations would have lower readings. Even though readings of positive samples whose Ct values are from 35 to 40 overlap with readings obtained from some negative samples, the negative samples were still able to be differentiated from positive human samples because of their higher digital readings for both monoclonal and polyclonal antibodies. These higher

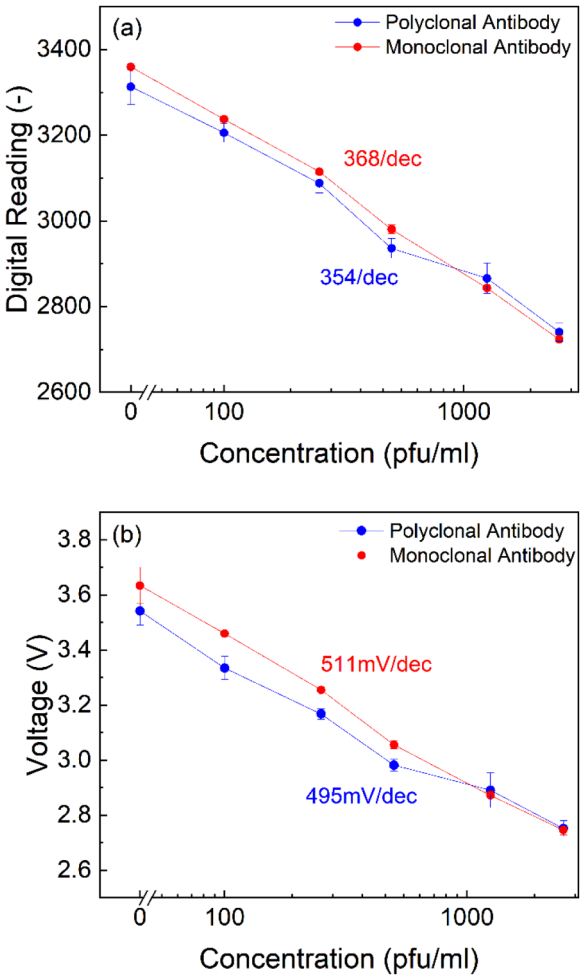

FIG. 3. Average (a) digital reading and (b) voltage extracted at 0.75 ms for each virus standard using strips functionalized with monoclonal and polyclonal antibodies.

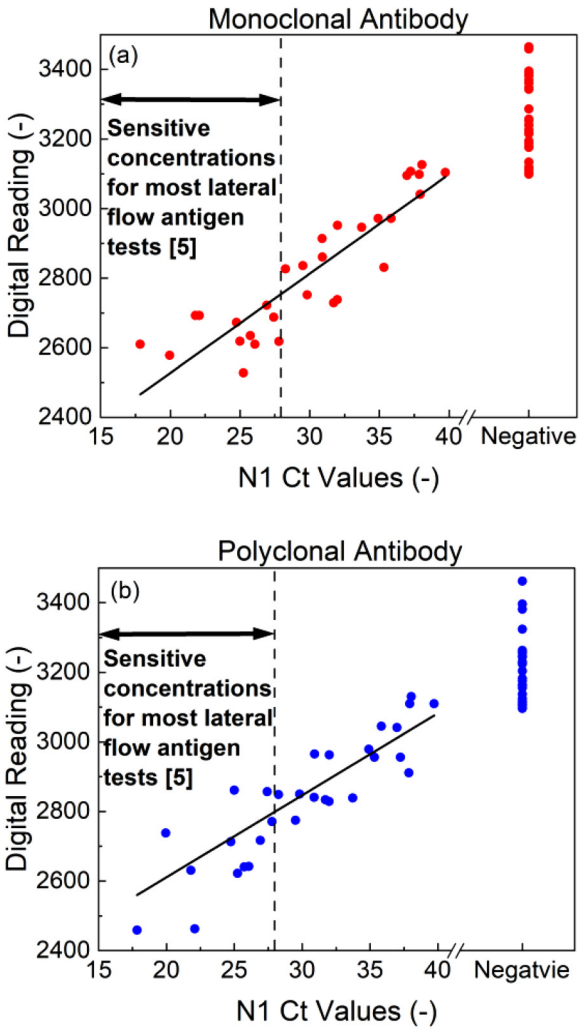

FIG. 4. Digital readings were measured in 30 s for all human saliva samples with different N1 Ct values using strips functionalized with (a) monoclonal antibody and (b) polyclonal antibody. Samples with Ct values larger than 40 are considered negative.

digital readings from negative samples were generated from the absence of the SARS-CoV-2 virus. The reduced amount of virus decreases antigen-antibody formation that decreases the conductance, therefore lessening the drop in drain voltage. Moreover, the results in Figs. 4(a) and 4(b) suggest that specimens with N1 Ct values close to 37 can still be differentiated from negative samples, which indicates that samples with virus concentration less than 1 PFU/ml could be detected.[5] This result demonstrates this sensor technology has a lower limit of detection compared with commercial lateral flow tests, which are only sensitive for samples that consist of high virus concentrations.[5]

