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Development and clinical evaluation of commercial glucose meter coupled with nanofiber based immuno-platform for self-diagnosis of SARS-CoV-2 in saliva

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\textbf{ABSTRACT}

Rapid diagnostics for the diagnosis of COVID-19 are urgently needed in offices, residences, and other public places due to the new Covid epidemic stages. A portable and easy-to-use immuno-sensing platform was developed and evaluated for a point-of-care and self-detection of SARS-CoV-2 spike protein without the need for extraction, separation, or amplification steps using clinically isolated samples (n = 40 samples). The sensing platform was fabricated based on functionalized nylon nanofibrous membranes and a commercial glucose meter to enable easy deployment of the sensing technology. The fabrication of the immunoreaction vial using nylon nanofibrous membranes as a support matrix for the tethering of antibodies significantly improved the sensitivity of the detection platform in contrast to the use of conventional nylon casted membranes. The sensitivity of the nanofibrous membrane attached antibody was at least an order of magnitude higher (~12 times) compared to the sensitivity of detection with regular casted membrane-based immunoreaction vial. The feasibility of the designed sensing platform was investigated using saliva as a non-invasive and self-administered sample for the diagnosis of SARS-CoV-2. With a detection limit of 9 ng mL\textsuperscript{-1} and no pretreatment processes required, the sensing platform demonstrated its suitability for the direct detection of SARS-CoV-2 spike protein in the spiked saliva samples. In addition, the developed platform depicted high agreement with RT-qPCR data in the analysis of the clinical samples with good stability over the storage time and reusability for three cycles with maintaining more than 95% of its original activity.

\textbf{1. Introduction}

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes Coronavirus Infectious Disease 2019 (COVID-19) is now causing one of the deadliest pandemics in history. The pandemic is difficult to control because of the rapid spread of the disease in human infection and a lack of sufficient vaccination for all nations or effective medicine to treat \cite{1,2}. On June 5, 2020, the disease’s basic reproduction number (R\textsubscript{0}), which refers to the number of persons infected by a single person, was between 2 and 4, with a case fatality ratio of around 7\% \cite{3}. This situation is supported by the average value of 3.17 obtained from 24 studies with 32 different R\textsubscript{0} values \cite{4}. As a result, the World Health Organization had tallied more than 133 million cases of COVID-19 and more than 2.9 million deaths by April 9th \cite{5}. Despite its high genetic similarity to the SARS coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MER-S-CoV), SARS-CoV-2 has a significantly higher infection rate than SARS-CoV and MERS-CoV \cite{6}.

Controlling the spread of SARS-CoV-2 has been difficult since most people are asymptomatic when they get COVID-19, making it difficult to detect instances of infection \cite{7}. As a result, the overall number of cases continues to be underestimated since the frequency of asymptomatic patients is unclear \cite{7,8}. For the time being, research has indicated that about 40\% of COVID-19 infections are asymptomatic \cite{9}.

One of the most difficult problems in avoiding SARS-CoV-2 transmission is the capacity to rapidly identify, detect, and isolate patients before they can spread the infection to others. This limitation results due to a lack of fast and accurate testing. Many depend on current COVID-19
screening methods, which generally involve a combination of symptom and travel-related survey questions and temperature measurements, as communities throughout the world begin to take steps to reopen businesses, schools, and other activities.

Although molecular diagnostic point-of-care devices are available \cite{10,11}, these tests are usually validated in labs using expensive and time-consuming procedures. The absence of testing centers in many countries, as well as the need for skilled staff and specialized equipment, as well as a global reagent scarcity, have hampered widespread testing, allowing the SARS-CoV-2 infection to continue and hinder the safe reopening of economic activities \cite{12}.

The most commonly used methods for diagnosing viral diseases are real-time polymerase chain reaction (RT-PCR) \cite{8,13–16}, lateral flow immunoassay (LFA) \cite{17–19} and electrochemical biosensors \cite{6,20–24}. Despite being RT-PCR is the principal technique for identifying such illnesses \cite{6,7,24}, the PCR technique needs highly skilled people, expensive equipment, complex chemicals, and a rather complex procedure, which limits its usage for regular testing (several times per week \cite{25} and in low-resource situations \cite{26–28}. Because of these drawbacks, PCR may not be suitable for real-time detection of asymptomatic infected persons in diverse environments, causing delays in the implementation of containment measures, and allowing the virus to spread further \cite{25}. On the other hand, LFA is a potential point-of-care (POC) option and an alternative to PCR. However, they have substantial drawbacks, such as qualitative reading and allegedly low sensitivity with large false-positive rates \cite{29–31}. As a result, there is still an urgent need for reliable and cost-effective diagnostic tests that can be widely used.

