An Internet-of-Disease System for COVID-19 Testing Using Saliva by an AI-Controlled Microfluidic ELISA Device

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Throughout coronavirus disease (COVID-19) outbreaks, the centers for disease control and prevention (CDCP) of a country require monitoring of particular territories to provide public health guidance. In this work, the Internet of Diseases (IoD) is suggested for continuous real-time monitoring of infectious diseases for public health. Because converging information and communication technologies (ICTs) with point-of-care (POC) devices to enable the IoD for continuous real-time health monitoring and processing of clinical records are crucial, an IoD platform associating a lab-on-a-chip (LOC) device to diagnose severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) from oropharyngeal saliva samples have been developed and uploaded the resultant diagnostic data into a cloud-based system to be connected with CDCP. Moreover, a choropleth IoD map to visualize provincial infection rate resulted diagnostic data into a cloud-based system to be connected with CDCP. Moreover, a choropleth IoD map to visualize provincial infection rate is proposed along with the IoD platform. The developed platform is applied for the quantification of SARS-CoV-2 N-protein antigen with a LOD as low as 0.013 ng mL\(^{-1}\) and the infection rate of various provinces is projected with the IoD map successfully. Thus, the proposed IoD system has the potential to become an imperative tool for the disease control and prevention centers to restrain COVID-19 outbreaks by identifying the severity of particular regions.

1. Introduction

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), identified as the causative agent of respiratory illness, primarily disperses from the coughs, sneezes, or saliva of symptomatic patients.[1–4] Transmission of this virus may also take place through contact with asymptomatic patients and contaminated surfaces.[5–7] The asymptomatic patients may convey a comparable amount of infection as the symptomatic resulting in silent transmission, as previously reported.[8–11] Thus, both the symptomatic and asymptomatic patients contribute as the carrier of the multiple transmittals of coronavirus disease (COVID-19) throughout the world.[12,13] In the initial phase of the outbreak of COVID-19, it controlled local transmission through symptomatic patients.[1,2] Successively, the transmission crossed the borders through asymptomatic patients having a travel history to the infected countries, and the infection advanced into the next stage ensuing in a pandemic.[3–6] Since the occurrence of the SARS-CoV-2 pandemic, more than 150 million human cases and 3.1 million deaths have been confirmed as of May 1, 2021 and more than half of the world has endured either full or partial lockdown with strong containment strategies for the first time in its history.[7,14,15] Therefore, comprehensive prevention measures draw the attention of global priorities to reduce the stress behind SARS-CoV-2.

Despite the variety of vaccines like Oxford/AstraZeneca, Pfizer/BioNTech, Moderna, Johnson & Johnson, Sinopharm, and a few additional vaccines have received approval in several countries, millions of people in many countries are still far away from receiving the vaccines. Additionally, one among many current challenges is the new variants of this virus that are materializing rapidly. Therefore, the approved vaccines may not be effective against such quick-spreading variants.[16–18] Since the transmission and fatality rates of SARS-CoV-2 are amazingly high, proper planning to forestall the spread of strains of COVID-19 by continuous real-time monitoring of the symptomatic and asymptomatic patients has been a big concern at this time. More significantly, because the symptomless patients apparently look sound having no usual clinical signs even in the computed tomography (CT) scan of the chest,[19,20] scrutiny of symptomless patients on larger scales needs important attention to manage the speedy transmission of SARS-CoV-2 virus.

To diagnose acute SARS-CoV-2 in asymptomatic and symptomatic patients, nucleic-acid amplification tests (NAA) test, spike (S)/nucleocapsid (N) protein tests, and antibody (AB) tests are the largely used tools so far.[8] Despite these tests for COVID-19 detecting infective agent ribonucleic acid (RNA) by reverse transcription-polymerase chain reaction (RT-PCR) are immensely sensitive, they bear high false-negative rates.[21,22] So far reported, serological tests for SARS-CoV-2 involve the lateral flow assay (LFA),[23–25] conventional enzyme-linked immunosorbent assay (ELISA),[26–28] and chemiluminescence immunoassay (CLIA).[27,28] Due to inherent constraints such as high false-positive rates including the visual interpretation and...
low sensitivity of LFA limits its applicability in such an urgent global issue as SARS-CoV-2.\textsuperscript{30–32} Whereas, the need for skilled hands, laboratory-based infrastructure, and long assay time in the case of conventional ELISA and CLIA make it difficult to be applied to resource-limited settings.\textsuperscript{33–36} However, these diagnosis methods fail to assist the centers for disease control and prevention (CDCP) of the territory to adopt necessary public health guidelines by determining the prevalence of the infection in a population, monitoring the transmission rate together with classified at-risk patients throughout the pandemic that is imperative. Enabling a rapid, effective, and reliable point-of-care (POC) testing method with remote monitoring can minimize these issues. In this situation, adopting non-invasive samples such as oropharyngeal saliva from the patients can be compared with other existing sample collecting procedures (i.e., blood serum, nasal and oral swab, plasma, etc.) for POC testing.

