A low-cost, portable, and practical LAMP device for point-of-diagnosis in the field

Sumeyra Kaymaz[1], Ali Ergenç [2], Ali Aytekin [3], Stuart Lucas[1], and Meltem Elitas[1]

[1]Sabanci University
[2]Istanbul Technical University
[3]Yeditepe Universitesi

November 15, 2021

Abstract

Transition of rapid, ready-to-use, and low-cost nucleic acid-based detection technologies from laboratories to points of sample collection has drastically accelerated. However, most of these approaches are still incapable of diagnosis starting from sampling, through nucleic acid isolation and detection in the field. Here, we developed a simple, portable, low-cost, colorimetric, and remotely controllable platform for reliable, high-throughput, and rapid diagnosis using loop mediated isothermal amplification (LAMP) assays. It consists of a thermally isolated cup, low-cost electronic components, a polydimethylsiloxane sample well, and a fast prototyped case that covers electronic components. The steady-state temperature error of the system is less than 1%. We performed LAMP, Colony-LAMP, and Colony PCR reactions using the yaiO2 primer set for Escherichia coli and Pseudomonas aeruginosa samples at 65@C and 30 min. We detected the end-point colorimetric readouts by the naked eye under day light. We confirmed the specificity and sensitivity of our approach using pure genomic DNA and crude bacterial colonies. We benchmarked our Colony-LAMP detection against Colony PCR. The number of samples tested can easily be modified for higher throughput in our system. We strongly believe that our platform can greatly contribute rapid and reliable diagnosis in versatile operational environments.

1. Introduction

Nucleic acid amplification is the first and main step for most gene-based detection methods and development of diagnostics technologies in life sciences. Polymerase chain reaction (PCR)-based nucleic acid amplification was conceived by Kary Mullis in 1983 [1]. PCR provides DNA amplification from a specific region of a DNA strand using a thermal cycler. It consists of 20-40 repeated thermal changes with discrete temperature steps (50 @C – 98 @C). Mostly, PCR reactions are carried out within a few hours. Also, PCR amplification products are usually visualized by gel electrophoresis. Although the PCR method is the gold standard for nucleic acid detection and feasible for laboratory conditions, it is labor and time consuming, requiring bulky and expensive instruments and trained personnel. Therefore, it is not convenient for on-field deployment or resource-limited settings. Here, the main purpose is to make nucleic acid-based diagnosis at the point of sample collection, starting from nucleic acid isolation to detection, using an easy-to-use, portable, low-cost platform with a reliable, precise, and rapid method.

To achieve this goal, various methods have been developed since 1990 [2-8]. Among these methods, Notomi and co-workers' Loop-mediated isothermal amplification (LAMP) technique succeeded in performing nucleic acid amplification and detection at the point of sample collection [2]. In comparison to PCR, LAMP is a rapid (~ 30 min) and robust technique uses a constant temperature (~ 65 @C) instead of a series of alternating temperature cycles. It does not require highly pure DNA isolates. Hence, LAMP methods decrease cost, time and the need of expensive instruments or trained personnel. It has a great potential for breaking through

the walls of laboratories to fields [9,10,11], clinics [12,13,14], farms [15,16,17], crime scenes [18,19] or even excavation sites.

To perform reliable, rapid, and robust nucleic acid detection outside laboratories, LAMP methods have been optimized and user-friendly, portable, low-cost devices to provide stable LAMP reactions have been developed [10,20-25]. Most of these optimization studies focused on primer design to achieve high specificity [20-22] or visualization methodology to obtain sensitive readouts [10,23-25]. Readouts were mostly determined by spectroscopic detection of turbidity either by the naked eye or cameras. Although the LAMP reactions were conducted in a simple to use, low-cost, and widely available equipment such as water baths, heat blocks, or ovens, still these instruments are bulky, and their usage is not practical for the point of sample collection. Moreover, a trained person is often required for nucleic-acid extraction, preparation of LAMP reactions, and interpretation of readouts in some circumstances. To benefit from the rapid, reliable, robust, and easy-to-use features of the LAMP technique, portable, low-cost, user-friendly platforms which can combine nucleic-acid extraction, LAMP reaction, and readout of detection are still very limited.

The recent urgency for the detection and diagnosis of COVID-19 cases has accelerated the transition of diagnostic assays and technologies from laboratories to points of sample collection for rapid, reliable, and ready-to-use tests [23,26-28]. Papadakis and co-workers developed the real-time colorimetric LAMP system to be used for COVID-19 diagnosis from clinical biopsy samples [23]. However, this device was not convenient to be used in resource limited settings or by non-trained personnel, since it requires digital image analysis using the images acquired by smartphone cameras [23]. Likewise, development of fully automated, low-cost, rapid, and portable LAMP assays for detection of bacterial pathogens are still at their crawling phase. Yan's group focused on food safety and implemented LAMP assay for bacterial culture and bacterial colonies of 116 strains of *Escherichia coli*(*E. coli*), *Salmonella typhimurium, Vibrio parahaemolyticus, Pseudomonas aeruginosa* (*P. aeruginosa*), and *Listeria monocytogenes* using laboratory equipment [14]. They achieved LAMP amplification of target DNA without any DNA extraction and purification from bacterial colonies or cultures. For laboratory-free LAMP reactions, Sayad and co-workers developed a centrifugal microfluidic platform to detect the genus *Salmonella*in spiked tomato samples [24]. They used a hot air gun to maintain constant temperature required for a LAMP reaction which might influence thermal stability of reactions and triggers non-repeatable results. Therefore, their method was not fully automated, rapid, reusable, or low-cost, in other words convenient for field studies. A comparable study was performed by Seo and co-workers. They used a colorimetric, high-throughput centrifugal LAMP microdevice for the multiplex pathogen detection using *E. coli* , *Salmonella typhimurium* , and*Vibrio parahaemolyticus* samples [25]. Like Sayad's development, fabrication, and operation of the centrifugal microdevice was very expensive and complex. Moreover, the entire genetic analysis on the microdevice was longer than 30 min (˜ 1 h). Boyle and co-workers developed a portable LAMP microfluidic device to detect*Streptococcus equi* subsp. *equi* in guttural pouch lavage specimens from convalescent horses [29]. Although this assay shows potential for field deployment, still DNA extraction requires standard laboratory protocols and laboratory equipment. Several microfluidic chips capable of generating and trapping emulsion droplets for digital loop-mediated isothermal amplification analysis have been developed [30,31]. The underlying motivation for these technologies were their low detection limits, however their complexity, high cost, and need of high-technical equipment are among their drawbacks.

