IoTs and Loop-Mediated Isothermal Amplification-Based Biosensing using Cloud-Enabled Features

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***Abstract*—Internet-of-Things technology (IoTs) have accelerated biosensor applications in all fields. Loop-mediated isothermal amplification (LAMP)-based biosensor technologies in conjunction with smartphone detection have been adequate to cover the demands of mobile diagnostics. The ease of use, affordability, portability, high sensitivity, flexibility, and specificity demands of point-of-care detection can be achieved by low-cost electronic components, 3-dimensional printing technologies, capturing images of calorimetrically detected readouts made our system a promising approach for real-time point-of-detection in the field. In this study, we implemented a cloud service to our LAMP-based biosensor. We previously performed bacteria detection using colony-based LAMP device and now distributed the optical readouts of the assay using smartphones. We transferred the obtained image and results of the assays through cloud. Our user-friendly interface simplifies the data processing, it directly digitized the readouts and eliminates the need of data interpretation.**

Keywords— biosensor; loop-mediated isothermal amplification; mobile; smartphone; analytical, cloud

I. INTRODUCTION

Development of new methods in biology and computer sciences enhanced the innovations in biosensor technologies. Implementation of cloud technology and Machine Learning Algorithms improved data storage, analyses and sharing capacity [1-4]. Besides, employment of smartphones has been accelerated the processes and provided user-friendly interfaces [5]. Interpretation of analyses results either can be remotely performed by experts and the final report is sent to the end-user or simply results do not require any interpretation. Therefore, know-how or experience has not been a bottleneck.

Along the advancement of data analytics, storage, and distribution, biological assays have been also developed. More robust, more sensitive, more specific assays have been achieved using low-cost, high-throughput, and automated processes outside the laboratories [6-9].

In nucleic acid research, invention of LAMP method has been a milestone that allowed amplification of nucleic acids at single temperature for 30 minutes without requiring rigorous sample preparation or robust reaction conditions [9]. Hence, LAMP techniques have been used for detection and diagnosis of several organisms in field, in clinics and in resource limited settings [6,7,10-14].

When these LAMP-based detection techniques [6-14] have been used with smart phones and IoTs, their assay readouts have been rapidly, reliably, and securely distributed among the users. Hence, molecular diagnostics could be combined with the spatiotemporal disease diagnostics [14-17].

Here, we implemented a smartphone application to digitalize and share the results of the colony LAMP reactions from field to central laboratories using OpenCV, Android Studio, Paho MQTT Client, Node-Red and MySQL platforms.

II. MATERIALS AND METHODS

A. Chemicals and cell culture

Bacterial strains of *Escherichia coli* (*E. coli,* ATCC10536) and *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC15442) were grown on Luria-Bertani (LB, Sigma-Aldrich Shanghai Trading Co., Ltd., China) agar plates at 37 °C for 17 h. The yaiO2 primer set was published in [6-9] and purchased from Oligomer® (Ankara, TURKEY). 2x WarmStart Colorimetric

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LAMP kit and Phusion High-Fidelity PCR kit were provided by New England Biolabs (MA, USA). Polydimethylsiloxane (PDMS) was ordered from Sylgard® 184 (Dow Corning, Midland, MI, USA).

B. LAMP device

We previously reported the LAMP device in [1], Figure 1. It consists of an aluminum sample holder, a thermally isolated mug, a lid, a temperature sensor, a resistor energized with a transistor, a display for real-time monitoring of the status of the process such as temperature and remaining time, and a 4x4 PDMS microwells (6.25 cm^2), and a printed circuit board and electronic components.

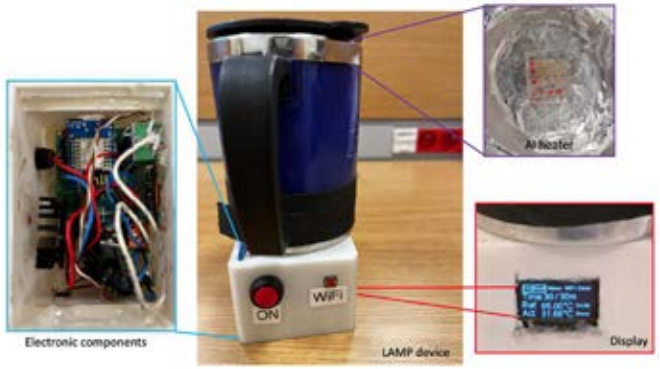

Fig. 1. LAMP device [1]

In the LAMP device, a microcontroller, ESP32 (Espressif), with Wi-Fi and Bluetooth capability is employed. A digital PI controller is utilized in the microcontroller to regulate the temperature of the aluminum sample holder using a 12 V power supply. The LM7805 reduces the voltage from 12 V to 5 V for energizing the ESP32 based module. The voltage of the resistance is controlled by Pulse Width Modulation (PWM) generated in the microcontroller. We used C++ algorithms for the device operation and temperature control. A SSD1306 OLED display is interfaced with the microcontroller over I2C protocol. In our system, the steady-state temperature error of the system remained less than %1 [1].