In this work, we present an Internet of Diseases (IoD) system employing the internet-of-things (IoT) feature as the backbone which can execute immunoassay effectively utilizing a microfluidic 96-well LOC device for detecting SARS-CoV-2 from a patient’s saliva. A cloud-database (DB) system associated with the IoD device will store patients’ e-healthcare records for the healthcare professionals and concerned government authorities to provide individual diagnostic information and thus an extensive survey of the COVID-19 pandemic. Since visualization is one of the critical needs for such IoD implementations for higher accessibility in clinical uses and adopting further health regulations, a choropleth map web application has been created with the IoD platform and deployed for addressing this need. At last, the platform has been tested to quantify SARS-CoV-2 N-protein antigen with a LOD as low as 0.013 ng mL\textsuperscript{-1}. The integration of an IoD module, 96-well LOC device, a cloud DB storage, and choropleth map offer certain exclusive advantages; such as i) the IoD device would be installed in different public places (e.g., hospitals, schools, restaurants, bus terminals, etc.) to facilitate SARS-CoV-2 testing for both asymptomatic and symptomatic patients; ii) since it is burdensome by the liable health department to collect the patient data from many places, IoD features of the proposed device would facilitate convenient and automated documentation of the infected patient’s information; iii) the choropleth IoD map would project the areas where more people are infected which can help healthcare authorities to decide necessary regulations to control the SARS-CoV-2 transmission; and iv) thus the general people can be aware of the situation of a certain area. The name IoD has been introduced as its core concept is developing a comprehensive system for the surveillance of infectious disease transmission rather than conventional IoT-based platforms used for remote health monitoring.\textsuperscript{37–40}

2. Results

2.1. Evaluation of AI-Based Image Recognition Performance

As demonstrated in Figure 1a–f, the accuracy, specificity, precision, sensitivity, false acceptance rate (FAR), false rejection rate (FRR), and F1 score of the AI-based image recognition using our proposed platform have been plotted. From the characterizations shown in Figure 1a–c, it can be seen that the image recognition performance such as accuracy, specificity, and precision suffer below a threshold value of 8. As shown in Figure 1b,c, the specificity and precision were improved up to almost 99.5% and plateaus above a threshold value of 8; whereas, the sensitivity experiences a significant decay in performance after a threshold value of 9. For the image recognition performance evaluation, 100 positive and 100 negative images of the reaction chamber had been collected during the fluidic operation of the device, and then image recognition was simulated in Raspberry Pi 4 to calculate the evaluating parameters (Figure 1d) that suggests a threshold value of 8–9 optimal for the platform. From Figures 1e,f, it can be seen that FAR and FRR values are the lowest (0.99%, and 0.0%, respectively) and the F1 value is the highest (100%) at a threshold value of 8; therefore, a threshold value of 8 has been set as an optimal value for the AI-image recognition of the platform.

These performance criteria have been characterized based on the equations given below.\textsuperscript{41–43}

\[
\text{Accuracy} = \frac{TP + TN}{\text{Number of total images}} \quad (1)
\]

\[
\text{Specificity} = \frac{TN}{TN + FP} \quad (2)
\]

\[
\text{Precision} = \frac{TP}{TP + FP} \quad (3)
\]

\[
\text{Sensitivity} = \frac{TP}{TP + FN} \quad (4)
\]

\[
\text{False acceptance rate (FAR)} = \frac{FP}{TP+FP} \quad (5)
\]

\[
\text{False rejection rate (FRR)} = \frac{FN}{TN + FN} \quad (6)
\]

\[
\text{F1 Score} = 2 \times \frac{\text{Sensitivity} \times \text{Precision}}{\text{Sensitivity} + \text{Precision}} \quad (7)
\]

where TP = true positives, FN = false positives, TN = true negatives, and FN = false negatives.