Most of the studies cited above showed that simple, portable, low-cost, robust, and field-deployable devices to perform LAMP-based diagnosis are still limited [32]. To overcome these limitations, we developed a low-cost (˜ 32 Euro), portable (˜ 70 g), remotely controlled (wireless), real-time, colorimetric LAMP device. We integrated a proportional-integral (PI) controller to maintain isothermal conditions. We showed that our device successfully conducts LAMP reactions without a prior DNA extraction step. It provides naked-eye detection of bacterial amplicons during the amplification reactions. We confirmed specificity of our method using *P. aeruginosa* colonies and its genomic DNA with yaiO_2primer set. We characterized the sensitivity of our system using*E. coli* and *P. aeruginosa* colonies in different sizes (1 mm – 2.5 mm) in the Colony LAMP and Colony PCR reactions. Moreover, we prepared serial dilutions of genomic DNA (1%, 10%, 100%) for *E. coli* and *P. aeruginosa* cells and performed conventional LAMP assays. In this study, the LAMP device is

tailored for Colony-LAMP reactions, however, it has a great potential to be used for rapid, reliable, simple, and high-throughput diagnosis in versatile operational environments.

2. Experimental section

2.1. Chemicals and reagents

Luria-Bertani (LB) medium was supplied by Sigma-Aldrich Shanghai Trading Co., Ltd. (Shanghai, China). 2x WarmStart Colorimetric LAMP kit and Phusion High-Fidelity PCR kit was provided by New England Biolabs (MA, USA). Bacterial strains of *E. coli* (ATCC10536) and *P. aeruginosa* (ATCC15442) were used. DNAzol® Reagent and Tris-HCl were purchased from ThermoFisher Scientific (Hayward, CA, USA). GelRed nucleic acid stain was ordered from Biotium (Hayward, CA, USA). Polydimethylsiloxane (PDMS) was ordered from Sylgard® 184 (Dow Corning, Midland, MI, USA). The yaiO2 primer set was purchased from Oligomer® (Ankara, TURKEY) [22].

2.2. Bacteria culture and genomic DNA extraction

Colonies of *E. coli* and *P. aeruginosa* cells were grown on LB agar plates at 37 degC for 17 h. A single colony was selected from the plates and inoculated into 5 ml LB broth medium for 16 h. Next, 1-ml culture was serially ten-fold diluted in distilled water and utilized for colony-forming unit (CFU) assay by the standard spread-plate technique.

In the genomic DNA extraction protocol, first suspension of cells was pelleted at 5000 g for 10 min. Next, the pellet was re-suspended in 1 ml of DNAzol(r) Reagent and incubated for 15 h at room temperature. Next, the samples were boiled at 10 min, and 500 µl of 100% Isopropanol were added with vigorous mixing. Then, samples were placed on ice for 10 min before being centrifuged at 12,000 g for 10 min. The DNA pellet was washed with 70% (v/v) ethanol, air dried and dissolved in 300 µl of DNase-free water. Next, we incubated the DNA samples at 65 °C for 10 min. Quality and concentration of the DNA samples were measured by spectrophotometric analysis at 260 and 280 nm.

2.3. LAMP assay using genomic DNA

LAMP reaction was performed in a 25- µl reaction mixture containing WarmStart Colorimetric LAMP 2X Master Mix, 2 µl DNA template, 8 µl nuclease free water, 1X primer mix. The LAMP primer mix consisted of 6 primers: two inner primers (1.6 µl, FIP and BIP), two outer primers (0.2 µl, F3 and B3), two loop primers (0.4 µl, LoopF and LoopB). The yaiO2 primer sequences were published in [22]. LAMP reactions were performed at 65 °C for 30 min. DNA integrity was checked using 1% agarose gel containing GelRed nucleic acid stain in 0.5X Tris-Borate-EDTA (TBE) buffer. Gel electrophoresis was run in the Mupid-One (Seraing, Belgium). Gel images were acquired using the BioRad GelDoc EZ Imaging Systems (California, USA).

2.4. Colony-LAMP assay

For the Colony-LAMP reaction, Warmstart Colorimetric LAMP 2X Master Mix, primer mix, and nuclease free water were prepared in a 25-µl volume. A colony was picked with a sterilized inoculating loop from the bacterial plate and directly transferred into the LAMP reaction mixture. We adjusted the pH of the reaction mixture to 8.8 with Tris-HCl in the microwells of the PDMS slab. Then, the PDMS slab was transferred into the LAMP device. The reactions were performed at 65 °C for 30 minutes. Results of 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.

2.5. Colony PCR reactions

We used the Phusion® High-Fidelity PCR Kit for the Colony PCR and followed the manufacturer's protocols. We carried out the LAMP reactions using the F3 and B3 primers. The medium-sized colonies (1.5 mm – 2 mm) were selected from the plates and transferred into the reaction mixture. Colony PCR was performed using a thermal cycler (Master cycler 384, Eppendorf AG, Hamburg, Germany). Thermocycling conditions

were set: 98 degC 30 s followed by 30 cycles of 98 degC 10 s, 56 degC and 72 degC 30 s, and final extension was at 72 degC 10 min. The PCR products were observed using 1.5 % agarose gel electrophoresis (Amplicon: 240 base pairs (bp)).