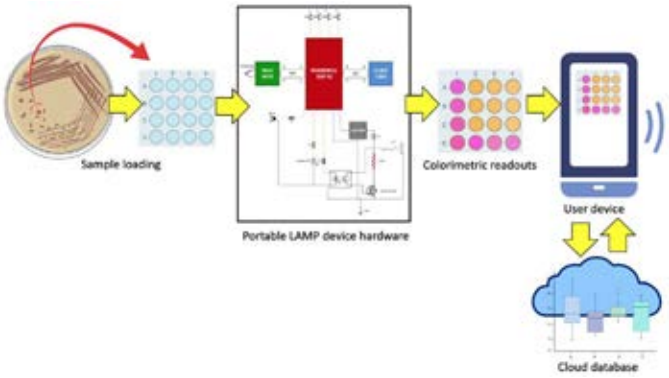

Fig.2. System architecture

The software of the system has an embedded software which is on the device and an event driven flow-based software which is on a PC or a single board computer. The embedded software makes the device independently run. The event driven flow-based software is a communication subroutine providing communication with a computer to record process temperature and to set the controller parameters such as reference temperature, temperature rise time and process duration. Communication between the device and the computer relies on Wi-Fi and MQTT protocol. The software on the computer is created in the Node-Red programming tool which is an open-source platform based on NodeJS. There is a MQTT broker on the computer which provides an interface between the software of the device and the Node-Red. Process data and parameters are transferred between the device and computer using JSON formatting on MQTT messaging.

C. Colony LAMP

We prepared a 25 µl reaction mixture containing Warmstart Colorimetric LAMP 2X Master Mix, 1X primer mix consisted of 3 primer pairs (1.6 µl, FIP and BIP, 0.2 µl, F3 and B3, 0.4 µl, LoopF and LoopB), 8 µl nuclease free water, a colony picked by a sterilized inoculating loop from the bacterial plate and directly transferred into the LAMP reaction mixture. The pH of the reaction mixture was adjusted to 8.8 with Tris-HCl in the PDMS microwells. The colony LAMP reaction was performed at 65˚C for 30 minutes. The results of the colony LAMP reactions were captured by a smartphone camera and the reactions were determined by the color change under the day light, while the positive reactions were in yellow (~orange), the negative ones were in pink colors.

D. Cloud and Web Application

The experiment results were detected and stored using the OpenCV, Android Studio, Paho MQTT Client, Node-Red and MySQL platforms. After the LAMP experiment, the experiment results were needed to be detected and stored. Using OpenCV, which is an open-source computer vision and machine learning library, first, the input test images were converted from BGR (blue, green, red) to HSV (hue, saturation, value). Next, the desired colors were searched in the detected district contours. When the colors were detected, they were labelled as positive for orange colors and negative for pink colors in the images.

In this library the contour and color detection were randomly performed, therefore neither the positive nor the negative results of the experiments were known (to eliminate bias). When the detection was performed, to address the readouts with the detected samples, we added a new feature to our code. First, we stored all the x and y values of the coordinates of the contours with their test results in an array. Next, we applied a sorting algorithm to determine the positive or negative test results. Finally, we saved this information in an array. To convert this process into a more user-friendly form, we developed a mobile application, namely LAMP App, via Android Studio software. In the mobile app, there are two pages; one is login page and the other one is the test analysis and sending page. The user first logs in to the mobile app and in the opening page, captures the images of the wells or chooses previously taken images and uploads it. Next, he or she clicks to "ANALYSE" button and triggers our OpenCV based algorithm which is embedded inside the app. Once the detection process ends, in the same page the result image and the result

array are shown. This enables the "SEND" button and user clicks to it for sending these outputs to the server.

At the server side, the output image and array data from the mobile app were converted to buffer arrays which was required for MQTT Communication. Then, these two arrays were sent to Node-Red tool through Paho MQTT Client. We developed a Node-Red Dashboard and showed the input user, test date and time, and result values on a table. Also, we put the processed input image on the dashboard. When this process happens a new test detail info is stored into the database via using MySQL. So that the data are protected.