2.2. Quantification of SARS-CoV-2 N-Protein Using the Proposed AI-Implemented IoD System

Based on the description in the “Operational mechanism of the device” section and optimized assay time and reagent volume for the binding of antigen with coated capture antibody in the wells/biotinylated detection antibody and HRP-conjugated streptavidin complex/enzyme-substrate (TMB) in the “Preparation of SARS-CoV-2 N-protein antigen” section, the developed immunoassay device has been implemented to quantify different concentrations of SARS-CoV-2 N-protein standard.\textsuperscript{44,45} As seen in Figure 2a, the corresponding hue (a parameter to distinguish one color from another)\textsuperscript{46} values translated from the chromogenic changes of TMB substrate were found to be proportional to the different concentrations of the biomarker with a limit of detection (LOD) as low as 0.013 ng mL\textsuperscript{-1}. The
measured LOD value is identical to the theoretically calculated LOD value of this experiment; hence, can be assumed to be correct.\textsuperscript{[45]} Here, the control signal value was realized in the absence of the target antigen, and the signal greater than the control signal was estimated as the LOD.

To validate the clinical trial, the detection of SARS-CoV-2 N-protein from oropharyngeal saliva was also simulated. The dilution effect of saliva using a concentration of 0.206 ng mL\(^{-1}\) of SARS-CoV-2 N-protein has been shown in Figure 2b. From the figure, it can be seen that the resultant hue values remain almost similar up to 20\% of diluted saliva, i.e., fivefold diluted by the assay diluent mixed with the biomarker, and then it tends to change with the increasing concentration of saliva. As higher the concentration of saliva, the lower the hue value of the biomarker. Thus, for the clinical trial, 20\% diluted saliva was observed to be optimal as the effect of saliva is reduced and the lytic effect of the diluent buffer cleaves the SARS-CoV-2 N-protein out of the saliva sample to be reacted with the capture antibody.\textsuperscript{[48]} Figure 2c shows the assay result of the clinical trial of SARS-CoV-2 N-protein of different concentrations using 20\% diluted saliva that shows a linear response as the raw antigen test. Finally, the immunoassay result for pure SARS-CoV-2 N-protein and SARS-CoV-2 N-protein mixed with 20\% saliva were compared and was shown in Figure 2d. From the figure, it is seen that the immunoassay results are identical for both the tests which validate the applicability of the platform for COVID-19 diagnosis in clinical applications.

2.3. Visualization of IoD System Web App

The primary concept of the proposed IoD platform is to place it in different public places to help the health authorities to conduct a mass diagnosis among the residents during pandemics. But documenting the diagnostic reports from such decentralized wide locations is literally burdensome requiring voluminous manpower.

The introduction of IoT features in the proposed IoD platform eases the challenges and facilitates automated and real-time diagnostic data acquisition and data transfer to cloud data storage. If someone is diagnosed with SARS-CoV-2 N-protein biomarker above/equal to the LOD value, then that person will be marked as COVID-19 positive when uploading the patient data to the server.

As seen from Figure 3a, when someone launches the IoD web application, it fetches the city-wise filtered data from the cloud data storage, plots the COVID-19 positive patient count on the pop-up boxes in each city, and set the tinting color parameters for each city in the choropleth map comparing with the

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Performance characterization of AI-image recognition. Here, a) accuracy, b) specificity, c) precision, d) sensitivity, e) false acceptance rate (FAR) and false rejection rate (FRR), and f) F1 score for a set of 100 positive and 100 negative images have been tested at different threshold values.}
\end{figure}
predefined threshold values representing the severity of disease spread. As shown in Figure 3b, if some new patients have been diagnosed in any city, the database is updated instantly with new patient counts and changes the patient count on the map in real-time. If a patient has been diagnosed previously with COVID-19 positive and diagnosed again with negative later on, then the patient count is being reduced with new results and thus a real-time overview of the current severity of the infection during a pandemic can be acknowledged by healthcare authorities and other users.

3. Discussion

Even though the RT-PCR test is highly specific, there is still a small chance for someone to issue a false positive, and several such disputes have also been reported\[50,51\]. The false-positive results may come up from laboratory errors such as off-target reaction and cross-contamination during manual operation during the test. Although some alternatives have been introduced to address this issue, a comparative assessment of the performance for SARS-CoV-2 detection using these current assays is challenging because of using different targets of SARS-CoV-2 RNA and the variability in their operational design to execute immunoassay. Above all, there are no standard guidelines for the validation of different assay protocols for the detection of SARS-CoV-2. Therefore, a performance comparison among various assay procedures together with our proposed methods in terms of sample type, assay time, and LOD has been shown (Table S1, Supporting Information). As shown in the table, our proposed method shows improved performance in the detection of SARS-CoV-2 compared to the other assay methods.