2.6. LAMP device

We developed a portable, microcontroller-controlled LAMP device consisting of affordable electronic and physical components. Aluminum sample holder, which is heated using a resistor, is housed in a thermally isolated mug and its lid (main body). Temperature is measured by a temperature sensor, and resistor is energized with a transistor. A display is utilized for real-time monitoring of status of the process such as temperature and remaining time of the LAMP reaction,**Figure 1** .

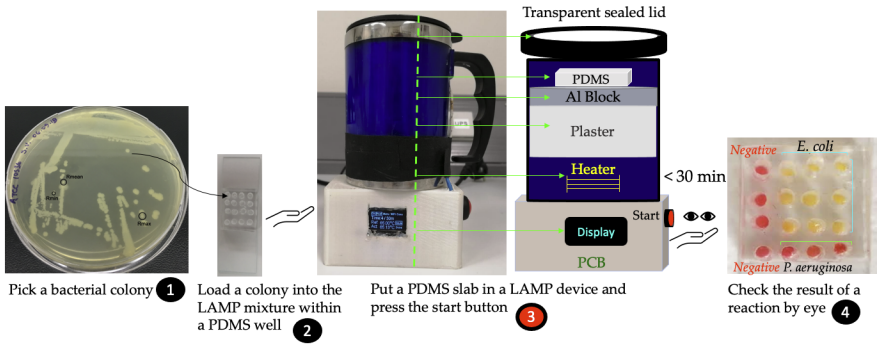


Figure 1. LAMP device and Colony LAMP in the device

The thermally isolated mug maintains the temperature (Length: 11 cm, radius: 7.5 cm, cost: 9 Euros). We designed a casing that houses the printed circuit board and electronic components using SOLIDWORKS software and manufactured it using a 3-dimensional (3D) printer and Acrylonitrile Butadiene Styrene (ABS) filament (Length: 5 cm, width: 9.5 cm, depth: 6.5 cm). Total weight of the device was 70 g. We made an array of PDMS microwells where LAMP reactions occurred (PDMS, Curing reagent (1): Base (10)). We cured PDMS in a petri dish at 75*C for 60 min. We punched 4x4 wells (6.25 cm^2) using a 2.5-mm biopsy puncher (Robbins Instruments, Chatham, MA, USA). The number of wells can be increased or decreased according to required number of samples (Total area: 176.7 cm^2). A PDMS slab was irreversibly bonded on a glass slides using a Corona system (BD20-AC, Electro-Technic Products Inc.). The components of the LAMP device with the steps of the experiment are shown in **Figure 1** . The principal block diagram for the circuit of the system is illustrated in **Figure 2.**

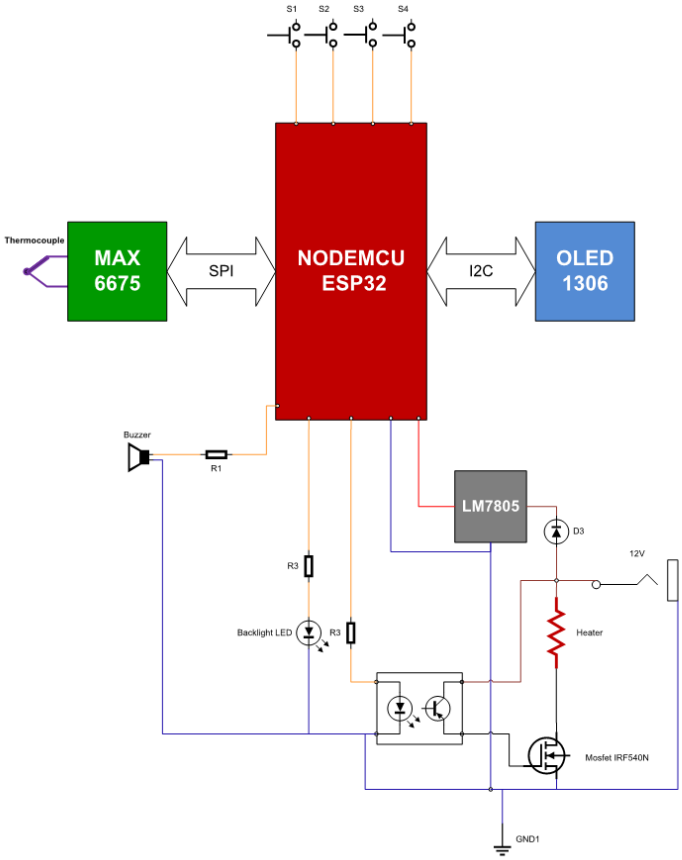


Figure 2 . Principal circuit diagram of the LAMP device. Max6675: Thermocouple Interface, NODEMCU ESP32: Microcontroller, OLED 1306: Display, LM7805: Voltage Regulator

A microcontroller, ESP32 (Espressif), with Wi-Fi and Bluetooth capability is employed as the controller. The temperature of the aluminum sample holder (Al block, **Figure 1**) is measured using K type thermocouple. The output of the thermocouple is processed by MAX6675 chip and transmitted to microcontroller via SPI interface. A 10 Ohm 15 Watts resistor is placed beneath the sample holder and the heating structure is surrounded by plaster for thermal insulation and integrity. The LM7805 reduces the voltage from 12 V power supply to 5 V for energizing the ESP32 based module. The resistor is energized by a mosfet transistor attached between 12V supply and resistor, IRF540, which is driven microcontroller insulated by an optocoupler. The voltage of the resistance is controlled by Pulse Width Modulation (PWM) generated in the microcontroller. A digital PI controller is utilized in the microcontroller to regulate the temperature of the system. A SSD1306 OLED display is interfaced with the microcontroller over I2C protocol,**Figure 1 - Figure 3** . It displays the reference temperature, the actual temperature of the sample holder, duration of the process, and the status of the process, **Figure 1** . The device supports Wi-Fi based internet of things (IoT) capabilities. The process parameters (reference temperature, heating temperature slope, duration of the process) and controller parameters may be adjusted. The process variables such as actual temperature and remaining time of the operation may be monitored and stored via web-based interface,**Figure 3** .