Given these disadvantages, rapid, cheaper, simpler, and more sensitive ways of identifying such a quickly spreading deadly illness are necessary. The development of quantitative, fast testing around infrastructure that has already been implemented at scale in the market might be a viable answer to these challenges. Commercial glucose meters have already been repurposed to detect several non-glucose-based targets, including cocaine, Ebola, hepatitis B, foodborne pathogens, and interferon-gamma \cite{32–39}. The glucose meter is the most widely used piece of diagnostic equipment in the world, with 422 million individuals using it daily to monitor their blood sugar \cite{40}. These meters are portable, affordable, easy-to-use, and extremely accurate \cite{41}. Many of these can be linked to smartphones through Bluetooth allowing detection findings to be integrated with contact-tracking applications and electronic health records.

Nanofibrous membranes (NFM) produced via electrospinning are promising materials, due to their ultrahigh specific surface areas, and have attracted an increasing interest for their applications in many fields \cite{42–51}. In addition to these characteristics, polycaprolactam (Nylon 6) fibers, commonly known as polyamide fibers, has outstanding mechanical strength, biocompatibility, flexibility, toughness, abrasion resistance as well as high resistance to solvents and oils \cite{52}. Thus, based on the ultra-high surface area, microporous network and other physico-chemical properties, nylon-based nanofibers have achieved higher sensitivity and lower limits of detection (LOD) for diverse analytes \cite{50,53}. However, compared with other polymers such as polyvinyl alcohol and cellulose, Nylon is less reactive to chemical modifications and biomolecule immobilization due to the relatively inert chemical structures. Thus, modified nylon fibers with chemically reactive groups for immobilization of biorecognition elements such as proteins are desired \cite{54}.

This study reports the fabrication of a novel designed, saliva-based platform test for the self-diagnosis of SARS-CoV-2 based on the spike antigen protein detection using a glucose meter. The detection of the spike protein as a target analyte was carried out based on sandwich immunological assay (Scheme 1) by tethering of SARS-CoV-2 spike protein antibodies as primary antibodies onto the surface of nanofibrous membranes as a sensor matrix with ultrahigh surface area and microporous structure. A secondary antibody labeled with β-galactosidase enzyme was used as detection antibodies creating sandwich-based detection system. Following that, the lactose was hydrolyzed as an enzymatic substrate into glucose, which was measured using a commercial glucose meter.
2. Materials and methods

2.1. Reagents

Polycaprolactam (Nylon 6, NY), formaldehyde, formic acid, lactose, and ethanolamine (EA), were supplied by Sigma (St. Louis, MO, USA) and used as received, O-phosphoric acid (85%) were purchased from Acros Chemical (Pittsburgh, PA, USA), cyanuric chloride (CC) were supplied by TCI Co. (OR, USA), Phosphate buffer solution (PBS) were purchased from Fisher Scientific, (Fair Lawn, NJ, USA), anti-SARS-CoV-2 spike protein primary and secondary (conjugate) antibodies were purchased from Absolute Antibody NA (Boston, MA, USA), SARS-CoV-2 spike protein was supplied by ABclonal INC. (Woburn, MA, USA). All other chemicals were of analytical grade and were supplied by Merck (Darmstadt, Germany). The glucose meter and glucose test strips were purchased from Nova Biomedical (Waltham, MA, USA).

2.2. Fabrication of nylon 6 nanofibrous membranes (Ny NFM)

A formic acid solution of Nylon 6 (15% w/v) was prepared with vigorous stirring in a water bath at 60 °C for 8 h. Electrospinning was carried out using a 10-mL plastic syringe with an 18-gauge tubular metal needle with a flat tip. The electrospinning process was carried out at room temperature using DXES-1 spinning equipment supplied with an applied voltage of 20 kV at a controllable propulsion velocity of 0.7 mL/h and 23 cm distance between the spinneret and collector surface. The resultant nanofibrous membranes (Ny NFM) were dried in a vacuum oven at 40 °C for 12 h. A Nylon solution (15% w/v) was used to cast a thin membrane film (Ny-CM) of similar thickness to the Ny NFM.