4. Conclusion

In this work, we investigated a multifunctional IoD system for convenient, fast, and automated diagnosis of contagious diseases evolving such as COVID-19 and quantify the severity of the disease. The device can execute immunodiagnostics for COVID-19 detection from saliva and add the test result in a cloud DB in real-time minimizing the hassle of the healthcare officials for data collection. Moreover, in striking contrast, the system can represent the patient count survey extensively by visualizing a choropleth map with live patient count feedback.

5. Experimental Section

Operational Mechanism of the Device: The conceptual demonstration of the proposed IoD system was shown in Figure 4a–e. The developed IoD microfluidic platform performed the COVID-19 immunoassay automatically by detecting the empty and filled reaction chamber using the developed AI-powered fluid detection application in the Raspberry Pi and analyzed the colorimetric assay outcome. The proposed IoD system had consisted of i) an IoD microfluidic platform including...
a linear actuator and a roller bar, ii) a 96-well LOC device along with a silicone pump, and valve, and iii) a cloud database (DB) storage. All the constituents of the IoD platform were accommodated into an enclosure made of polymethyl methacrylate (PMMA) sheet. To explicate the immunoassay procedures executed by the 96-well LOC device of the platform at first the washing buffer and TMB substrate were loaded into the reagent reservoir of the LOC device and then the diluted saliva sample containing SARS-CoV-2 antigen was applied to the detection antibody and enzyme coated sample pad was placed on the LOC device that made an antigen + detection antibody + enzyme conjugate (Figure 4a,b). After that, the start button was pressed on the touchscreen to initiate the ELISA procedure that started the detection of the fluid filling/emptying in the reaction chamber using AI-based image recognition and operated the linear actuator according to the predefined assay protocol (Figure S1, Supporting Information). The linear actuator was attached to the LOC device containing the slidable stage and was capable of moving the stage under the roller bar. Eventually, the silicone-made pump and valve were pressed and released accordingly by the roller bar for controlling the fluid movement for filling/emptying the reaction chamber sequentially with the conjugated saliva sample, washing buffer, and TMB substrate. Detail structure of the platform was depicted in the “IoD Platform Preparation” section. The solution loading mechanism in the reaction chamber was further described (Figure S2, Supporting Information).

When the reaction chamber was loaded with the saliva sample, it reacted and was captured by the coated capture antibody inside the 96-well microplate and made (antigen + detection antibody + enzyme) + capture antibody complex (Figure 4c). After that, the reaction chamber was washed by flowing the washing buffer solution through the reaction chamber. Finally, the reaction chamber was loaded with TMB substrate, and the post-assayed hue value was measured to quantify the assay result (Figure 4d). The assay result was then sent to the server to typify the assay result and was stored in the cloud DB storage (Figure 4e). If the individual sample was tested positive, then it was added to the total COVID-19 affected patient counter with regarding patient’s information (e.g., patient’s ID, patient’s cities/locations, test results, etc.). In addition, a choropleth map web application was associated with the counter DB as given in the “Visualization of IoD system web app” section visualizing the patient’s count for each city using toasts with a dynamic tinting appearance throughout the city map area demonstrating the severity of the disease propagation. The application layout could be accessed using a web browser. From the developed web application, it was also possible to search for individual’s test results by using the patient’s ID. If a COVID-19 positive patient was tested negative after curing using the IoD system, then the platform automatically reduced the patient count, and the choropleth map was then updated accordingly.

**Design and Implementation of AI-Implemented IoD System:** The IoD had interrelated the diagnosis of diseases with the internet utilizing the internet-of-things (IoT) as the backbone. Here, Figure 5a,b shows the workflow and architecture of the proposed IoD platform. As shown in Figure 5a, the developed IoD system had uploaded the COVID-19 test result to the DB in the cloud data storage. When a user was entered into the IoD web application, the web server fetched data from the DB in cloud data storage, filtered the data by patient’s locations, and projected the patient counts on each city in the IoD map with corresponding tinting color representing the severity of the disease propagation. Figure 5b explains the implemented IoD gateway scheme architecture that included an edge physical layer, transport layer, cloud data storage, and application layer. Figure 5b extensively outlines the designed...
gateway scheme and middleware used in this IoD-based biomedical application.