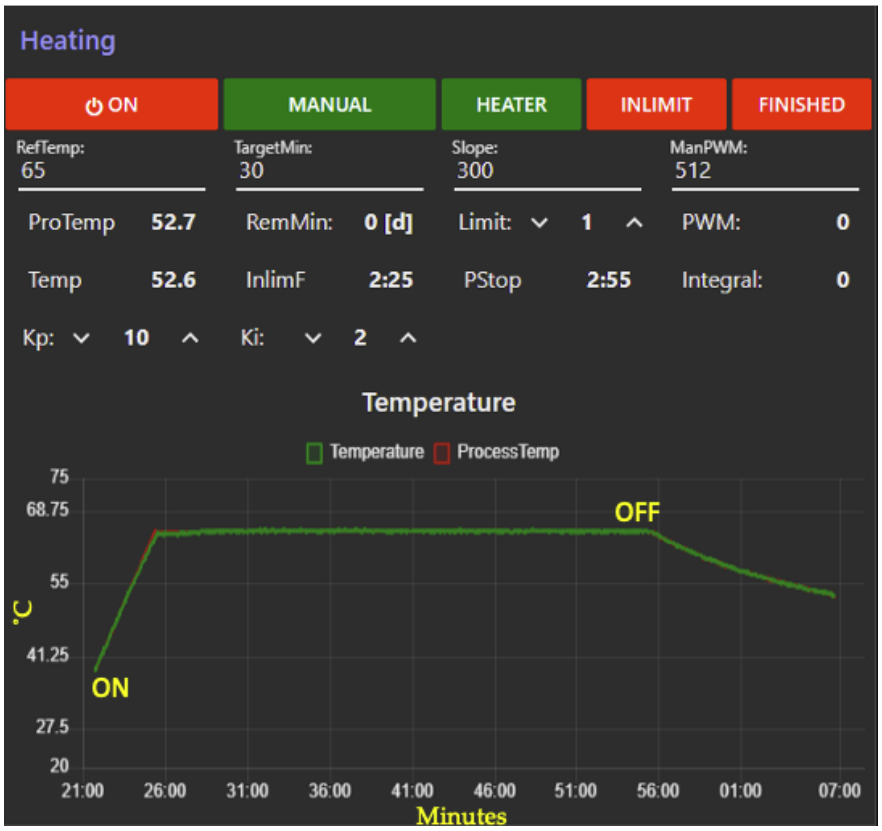


Figure 3 . Interface of the LAMP device through the server

Since the LAMP reactions were sensitive to thermal changes, the controller parameters of the system were adjusted to provide precise temperature control. The steady-state temperature error of the system remained less than %1, **Figure 3** .

3. Results and Discussion

Here, we conducted Colony-LAMP reactions directly using *E. coli*and *P. aeruginosa* bacterial colonies in our LAMP device. We used the yaiO_2 primers to amplify the *yaiO* gene region, which is conserved across diverse lineages of *E. coli* , but not in anther foodborne pathogen such as *P. aeruginosa.* We evaluated the performance of the device and compared the obtained Colony-LAMP results to a conventional LAMP assay that is performed in the device using genomic DNA extracted from bacteria, and a Colony PCR in the thermal cycler. All results were determined through real-time monitoring of the color change of the reactions by naked eye and confirmed by electrophoresis.

3.1 Colony LAMP

Figure 4 demonstrates the results of the Colony-LAMP assay using colonies in different diameters of *E. coli* : R_{min} [?] 1.437 mm, R_{mean} [?] 1.744 mm, R_{max} [?] 2.281 mm, and *P. aeruginosa* : R_{min} [?] 1.537 mm, R_{mean} [?] 1.853 mm, R_{max} [?] 2.012 mm (**Figure 4a**). Here, we picked the colonies, transferred them into the LAMP reaction mixture in the PDMS wells (4 x 4 wells), and carried out the LAMP reaction at 65 @C for 30 min. We monitor the color change of the LAMP reactions in real time through the transparent, sealed lid of the LAMP device (**Figure 1**). The *yaiO* genomic region was amplified in reaction wells where the *E. coli* colonies were transferred and the color change of these wells became yellow, **Figure 4b** . Whereas the LAMP reactions did not work for *P. aeruginosa* colonies. These reactions were pink in color as the negative

control (N) that does not contain any bacterial material. **Figure 4c** shows the ladder-like pattern bands for *E. coli* colonies but not for the *P. aeruginosa* colonies. Hence, specificity of our method was confirmed.

To evaluate the sensitivity of Colony-LAMP reactions, we performed three independent LAMP reactions on the same PDMS slab with the size of R_{min}, R_{mean}, R_{max} both for *E. coli* and *P. aeruginosa* strains. Since we developed the LAMP device mostly for field studies or resource-limited settings, we studied the sensitivity of our method according to size of the colonies. We picked colonies that a person can easily identify and pick, approximately 1 mm – 2.5 mm in diameter. The Colony-LAMP assay worked for all different diameter colonies of *E. coli* but not for the*P. aeruginosa* . Also, our result showed that we can run independent reactions in the same PDMS slab without any cross contamination.