III. Results and Discussion

Differently from previously reported studies in the literature, here, we implemented cloud service. We conducted the colony LAMP reactions as reported in [7]. We used both *E. coli* and *P. aeruginosa* bacterial colonies in the experiments. The yaiO_2 primers designed specifically to amplify the *yaiO* gene region across diverse lineages of *E. coli*, but not in *P. aeruginosa*. Therefore, the positive readouts present the amplicons of the *yaiO* gene only in the colony LAMP reactions using the *E. coli* colonies. The color of the positive LAMP reactions become orange, Figure 3a. The results of the colony LAMP using the *P. aeruginosa* colonies were pink, which indicates the negative readouts [7]. In this study, we implemented a smartphone application to digitalize and share the results of the colony LAMP reactions from field to central laboratories, Figure 3c.

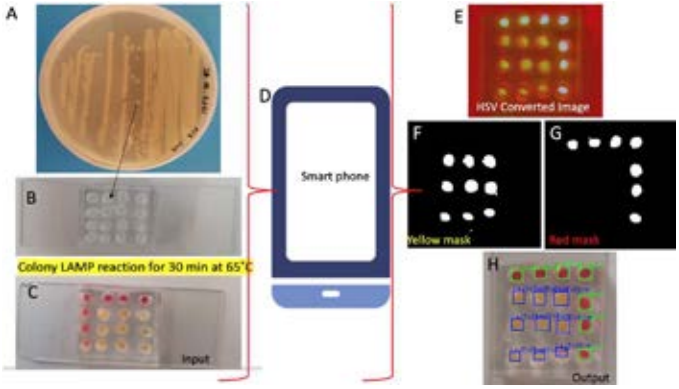

Fig.3. LAMP assay in the device [1]. Single colony is picked A) and placed in a microwell in the PDMS slab. LAMP reaction wells before B) and after C) the LAMP reaction is performed. D) Images by smart phones, E) HSV converted images, F) Yellow and G) Red masks were implemented, H) Output.

First, the user runs the "LAMP App" and is asked to be logged in, Fig.4a. Next, the image of the PDMS slab captured by a smartphone camera, Fig.3c, Fig.4a. When the analysis is performed, the image is processed using OpenCV algorithm to identify the color difference between the orange and the pink wells. To create the address of the reactions, the coordinates of the wells were stored in an array. To clearly distinguish the wells, the pink wells were contoured by a green boarder while the orange ones were blue. Afterwards, the readouts were digitally shown, "1" stands for the positive results, while "0" indicates the negative results. The user ID, date and time of the analysis, corresponding analysis results, and the image of the readouts are stored in the cloud database, Fig. 4b. Besides, using this application, previously stored images of the analysis results can be reviewed, reanalyzed, and sent to other users. The analyzed and sent test results were stored in the cloud service.

Fig.4. Mobile application with data visualization. A) LAMP application interface on a smartphone, B) Log in, C) Capture Image, D) Analyze, E) Send, F) Overview of the stored readouts.

When different color indicators in the LAMP reactions are used, the setting parameters of the defined colors can be adjusted.

In the previous system, data was collected and stored manually [6,7]. When the data will be large, collecting and storing this data can be a problem. As a solution to this problem, a mobile application and an additional cloud system are proposed. Advantages over the previous system are as follows: collecting data with a user-friendly application, storing this data in a cloud environment, sorting these collected results by date, and thus performing the analysis of the results more efficiently.

IV. Conclusion

This paper presents one of the best candidates for a portable, smart, and cloud-based data delivered LAMP-based biosensor technologies. The contribution of this study is to develop a system that can immediately deliver the obtained test data for different types of assays. Here, we implemented our communication algorithm for the data obtained in our previous study where bacteria detection was performed using the LAMP reaction. However, several different LAMP-based nucleic acid detections can be performed in our system.

Our easy-to-use interface simplifies the data processing, it directly digitized the readouts and eliminates the interpretation of the data. It allows easy access to tested samples, both original and processed data are saved on database. It can be accessed by smartphones and people without any expertise can easily use it. The cloud service allows historical record of the analysis results and provides subsequent download. The user and his/her measurements were automatically added.

The developed biosensor technology opens the doors of the laboratories into the field. Not only the assays become laboratory-independent, but also analysis of the data and sharing results become reliable, rapid, easy, and user-friendly.

Our future goal is to implement a machine learning algorithm to determine different test results using different samples or colorimetric readouts. Moreover, we plan to quantify the readouts to determine the density of the samples. Also, a calibration process can be integrated into our system.

References

[1] V. Bianchi, A. Boni, M. Bassoli, M. Giannetto, S. Fortunati, M. Careri, I. De Munari, "IoT and Biosensors: A Smart Portable Potentiostat With Advanced Cloud-Enabled Features," IEEE Acess, vol. 9, pp. 141544-14154, October 2021.