The edge layer for immunodiagnostics was comprised of a physical layer and the edge gateway scheme. A Raspberry Pi 4 minicomputer associated with a camera module and the linear actuator had been employed as the physical layer, which could execute immunodiagnostics and analyze the assay results in real-time. The monitoring layer within the edge gateway had received the results and the pre-processing layer filtered the results before assigning them to the different temporary variables in the storage layer. The security layer assured a secure pathway to upload the result to the cloud data storage over the transport layer using MySQL Open Database Connectivity (ODBC). A detailed connection diagram within the edge layer was shown (Figure S3a, Supporting Information). In the transport layer, a secure and stable 30 Mbps internet connection had been provided to the IoD platform over Wi-Fi using the Raspberry Pi 4 built-in WLAN connectivity.

Personal cloud storage had been set up on a Linux OS-based personal mini server PC (ProLiant MicroServer Gen10, HP Enterprise, USA). This PC was the main web server of the system and was switched to the main internet gateway using the port forwarding method from the internet router. A MySQL database had also been configured as the Amazon RDS in the server PC. This server PC had been employed as the main cloud storage of the IoD system. For the data visualization in the application layer, the Python Django environment had been installed in the server PC that had been configured as the EC2 environment of the Amazon Web Service (AWS).

The backend had been developed using the MVT-based Python Django framework and had been linked with the MySQL database created in the personal cloud storage over the network layer using a security layer (Django ORM). For better data management, an API had been created using Django REST framework-based API gateway that had used a gateway scheme like Amazon’s HTTP signature scheme providing a high level of availability, privacy, security, and robustness. An AJAX layer had been created to communicate with the IoD platform user interface using the JavaScript AJAX library that had translated Semantic Web data into machine-readable data. The Scalable Vector Graphics (SVG) tags had been occupied to visualize the country map and the data processing and map color-changing algorithm had been developed using native JavaScript. COVID-19 IoD dashboard connection structure with API and DB was shown (Figure S3b, Supporting Information). The developed web application had been deployed to the personal webserver. When a user entered the web app, it fetched the patients’ information from the server database and visualized the user interface on the user’s browsing device.

Preparation of Silicone Pillar and Valve Integrated Pump: The silicone pillar containing the reaction chamber on the top of the pillar for a single 96-well capping (Figure 6a) and the mechanical micropump with an in-built valve was designed using Rhino 3D software. Then, the design had been translated into a mold by a 3D printer that had been used for making silicone-made pillars and micropumps with an in-built valve.
Preparation of LOC Device: The LOC device included a sample inlet/outlet, a reagent reservoir, and the silicone-made micropillar accommodating reaction chamber, as shown in Figure 6b. The inlet/outlet, reagent reservoir, and holes for the micropillar were designed using Corel Draw 2021 and made on a transparent PMMA sheet of 3 mm thickness that served as the upper layer of the LOC device engraving by the laser machining (C30, Coryart Inc., Korea). After that, microfluidic channels were cut on a 200 µm clear type double-sided tape (Yon Woo Corp, Korea) using a paper cutter (Silhouette Portrait 2, USA) that was attached under the PMMA made the upper part maintaining the alignment. Finally, the bottom part of the chip was made using 100 µm clear PET film that was then attached underneath sealing the microfluidic channels/inlets from the bottom. At last, the pillar and micro-pump were attached to the chip using highly adhesive transparent double-sided tape.

Figure 5. IoD system implementation. a) Proposed IoD system workflow. b) IoD gateway scheme implemented based on edge gateway architecture.

Figure 6. Images of the real platform. a) The fixation of a 96-well piece with a silicone pillar separating it from the 96-well cartridge. b) Thermoplastic-made LOC device accommodating a silicone pillar with the 96-well plate and silicone actuator. c) Mechanical frame that connects the linear motor with the LOC device loaded stage. d) The proposed AI app-based ELISA platform.
tape (3M double-sided, Korea). The step-by-step LOC device-making procedure was shown (Figures S4a–i, Supporting Information).