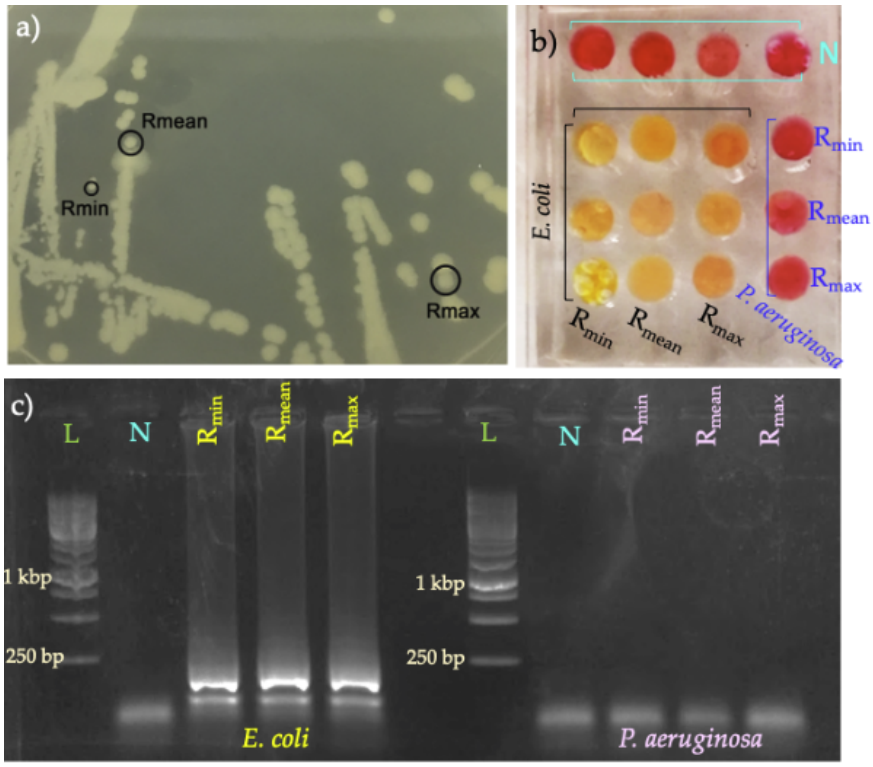


Figure 4 . Colony-LAMP assay for *E. coli* and *P. aeruginosa* colonies. **a)** Images of the colonies picked from*E. coli* plate. R: Colony diameter, *E. coli* : R_{min} [?] 1.437, R_{mean} [?] 1.744, R_{max} [?] 2.281 mm, *P. aeruginosa* : R_{min} [?] 1.537, R_{mean} [?] 1.853, R_{max} [?] 2.012 mm. **b)** Colorimetric Colony-LAMP reactions in the PDMS wells. Pink color indicates the negative, yellow color shows the positive results. Electrophoresis results for**c)** *E. coli,* **d)** *P. aeruginosa* colonies. L: DNA ladder, N: Negative control.

3.2 LAMP using genomic DNA

Figure 5 presents the sensitivity and specificity of the LAMP reactions using yaiO_2 primers and genomic DNA of bacterial strains. We set 3 concentrations with 1% dilution with 5.68×10^{10}, 50% dilution with 2.87×10^{11}, 100% with 5.34×10^{11} copy numbers for *E. coli* and 1% dilution with 6.82×10^{10}, 50% dilution with 2.72×10^{11}, 100% with 5.74×10^{11} copy numbers for *P. aeruginosa* (100% dilution [?] bacterial culture of a mean size colony). We conducted the LAMP assay with the same parameters used for the Colony-LAMP assay. **Figure 5a**demonstrates the LAMP reactions using genomic DNA in the PDMS wells. The first row of the PDMS slab were pink, which presents the negative controls both for *E. coli* and *P. aeruginosa* strains. All the wells belong to the dilutions of *P. aeruginosa* genomic DNA were also negative. The rest of the wells were in yellow color and containing the serial dilutions of the *E. coli* genomic DNA in triplicate. We

evaluated the results of the LAMP assay using electrophoresis, **Figure 5b** . We obtained the ladder-like patterns for all the dilutions of *E. coli* genomic DNA in the gel electrophoresis. We did not observe similar bands for the genomic DNA dilutions of *P. aeruginosa* , **Figure 5b** . LAMP assay using genomic DNA also confirmed the specificity, sensitivity of the LAMP method and robustness of the LAMP platform.

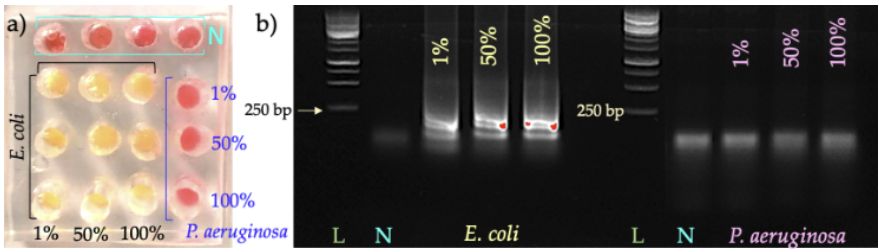


Figure 5 . Sensitivity and specificity of LAMP reactions using genomic DNA. **a)** Colorimetric LAMP reactions in the PDMS wells.*E. coli* cells: 1% = 5.68×10^{10}, 50% = 2.87×10^{11}, 100% = 5.34×10^{11} copy numbers. *P. aeruginosa* cells: 1% = 6.82×10^{10}, 50% = 2.72×10^{11}, 100% = 5.74×10^{11}copy numbers. Pink color indicates the negative, yellow color shows the positive results. **b)** Electrophoresis results for *E. coli*and *P. aeruginosa* strains. L: DNA ladder, N: Negative control.