2.3. Chemical modifications of nylon nanofibrous membranes

Ny NFMs were chemically modified for the antibodies immobilization according to reactions shown in Scheme 2. Firstly, Ny-based NFMs were reacted with formaldehyde to convert the amide (N–H) groups to N-methylol groups to form Ny-OH NFMs [55,56]. Briefly, 2 g of Ny NFM were immersed in 25 mL of formaldehyde (36.5% w/v) and 0.2 mL phosphoric acid (85% w/w) at a temperature of 60 °C for about 1 h. Then the prepared Ny-OH NFMs were washed several times with distilled water.

The hydroxyl groups on the Ny-OH NFMs were then activated with cyanuric chloride (CC) for immobilization of the antibodies onto the nanofibrous membrane surface. After Ny NFM hydroxyl groups activation using an alkaline aqueous solution for 1 h, the nanofibrous membrane was activated with CC at a concentration of 4% (w/v) in a dioxane solution for 3 h. The resulting membrane (Ny-CC NFM) was washed with dioxane, deionized water and acetone, and dried under vacuum. A 10 mm Ny NFM disc with 0.05 mm thickness was fixed in the bottom of the cryovial (2 mL) as shown in Scheme 2 and similarly, a vial was prepared in parallel by using a Ny casted disc.

2.4. Immobilization of anti-COVID antibodies

25 μL of anti-COVID (25 μg mL⁻¹) antibodies were dropped onto the surface of the CC-activated Ny NFMs disc and kept for about 3 h at 4 °C. After being extensively rinsed with PBS to remove any free antibodies, followed by blocking of the remaining active sites using 100 μL of 2% ethanolamine at room temperature for 1 h, then rinsed again with PBS and stored at 4 °C to use.

Scheme 2. Modification of Nylon NFM with cyanuric chloride and antibodies immobilization. Photograph of the immunoreaction vial with antibodies-immobilized Ny NFM at the bottom of the vial.
2.5. SARS-CoV-2 spike protein detection

One mL of PBS buffer spiked with different concentrations of SARS-CoV-2 spike protein was added to the immunoreaction vial and incubated for 20 min at the room temperature. After wash 3 times with 1 mL with PBST (PBS containing 0.05% Tween 20), and one time with PBS, 100 μL of secondary antibody solution was added and incubated for additional 20 min at the room temperature. Then, the immunoreaction vial was washed as previously described. 100 μL of lactose solution at concentration of 50 mM was added to the vial and incubated for 20 min. Finally, the electrochemical measurements were carried out using a new glucose meter test strip and read out using a glucose meter. All measurements were repeated in triplicates.

2.6. Simulated and clinical samples

Healthy volunteer saliva was spiked with different concentrations of SARS-CoV-2 spike protein to create simulated samples. Clinical samples were collected from adults between 21 and 60 years old and were previously tested for SARS-CoV-2 by RT-qPCR. The sample collection and analysis steps followed the IRB-approved protocol (Faculty of Medicine Ethical Committee- Alexandria University, IRB approval No: 00012,098) and followed the principles outlined in the Declaration of Helsinki for all human experimental investigations. The detection was carried out as previously described.

3. Results and discussion

3.1. Physicochemical characterization of the prepared nanofibrous membranes

The immunoreaction vial was fabricated with a nanofibrous membrane immobilized on the bottom of the vial, as shown in Scheme 2. The nanofibrous membrane was a chemically modified Nylon membrane with immobilized antibodies, as illustrated in Scheme 2. Nylon-based nanofibrous membrane was selected based on the following properties: (I) the hydrophilicity of the matrix to improve the detection performance using aqueous samples, (II) the physical and chemical properties of Nylon as a robust matrix for fabrication of the detection platform, and (III) the ultrahigh surface and microporous areas that can increase the efficiency and the accessibility of the active sites to the targets.

The successful activation of Ny NFM was evaluated using FT-IR spectroscopy. Fig. 1a depicts the FTIR spectra of pristine Nylon nanofibers before and after modification. The appearance of a new peak at 1042 cm\(^{-1}\) for the C–O of a primary alcohol, broadening band in the

![Fig. 1](image-url)
The morphologies of the Ny NFMs during the different steps of chemical modifications were characterized by scanning electron microscopy (SEM). As shown in Fig. 1 (b and c), the Ny-NFMs revealed randomly oriented three-dimensional non-woven membrane structures with an average fiber diameter of 75 nm. The average diameter of the nanofiber increased to 90 nm after the reaction with formaldehyde in an acidic medium (Fig. 1 c and f). Consequently, the nanofiber diameter was further increased to 120–130 nm after the activation by CC, as illustrated in Fig. 1 (d and g). The increase in the nanofiber diameters after the modifications were possibly due to increased hydrophilicity and swelling behavior of the methylolated and activated nanofibers and the impregnations of the fibers with the different aqueous solutions through the modifications [57]. This observation agreed well with the water contact angle properties of the Ny NFMs before and after the modification. The contact angle measurements of non-modified Nylon NFM decreased from 91° to 57° for the CC-activated Ny NFM during a contact time of 30 s, attributed to the modifications by formaldehyde and cyanuric chloride.