[2] A. Boni, V. Bianchi, A Ricci, I. de Munari, "NB-IoT and Wi-Fi technologies: An integated approach to enchance portability of smart sensors," IEEE Access, vol.9, pp. 74589-74599, 2021.

[3] Y. Yuan, S. Feng, M. Alahi, A. Nag, N. Afsarimabesh, H. Zhang, and S. He, "Development of an Internet of Things based electrochemical microfluidic system for free calcium detection," Appl., Sci., vol. 8, pp. 1347, Aug. 2018.

[4] D. Verma, K. R.B. Singh, A.K. Yadav, V. Nayak, J. Singh, P. R. Solanki, R.P. Singh, "Internet of Things (IoT) in nano-integrated wearable biosensor devices for healtcare applicatios," Biosensors and Bioelectronics: X, vol. 11, pp. 100153, May 2022.

[5] D. Zhang, Q. Liu, "Biosensors and bioelectronics on smartphone for portable biochemical detection," vol. 75, pp. 273-284, 2016.

[6] S.V. Kaymaz, A. F. Ergenc, A.O., Aytekin, S. J. Lucas, M. Elitas, "A low-cost, portable, and practical LAMP device for point-of-diagnosis in the field," Biotechnology and Bioengineering, vol. 119, pp. 994-1003, 2022.

[7] D. Kaygusuz, S.Vural, A. O. Aytekin. S. J. Lucas, M. Elitas, "DiamonDNA: A portable, low-cost loop-mediated isothermal amplification platform for naked-eye detection of genetically modified organisms in resource-limited settings," Biosensors and Bioelectronics, vol. 141, pp. 111409, 2019.

[8] W. Jaroenram, P. Cecere, P. P. Pompa, "Xylenol organe-based loop mediated DNA isothermal amplification for sensitive naked-eye detection of *Escherichia coli*," Journal of Micorbiological Methods, vol. 156, pp. 9-14, 2019.

[9] T. Natomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanable, N. Amino, T. Hase, "Loop-mediated isothermal amplification of DNA," Nucleic Acid Research, vol. 28, pp. e63, 2000.

[10] S. Li, Y. Liu, Y. Wang, H. Chen, C. Liu, Y. Wang, "Lteral flow biosensor combined with loop-mediated isothermal amplification for simple, rapid, sensitive, and reliable detecion of *Brucella spp*," Infect Drug Resist. Vol. 12, pp. 2343-2353, 2019.

[11] A. Ganguli, A. Mostafa, J. Berger, M. Y. Aydin, F. Sun, S. A. S. de Ramirez, E. Valera, B. T. Cunningham, W. P. King, R. Bashir, "Rapid isotehrmal amplificaton and portable detection system for SARS-CoV-2," Proc Natl Acad Sci U S A, vol 117, pp. 22727- 22735, 2020.

[12] J. Rainbow, E. Sedlackova, S. Jiang, G. Maxted, D. Moschou, L. Richtera, P. Estrela, "Integrated Electrochemical Biosensors for Detection of Waterborne Pathogens in Low-Resource Setting," Biosensors, vol.10, pp. 36, 2020.

[13] M. Rezaei, S. R. Bazaz, D. M. Rad, O. Shimoni, D. Jin, W. Rawlinsin, M. E. Warkiani, "A Portable RT-LAMP/CRISPR Machine for Rapid COVID-19 Screening," Biosensors, vol. 11, pp. 369, 2021.

[14] M. Zhang, J. Liu, Z. Shen, Y. Liu, Y. Song, Y. Liang, Z. Li, L. Nie, Y. Fang, Y. Zhao, "A newly developed paper embeded mcirochip based on LAMP for rapid multiple detections of foodborne pathogens," BMC Microbiol., vol. 21, pp. 197, 2021.

[15] L. Kong, H.-b. Wang, S.-s.Wang, P.-p. Xu, R.-f. Zhang, S. Dong, X.-b. Zheng, "Rapid detection of patato late blight using a loop-mediated isothermal amplification assay," Journal of Integrative Agriculture, vol. 19, pp. 1274-1282, 2020.

[16] K. Yin, V. Pandian, K. Kadimsetty, X. Zhang, C. Ruiz, K. Cooper, C. Liu, "Real-time Colorimetric Quantitative Molecular Detection of Infectious Diseases on Smartphone-based Diagnostic Platform," Scientific Reports, vol. 10, pp. 9009, 2020.

[17] J. Song, V. Pandian, M. G. Mauk, H. H. Bau, S. Cherry, L. C. Tisi, C. Liu, "Smartphone-Based Mobile Detection Platform for Molecular Diagnostics and Spatiotemporal Disease," Analytical Chemistry, vol. 90, pp. 4823-4831, 2018.