3.3 Colony PCR

Finally, we performed a conventional Colony PCR using the F3 and B3 primers from the LAMP primer set. We selected medium-sized colonies of*E. coli* (R_{min} [?] 1.5 mm, R_{mean} [?] 1.8 mm, R_{max} [?] 2.5 mm) and *P. aeruginosa* (R [?] 2 mm) strains. The Colony PCR reactions were performed in thermal cycler for 80 min (Experimental section, *2.5. Colony PCR*). We evaluated the results using electrophoresis, **Figure 6** . The amplified*yaiO* gene sequence was 240 bp for *E. coli* samples, as expected, there was not any bands for the *P. aeruginosa* sample.

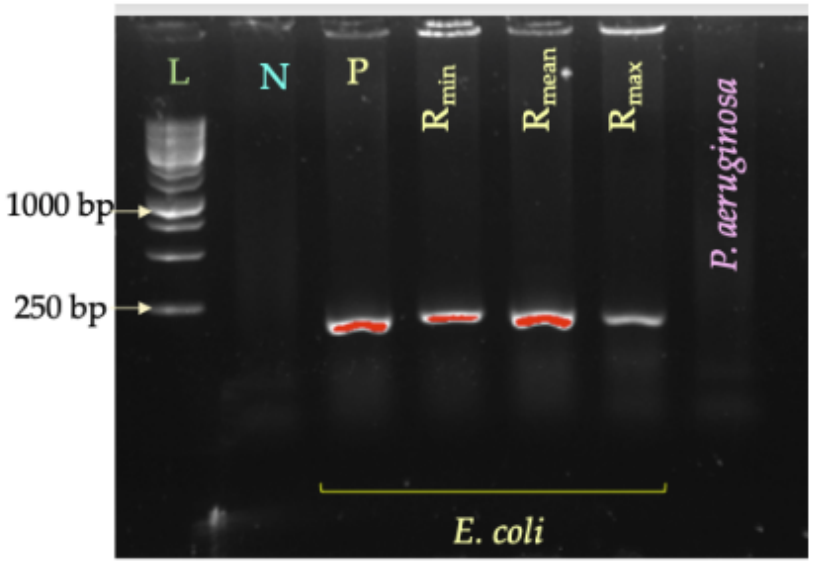


Figure 6 Sensitivity and specificity of Colony PCR reactions. L: DNA ladder, N: Negative sample, P: Positive sample, R: Colony diameter. *E. coli:* R_{min} [?] 1.5 mm, R_{mean} [?] 1.8 mm, R_{max} [?] 2.5 mm. *P. aeruginosa* : R [?] 2 mm.

Here, for the first time we presented a Colony-LAMP assay performed in the LAMP device that takes the samples and provides real-time colorimetric readouts in 30 minutes. While an early work by Yan and co-workers implemented a Colony-LAMP assay for 116 strains of food pathogens using analytical laboratory setups, this is the first work that developed a LAMP device capable of conducting both conventional and Colony-LAMP reactions [14]. Besides, this work is one of the first examples of field-ready modern LAMP device that is low-cost, portable, user-friendly, and robust to rapidly provide specific and sensitive readouts without false positives. In our previous studies, we developed a simple, low-cost, and portable LAMP device for GMO detection in the field [10]. Next, similar technologies have been developed to perform LAMP reactions either for COVID-19 diagnosis [23, 26-28] or food pathogen detection [24, 25, 29-31]. Still, there is a pressing need for a sensitive, robust, and reliable integrated method of nucleic acid extraction and detection on the same diagnostic devices. In other words, nucleic acid-based diagnostic technologies should be capable of utilizing the entire process from sample preparation to visualization of the results at once.

The major hurdles for on-site nucleic acid detection can be classified as method- and technology-based limitations. Most of the nucleic acid detection methodologies involves nucleic acid extraction and amplification steps requiring tedious liquid handling steps with high-cost reagents, specialized equipment, and trained technicians [1-8]. These hurdles limit their usefulness for field-based studies. LAMP technique has overcome majority of these limitations [2]. Improved and more sample-specific LAMP protocols have also been developed and published in recent decade [9-19]. In contrast to LAMP methods, technology development for LAMP-based detection has not been completely accelerated yet. In most studies, the focus is developing microfluidic devices for LAMP-based assays. However, microfluidic devices are expensive to fabricate, complex to use, and require trained personnel and stable operation conditions, which makes this technology not feasible for field deployable real-time diagnosis [30-33]. Therefore, portable, rapid, cost-effective, automated, and remotely controllable LAMP platforms with colorimetric readouts will be future of field-ready modern nucleic-acid based detection platforms.

4. Conclusion

This study reported the first LAMP device that conducts Colony-LAMP reactions at 65 @C for less than 30 min, allows carrying out 16 or more reactions at the same time, operation parameters can be remotely monitored, and results can be real-time visualized by naked eye while the reactions are running. We performed LAMP, Colony-LAMP, and Colony PCR reactions using *E. coli* and *P. aeruginosa* samples. We presented detection of the *yaiO* gene which is specific to*E. coli* using the yaiO_2 primer set. We presented specificity and sensitivity of our approach using pure genomic DNA and crude bacterial colonies. We benchmarked our Colony-LAMP detection against Colony PCR. We strongly believe that our portable, remotely controlled colorimetric-LAMP device with its low-cost, user-friendly interface can greatly contribute rapid and reliable diagnosis in versatile operational environments as clinics, fields, farms, and resource-limited areas. Furthermore, our approach can be used for on-field detection of pathogens without requiring a trained personnel or expensive equipment. Considering the significant success of LAMP assays in diagnosis sciences, we are inspired to integrate an automated nucleic acid extraction module to this platform regardless of species of the samples. To address this aim, we work on rapid and reliable platforms for conducting nucleic acid extraction assays using mechanical and chemical isolation methods those are safe not only for DNA amplification and detection reactions but also to the user and environment.