3.2. Optimization of the experimental factors for COVID-19 detection

The performances of the assembled detection platform were adjusted by optimizing several parameters, namely cyanuric chloride concentration, antibodies immobilization, time necessary for antibodies immobilization, and substrate concentration.

The Ny-OH NFM was activated using different concentrations of CC ranging from 1 to 12% (w/v) to study the impact of CC concentration on the glucose meter readout at a SARS-CoV-2 spike protein level of 250 ng ml⁻¹. The results illustrate that the readout response increased linearly with an increase in CC concentration and achieved a plateau at concentrations higher than 4% (Fig. S1a).

As the antibody concentration implicitly influences the performance of sensors, different concentrations of the SARS-CoV-2 spike antibodies at the range of 1–50 μg ml⁻¹ were tested at a SARS-CoV-2 spike protein level of 250 ng ml⁻¹. The glucose meter reading increased progressively with an increase in antibody concentration, as illustrated in Fig. S1b, with the highest readout response obtained at 25 μg ml⁻¹ antibodies. The further increase in antibody concentration over 25 μg ml⁻¹ led to a reduction in the response. This behavior might be connected to antibody steric hindrance, which could diminish the accessibility of SARS-CoV-2 spike protein to antibody binding sites in nanofibrous membranes [58].

Finally, the optimum antibody concentration for the best readout response was determined to be 25 μg ml⁻¹.

The optimum time of SARS-CoV-2 spike antibodies tethersing onto the activated Ny NFM was evaluated by carrying out the antibody immobilization process with an antibody concentration of 25 μg ml⁻¹ at 4°C at different times between 0.5 and 6 h. The glucose meter readout at a SARS-CoV-2 spike protein level of 250 ng ml⁻¹ increased with an increase in the immobilization time of the antibodies up to 3 h (Fig. S1c). That notwithstanding, additionally prolonged immobilization times did not result in a higher readout value, indicating that immobilization sites of the nanofibrous membrane were saturated.

The response of the detection platform was investigated at different concentration points of the immuno-reaction time. Fig. S1d clearly shows that the detection platform response at a SARS-CoV-2 spike protein level of 250 ng ml⁻¹ increased linearly with incubation time and reached a plateau after 30 min. Since one of the critical needs of the SARS-CoV-2 sensing platforms is rapid detection, the immuno-reaction time of 20 min was adopted as the ideal time point instead of 30 min as it provided around 93% of the highest readout at 30 min.

The concentration of the substrate is another critical factor affecting the enzyme-catalyzed reaction [59]. With the other parameters fixed, the sensing platform response increased continuously with the concentration of lactose and reached the maximum value at the concentration of 50 mM (Fig. S1e). No further obvious increase in the response with an increase in the substrate concentration was observed. So, 50 mM was used as the optimum concentration for the best analytical performance.

Beyond that, the incubation time of the substrate was varied between 5 and 40 min, and 20 min was considered for further measurements (Fig. S1f).

3.3. Analytical performance of the detection platform

Based on the critical need for rapid, accurate, and easily scalable tests that can enable the self-diagnosis of acute SARS-CoV-2 infection and provide widespread testing [60], the nucleocapsid and spike proteins share a highly conserved structure with SARS-CoV-2, while spike protein is less conserved, more highly specific to SARS-CoV-2, and more suitable for the point-of-care diagnosis platforms. A novel immuno-sensing platform capable of sensitive detection of SARS-CoV-2 in human saliva based on the spike protein was developed and validated using only low-cost detector that is inexpensive and widespread across the world. The assay design required the integration and optimization of three novel components: to fabricate an immunoreaction matrix for binding antigen in human samples, to transduce binding into a signal, and to detect that signal.