Fabrication of the IoD Platform: As the immunoassay was carried out on the 96-well LOC device, the microfluidic operation to conduct the immunoassay was performed using a silicone-made pump with an in-built valve (Figure 6b). As shown in Figure 6c, a chip loading stage accommodating the LOC device connected with a linear actuator (Firgelli Technologies Inc., Canada) had been employed. A roller bar associated with the linear motor using bearing and shaft had moved over the silicone pump as previously reported.[34]

For controlling the linear actuator, a Raspberry pi 4 single-board mini-computer had been used as the main circuitry, and a Raspberry Pi camera module for capturing images for the detection of solution in the reaction chamber using AI. The detailed AI training procedure using the OpenCV machine learning-based Haar Cascade Classifier had been discussed (Figure S5 and Table S2, Supporting Information). For better visibility, an LED array associated with a relay module had also been installed inside the enclosure. The platform enclosure and LOC device stage for the IoD system had been designed using Corel Draw Graphics Suit 2021 and fabricated using PMMA sheets by laser machining (C30, Coryart Inc., Korea).[35] To cut the PMMA sheets, the laser machine was operated with a cutting speed of 10 mm s⁻¹ at 50% power, and the sheets were thus cut and glued together to prepare the enclosure that was shown in Figure 6d. A demonstration of the microfluidic operation of the platform was given (Figure S6 and Movie S1, Supporting Information).

For demonstration purposes, here color dye solutions of different colors were used. For the development of the AI-powered controlling software, OpenCV Haar Cascade Classifier had been occupied and was made using Qt Creator and C++ programming language. The software was then installed on the raspberry pi 4. The UI of the controlling software with the operational procedure was given (Figure S7, Supporting Information).

Preparation of SARS-CoV-2 N-Protein Antigen Sample: SARS-CoV-2 (COVID-19) N-protein antigen ELISA kit was purchased from RayBiotech, USA. The sample diluent provided with the ELISA kit was diluted fourfold using 1× distilled (DI) water. The Biotinylated detection antibody and HRP-streptavidin were prepared by 80-fold, and 100-fold dilution, respectively with 1× sample diluent. The washing buffer was prepared by diluting it 20-fold with DI water. The Biotinylated detection antibody and HRP-streptavidin were then mixed at a 1:1 ratio and incubated at room temperature for 120 min. This mixture was then used to conjugate antibodies on a glass fiber membrane filter at 4°C overnight. This filter had been used underneath the cellulose filter papers on the sample inlet of the LOC device. The standard stock solution of 200 ng mL⁻¹ sample antigen reagent was prepared using 600 μL 1× sample diluent and then serially diluted at 1:2 ratios to prepare 50, 16.67, 5.556, 1.852, 0.617, and 0.206, and 0.069 ng mL⁻¹ samples. Again, the 0.069 ng mL⁻¹ sample was diluted at a 1:1 ratio to prepare 0.0343, 0.0171, and 0.0086 ng mL⁻¹ samples. Later, the 0.011 ng mL⁻¹ sample was prepared by mixing 5.56 ng mL⁻¹ and 16.67 ng mL⁻¹ samples. For preparing saliva samples, human saliva was collected and diluted at 10%, 20%, 30%, 50%, 75%, and 100% concentrations using 1× sample diluent and used for serial dilution of the SARS-CoV-2 N-protein standard. For the saliva experiment, saliva from some healthy and sound persons was collected following local regulations and diluted with the antigen in different ratios mixing it with the assay diluent.

Statistical Analysis: Each concentration had been measured for n = 3 samples and the mean resultant hue values were plotted with error bars address ± standard deviation. The detection of outliers used the method of mean square error. If the hue values of certain concentrations exceeded three times the mean standard deviation, then these values were defined as outliers. If there were missing values or outliers for certain concentrations in one set of data, then those concentrations were redundant. However, no outlier data was found during the experiment. All the data were plotted and the LOD was validated using Microsoft Excel. The open-source IDEs such as “Qt Creator” and “Thonny” was utilized for app making and statistical performance analysis using C++ and Python 3.7 programming languages.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
N.H.B. and J.S.S. conceived the concept of this approach. N.H.B. designed the proposed LOC device and software and implemented the proposed method. N.H.B. collaborated in developing the software. N.H.B. carried out the experiments. J.L. collaborated while experimenting. N.H.B. and M.J.U. wrote the manuscript. N.H.B. prepared the data, and figures and wrote the review responses. J.S.S. critically revised the manuscript. J.S.S. supervised the entire project. All authors had read and approved the final manuscript.

Data Availability Statement
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Keywords
COVID-19, internet-of-disease, lab-on-a-chip, N-protein, point-of-care

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