Conflict of interest

The authors declare no competing interests.

Acknowledgements

This work was conducted by the partial support from the H2020-MSCA-IF-2017, 786645—MMED.

References

1. Mullis, K. B. (1990). Recombinant DNA technology and molecular cloning. *Scientific American* , *262,* 181-231.

2. Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic acids research* , *28* , E63. doi:10.1093/nar/28.12.e63
3. Walker, G. T., Fraiser, M. S., Schram, J. L., Little, M. C., Nadeau, J. G., Malinowski, D. P. (1992). Strand displacement amplification–an isothermal, in vitro DNA amplification technique. *Nucleic acids research* , *20* , 1691–1696. doi:10.1093/nar/20.7.1691
4. Compton J. (1991). Nucleic acid sequence-based amplification. *Nature* , *350* , 91–92. doi:10.1038/350091a0
5. Guatelli, J. C., Whitfield, K. M., Kwoh, D. Y., Barringer, K. J., Richman, D. D., Gingeras, T. R. (1990). Isothermal, in vitro amplification of nucleic acids by a multienzyme reaction modeled after retroviral replication. *Proceedings of the National Academy of Sciences of the United States of America* , *87* , 1874–1878. doi:10.1073/pnas.87.5.1874
6. Walker G. T. (1993). Empirical aspects of strand displacement amplification. *PCR methods and applications* , *3* , 1–6. doi:10.1101/gr.3.1.1
7. Lizardi, P. M., Huang, X., Zhu, Z., Bray-Ward, P., Thomas, D. C., Ward, D. C. (1998). Mutation detection and single-molecule counting using isothermal rolling-circle amplification. *Nature genetics* , *19* , 225–232. doi:10.1038/898
8. Wiedmann, M., Wilson, M.J., Czajka, J., Luo, J., Barany, F., Batt, C. A. Ligase chain reaction (LCR)-overview and applications. *PCR Methods Appl., 3* , 51–64. doi:10.1101/gr.3.4.s51
9. Prasannakumar, M. K., Parivallal, P. B., Pramesh, D., Mahesh, H. B., Raj, E. (2021). LAMP-based foldable microdevice platform for the rapid detection of Magnaporthe oryzae and Sarocladium oryzae in rice seed. *Scientific reports* , *11,* 178. doi:10.1038/s41598-020-80644-z
10. Kaygusuz, D., Vural, S., Aytekin, A. Ö., Lucas, S. J., Elitas, M. (2019). DaimonDNA: A portable, low-cost loop-mediated isothermal amplification platform for naked-eye detection of genetically modified organisms in resource-limited settings. *Biosensors & Bioelectronics* , *141* , 111409. doi:10.1016/j.bios.2019.111409
11. Martzy, R., Kolm, C., Brunner, K., Mach, R. L., Krska, R., Šinkovec, H., Sommer, R., Farnleitner, A. H., Reischer, G. H. (2017). A loop-mediated isothermal amplification (LAMP) assay for the rapid detection of Enterococcus spp. in water. *Water research* , *122* , 62–69. doi:10.1016/j.watres.2017.05.023
12. Garg, N., Sahu, U., Kar, S., & Ahmad, F. J. (2021). Development of a Loop-mediated isothermal amplification (LAMP) technique for specific and early detection of Mycobacterium leprae in clinical samples. *Scientific reports* , *11* , 9859. doi:10.1038/s41598-021-89304-2
13. Xun, G., Lane, S. T., Petrov, V. A., Pepa, B. E., Zhao, H. (2021). A rapid, accurate, scalable, and portable testing system for COVID-19 diagnosis. *Nature communications* , *12* , 2905. doi:10.1038/s41467-021-23185-x
14. Yan, M., Li, W., Zhou, Z., Peng, H., Luo, Z., Xu, L. (2017). Direct detection of various pathogens by loop-mediated isothermal amplification assays on bacterial culture and bacterial colony. *Microbial pathogenesis* , *102* , 1–7. doi:10.1016/j.micpath.2016.10.025.
15. Bosward, K. L., House, J. K., Deveridge, A., Mathews, K., Sheehy, P. A. (2016). Development of a loop-mediated isothermal amplification assay for the detection of Streptococcus agalactiae in bovine milk. *Journal of dairy science* , *99* , 2142–2150. doi:10.3168/jds.2015-10073
16. Pascual-Garrigos, A., Maruthamuthu, M. K., Ault, A., Davidson, J. L., Rudakov, G., Pillai, D., Koziol, J., Schoonmaker, J. P., Johnson, T., Verma, M. S. (2021). On-farm colorimetric detection of Pasteurella multocida, Mannheimia haemolytica, and Histophilus somni in crude bovine nasal samples. *Veterinary research* , *52* , 126. doi:10.1186/s13567-021-00997-9
17. Garrido-Maestu, A., Azinheiro, S., Carvalho, J., Abalde-Cela, S., Carbó-Argibay, E., Diéguez, L., Piotrowski, M., Kolen'ko, Y. V., Prado, M. (2017). Combination of Microfluidic Loop-Mediated Isothermal Amplification with Gold Nanoparticles for Rapid Detection of *Salmonella* spp. in Food Samples. *Frontiers in microbiology* , *8* , 2159. doi:10.3389/fmicb.2017.02159
18. Jackson, K. R., Layne, T., Dent, D. A., Tsuei, A., Li, J., Haverstick, D. M., Landers, J. P. (2020). A novel loop-mediated isothermal amplification method for identification of four body fluids with smartphone