In Figs. 5(a) and 5(b), we demonstrate the results from human samples that were subdivided into three categories: positive specimens, low positive specimens, and negative specimens. Positive specimens are defined as saliva samples with both N1 and N2 Ct values smaller than 40, whereas negative samples have Ct values over 40 for both N1 and N2 targets.[17] Low positive specimens indicate saliva samples with either N1 or N2 Ct values lower than 40, but not both.[17] Figures 5(a) and 5(b) illustrate that despite the fact that readings of low positive specimens overlap with readings of positive and negative samples, positive samples were still differentiated from negative samples. This is because the highest reading of positive samples (2952 for monoclonal antibody and 2965 for polyclonal antibody) does not overlap with the lowest reading of negative samples (3103 for monoclonal antibody and 3096 for polyclonal antibody). These results also demonstrate that all positive samples with N1 Ct values lower than 33.7 can be easily verified since their readings are much smaller than negative samples, whereas current lateral flow antigen tests have difficulty detecting positive samples with over Ct values over 28.[5]

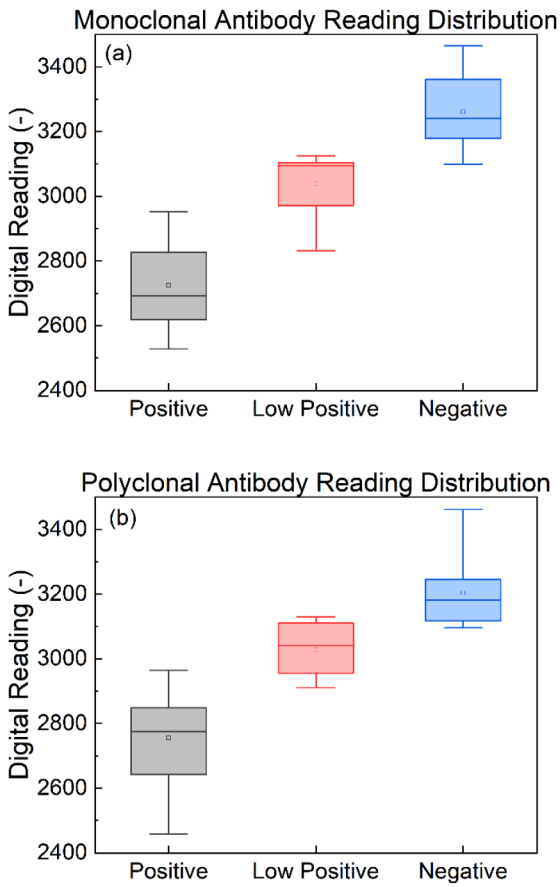

FIG. 5. Box plot of reading distribution of positive, low positive, and negative samples using strips functionalized with (a) monoclonal antibody and (b) polyclonal antibody. The top and bottom horizontal lines of each box represent maximum and minimum digital readings for each category. The second, third, and fourth horizontal lines represent the first quartile, median, and third quartile. The square inside the box plot is the mean.

TABLE I. Comparison of various available detection methods for SARS-CoV-2.

Method	Detection limit in Ct values	Average waiting time	Reference
Polymerase chain reaction (PCR)	Ct values 35–40	More than an hour to a day	3, 4, and 17
Lateral flow tests	Ct values 28 or less	Between 10 and 30 min	5
Double-pulse measurement	Ct values 35–37	Less than 1 min	This study

Although monoclonal antibodies and polyclonal antibodies show similar sensitivity when testing virus standards and human samples in Figs. 4(a) and 4(b), there are some differences that could be discussed. Polyclonal antibodies are cost-efficient; however, their high tolerance to similar epitopes could cause them to bind to another virus. Figure 5(a) shows that monoclonal antibodies have slightly better sensitivity, and the slope of the regression line is steeper in Fig. 4(a), but they are more expensive. A summary table for comparison across different detection methods for SARS-CoV-2 is shown in Table I, showing the advantage of this double-pulse measurement for SARS-CoV-2 detection.

IV. CONCLUSION

Due to the ongoing spread of SARS-CoV-2, the world is in urgent need of a rapid and sensitive detection method. Our findings demonstrate that this cost-effective technology has more sensitive detection at higher Ct value samples and produces rapid results compared with the currently available rapid tests and PCR. Based on our results, samples with N1 Ct values less than 37 could be detected, whereas the current commercial SARS-CoV-2 rapid antigen tests have trouble verifying positive samples with N1 Ct values over 28.[5] The price for each disposable strip after functionalization is less than a dollar and the time required waiting for results is less than a minute. Most of the current onsite tests use PCR that takes more than an hour to obtain results.[18] In this situation, patients could have come into contact with other people before receiving positive results. By contrast, our rapid test can recognize positive cases within a minute. Therefore, patients could be put in quarantine immediately to prevent further infection. Moreover, this

technology can be adapted for other diseases by attaching different antibodies to the same basic sensor platform, which can help in developing rapid testing for future pandemics.

ACKNOWLEDGMENTS

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DATA AVAILABILITY

The data that support the findings are available from the corresponding author upon reasonable request.

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