The analytical performance of the fabricated detection platform was investigated through a correlation between glucose readout and antigen concentration by the spiking of SARS-CoV-2 spike protein in buffer (Fig. 2a). Using the commercial glucose test strips and a glucose meter, the detection platform depicted a broad linear dynamic range of 5 ng ml⁻¹–1 μg ml⁻¹ and the detection limit was calculated to be 5 ng ml⁻¹ based on the slope method (σₚ/θ/slope), where σₚ was the standard deviation of the blank samples [61,62]. The construction of the detection platform based on the regular polymeric casted membrane as a matrix for immobilization of the antibodies exhibited significantly lower sensitivity with LOD of 60 ng ml⁻¹. In contrast, the high sensitivity and performance of the diagnosis platform could be due to the robust structure of the nanofibrous membranes with the high surface area and high porosity structure, which improves the accessibility of antigens to the recognition sites [50,56,57]. In addition, the employment of the β-galactosidase as an enzymatic label has an advantage in improving the sensitivity of the diagnosis platform through the hydrolysis of lactose-generating galactose as a by-product and acts as an interference to the glucose. In this specific case, the presence of galactose helps in increasing the achieved signal, consequently, enhancing the sensitivity of the sensing platform.

The specificity of a diagnosis tool toward the desired target is regarded as one of the critical challenges. The cross-reactivity of the developed sensing platform was investigated against non-SARS-CoV-2 respiratory viruses, specifically the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Influenza A (H1N1). As depicted in Fig. 2b, no significant cross-reactions or interferences toward SARS-CoV-2 spike protein detection were caused by the spike proteins of the tested respiratory viruses at high concentration (100 ng ml⁻¹). The achieved results show that the developed detection platform is highly specific toward the SARS-CoV-2 diagnosis.

The reusability of the diagnosis platform was investigated using SARS-CoV-2 spike protein at a concentration of 500 ng ml⁻¹ for reusing
the immunoreaction vial for multiple diagnosis cycles. The regeneration step was carried out by adding 1 mL of 0.1 M glycine hydrochloric acid buffer at a pH value of 2.8 to the immunoreaction vial for 5 min followed by washing three times with ultrapure water. The achieved results revealed that the immunoreaction vial can perform three diagnosis cycles using a new glucose test strip at each diagnosis cycle, as the detection platform maintained more than 95% of its initial glucose meter readout in the second and third cycles, while at the fourth diagnosis cycle, the detection platform lost about 40% of its initial detection accuracy (Fig. 2c). The activity loss may be due to denaturation of the immobilized antibodies or destruction of nanofibrous membranes with repeating regeneration using the acidic glycine buffer [64]. Given that tens of millions of COVID-19 tests are performed every day throughout the world, the wash-reuse approach is expected to decrease the diagnosis and tracking costs by more than three times, potentially resulting in a financial advantage for COVID-19 testing.

The immunoreaction vial exhibited good stability when stored at 4 °C retaining more than 94% of the initial response after 60 days of storage. The high stability was probably owing to the robust design of the detection platform based on the nanofibrous membranes as a supporting matrix for the tethering of the antibodies.

3.4. Feasibility of the sensing platform for SARS-CoV-2 detection in saliva

The selection of saliva as a promising non-invasive specimen for SARS-CoV-2 detection has challenges and opportunities. Saliva is the most promptly obtained human specimen, being especially advantageous in certain populations such as children. In addition, it exhibits greater sensitivity with the huge advantage of a self-sample collection of saliva with less variability compared to the nasopharyngeal samples [65]. To et al. (2020) reported that also SARS-CoV-2 is present in the saliva of around 92% of patients [66] as well as Wylie et al. examined SARS-CoV-2 detection in patient-matched nasopharyngeal and saliva samples, and it was discovered that saliva had higher detection sensitivity and consistency throughout the infection with less variation in terms of nasopharyngeal [67]; however, it is also a complex and viscous sample contains various components [68,69] presenting difficulties for the specific biomarker detection [60].

Human saliva samples were collected from a healthy person after mouth rinsing with 1 mL of ultrapure water and spiked with known concentrations of SARS-CoV-2 spike protein to investigate the application of a fabricated diagnosis platform for the detection of SARS-CoV-2 in human samples. One of the key steps is the washing step after incubation the saliva sample in the immunoreaction vial and before adding the secondary antibody to reduce the effect of endogenous glucose content or other biomolecules in saliva that could interfere with the assay. The immunosensing vial was extensively washed using PBST and PBS before adding the secondary antibodies. Non-spiked saliva sample was served as a control sample. No signal was achieved with the blank saliva sample, which confirmed the elimination of the endogenous enzymes or glucose in saliva. The blank and each spiked concentration of SARS-CoV-2 spike protein were tested in triplicate. The matrix effect due to the analysis in saliva without any pretreatment was evaluated by constructing the calibration curve in untreated saliva, observing well-defined sigmoidal behavior with lower intensity of glucose meter signal due to the matrix effect with a detection range of 18 ng mL\(^{-1}\) – 1 μg mL\(^{-1}\) and LOD of 18 ng mL\(^{-1}\) (Fig. 3a). While adding 0.5 mL PBS into the immunoreaction vial reduced the matrix effect with a detection range of 9 ng mL\(^{-1}\) – 1 μg mL\(^{-1}\) and detection limit of 9 ng mL\(^{-1}\) (Fig. 3b). So, it is recommended to add 0.5 mL PBS during the fabrication of the immunoreaction vial to reduce the matrix effect and enhance the stability of the platform over the storage time.

Then, human saliva samples were spiked with known concentrations of SARS-CoV-2 spike protein from 15 ng mL\(^{-1}\) to 1 μg mL\(^{-1}\). The structural studies on SARS-CoV-2 reported in literature until now are not able to furnish unequivocal information related to the number of S proteins present on each virus [70,71], thus it is difficult to calculate the number of viruses detected using the calibration curve for S protein. As depicted in Table S1, the recovery rate was in the range of 94.9%–98.3%, and the relative standard deviation (RSD%) was about 4.97%.

Furthermore, the developed sensing platform was examined to discriminate between SARS-CoV-2 infected and healthy individuals using clinical samples. Saliva samples were collected from 5 healthy individuals, 30 SARS-CoV-2 infected individuals during the first week of the positive test and 5 SARS-CoV-2 infected individuals during the second week of the positive test based on the IRB-approved protocol. RT-qPCR was used to confirm the negative and positive infection of SARS-CoV-2. The developed sensing platform exhibited 100% agreement with the RT-qPCR data performed for the same negative samples, while 90% sensitivity was achieved for the infected individuals during the first week of the positive test (Table 1). Unsurprisingly, the sensitivity of the diagnosis platform was lower for the infected individuals during the second week of the positive test. This could be that the viral loads in the saliva samples peaked during the first week of SARS-CoV-2 symptoms and significantly decreased in the second week [72,73]. The above-mentioned results verify the applicability, accuracy, and reproducibility of the fabricated detection platform for an early, rapid and self-diagnosis of SARS-CoV-2 in the saliva without any pre-cleaning, lysing, enrichment, or separation steps for the samples to match the requirements for the point of care.

Additionally, the quantitative readout may be sent electronically so that test results can be reported and tracked more effectively. In ambulatory settings with support for remotely observed testing, digital reporting, and results in the notification through telemedicine or a
smartphone app, such a test might be done with modest process changes. One of the key advantages of this approach is the simplicity of detecting Covid-19 without any sample pretreatment such as cleanup, lysis, enrichment, separation, or amplification steps. The developed diagnosis platform also demonstrated high specificity for detecting Covid-19 during the early stages of the infection. In comparison with previously developed sensing platforms (Table S2), the LOD of this sensor was comparable to many of the lab-based electrochemical sensors but lower than the high-end FET sensors. However, it is important to note that the LOD detection achieved using a commercially available glucose sensor meets the clinical needs for early stage detection of the infection. Thus, this sensing platform has the potential to meet the ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users) criteria proposed by the World Health Organization [74] for developing point-of-care diagnostic devices. In summary, this study demonstrates the development and clinical validation of a portable, easy-to-use and low-cost sensing platform for the detection of SARS-CoV-2 in early-stage clinical samples (week 1 of the infection) with high sensitivity.

4. Conclusion

Several scientific communities have been working hard to develop sensitive and portable assays for SARS-CoV-2 since the World Health Organization announced the COVID-19 pandemic in March 2020. Herein, a friendly-use immuno-sensing platform was designed based on nanofibrous membranes and the most widespread diagnostic device “glucose meter” for the sensitively and precisely self-diagnosis of SARS-CoV-2 in human saliva. The constructed sensing platform based on nylon nanofibrous membranes exhibited high sensitivity for the direct detection of the SARS-CoV-2 spike protein in the saliva sample with a detection limit equal to 9 ng mL$^{-1}$ without any pretreatment. Evaluation with clinical samples demonstrated high specificity and adequate sensitivity of the approach to enable detection of the early-stage infection (1st week of the infection). The novel designed diagnosis platform could be easily combined with Bluetooth wireless-enabled software technologies to support “sample-to-answer” reporting and screening of SARS-CoV-2 infection.
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