detection. *Forensic science international. Genetics* , *45* , 102195. doi:10.1016/j.fsigen.2019.102195
19. Watthanapanpituck, K., Kiatpathomchai, W., Chu, E., & Panvisavas, N. (2014). Identification of human DNA in forensic evidence by loop-mediated isothermal amplification combined with a colorimetric gold nanoparticle hybridization probe. *International journal of legal medicine* , *128* , 923–931. doi:10.1007/s00414-014-1018-9
20. Goto, M., Honda, E., Ogura, A., Nomoto, A., & Hanaki, K. (2009). Colorimetric detection of loop-mediated isothermal amplification reaction by using hydroxy naphthol blue. BioTechniques, *46* , 167–172. doi:10.2144/000113072
21. Mori, Y., Nagamine, K., Tomita, N., & Notomi, T. (2001). Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochemical and biophysical research communications* , *289* , 150–154. doi:10.1006/bbrc.2001.5921
22. Jaroenram, W., Cecere, P., & Pompa, P. P. (2019). Xylenol orange-based loop-mediated DNA isothermal amplification for sensitive naked-eye detection of Escherichia coli. *Journal of microbiological methods* , *156* , 9–14. doi:10.1016/j.mimet.2018.11.020
23. Papadakis, G. et al. (2020). Real-time colorimetric LAMP methodology for quantitative nucleic acid detection at the point-of-care bioRxiv. doi:10.1101/2020.07.22.215251
24. Sayad, A. A., Ibrahim, F., Mukim Uddin, S., Cho, J., Madou, M., Thong, K. L. (2018). A microdevice for rapid, monoplex and colorimetric detection of foodborne pathogens using a centrifugal microfluidic platform. *Biosensors & Bioelectronics* , *100* , 96–104. doi:10.1016/j.bios.2017.08.060
25. Seo, J. H., Park, B. H., Oh, S. J., Choi, G., Lee, E. Y., & Seo, T. S. (2017). Development of a high-throughput centrifugal loop-mediated isothermal amplification microdevice for multiplex foodborne pathogenic bacteria detection. *Sensors and Actuators B: Chemical* , *246* , 146-153. doi:10.1016/j.snb.2017.02.051
26. Budd, J., Miller, B. S., Manning, E. M., Lampos, V., Zhuang, et al. (2020). Digital technologies in the public-health response to COVID-19. *Nature medicine* , *26* , 1183-1192. doi:10.1038/s41591-020-1011-4
27. Poschenrieder, A., Thaler, M., Junker, R., Luppa, P. B. (2019). Recent advances in immunodiagnostics based on biosensor technologies—from central laboratory to the point of care. *Analytical and bioanalytical chemistry* , *411* , 7607-7621. doi:10.1007/s00216-019-01915-x.
28. Vandenberg, O., Martiny, D., Rochas, O., van Belkum, A., Kozlakidis, Z. (2021). Considerations for diagnostic COVID-19 tests. *Nature Reviews Microbiology* , *19* (3), 171-183.doi:10.1038/s41579-020-00461-z
29. Boyle, A. G., Rankin, S. C., O'Shea, K., Stefanovski, D., Peng, J., Song, J., Bau, H. H. (2021). Detection of Streptococcus equi subsp. equi in guttural pouch lavage samples using a loop-mediated isothermal nucleic acid amplification microfluidic device. *Journal of veterinary internal medicine* , *35* , 1597–1603. doi:10.1111/jvim.16105
30. Ma, Y. D., Luo, K., Chang, W. H., Lee, G. B. (2018). A microfluidic chip capable of generating and trapping emulsion droplets for digital loop-mediated isothermal amplification analysis. *Lab on a chip* , *18* , 296–303. doi:10.1039/c7lc01004d
31. Wan, L., Gao, J., Chen, T., Dong, C., Li, H., Wen, Y. Z., Lun, Z. R., Jia, Y., Mak, P. I., Martins, R. P. (2019). LampPort: a handheld digital microfluidic device for loop-mediated isothermal amplification (LAMP). *Biomedical microdevices* , *21* , 9. doi:10.1007/s10544-018-0354-9
32. Wan, L., Chen, T., Gao, J., Dong, C., Wong, A. H., Jia, Y., Mak, P. I., Deng, C. X., Martins, R. P. (2017). A digital microfluidic system for loop-mediated isothermal amplification and sequence specific pathogen detection. *Scientific reports* , *7* , 14586. doi:10.1038/s41598-017-14698-x
33. Almassian, D. R., Cockrell, L. M., & Nelson, W. M. (2013). Portable nucleic acid thermocyclers. *Chemical Society reviews* , *42* , 8769–8798. doi:10.1039/c3cs60144g

Figure Legends

Figure 1. LAMP device and Colony LAMP in the device

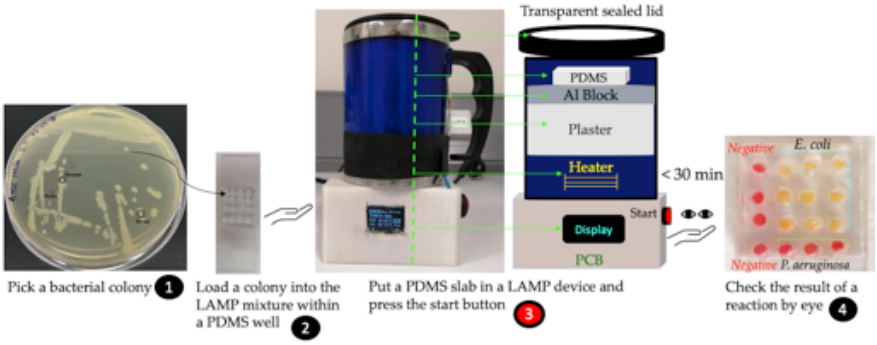
Figure 2 . Principal circuit diagram of the LAMP device. Max6675: Thermocouple Interface, NODEMCU ESP32: Microcontroller, OLED 1306: Display, LM7805: Voltage Regulator

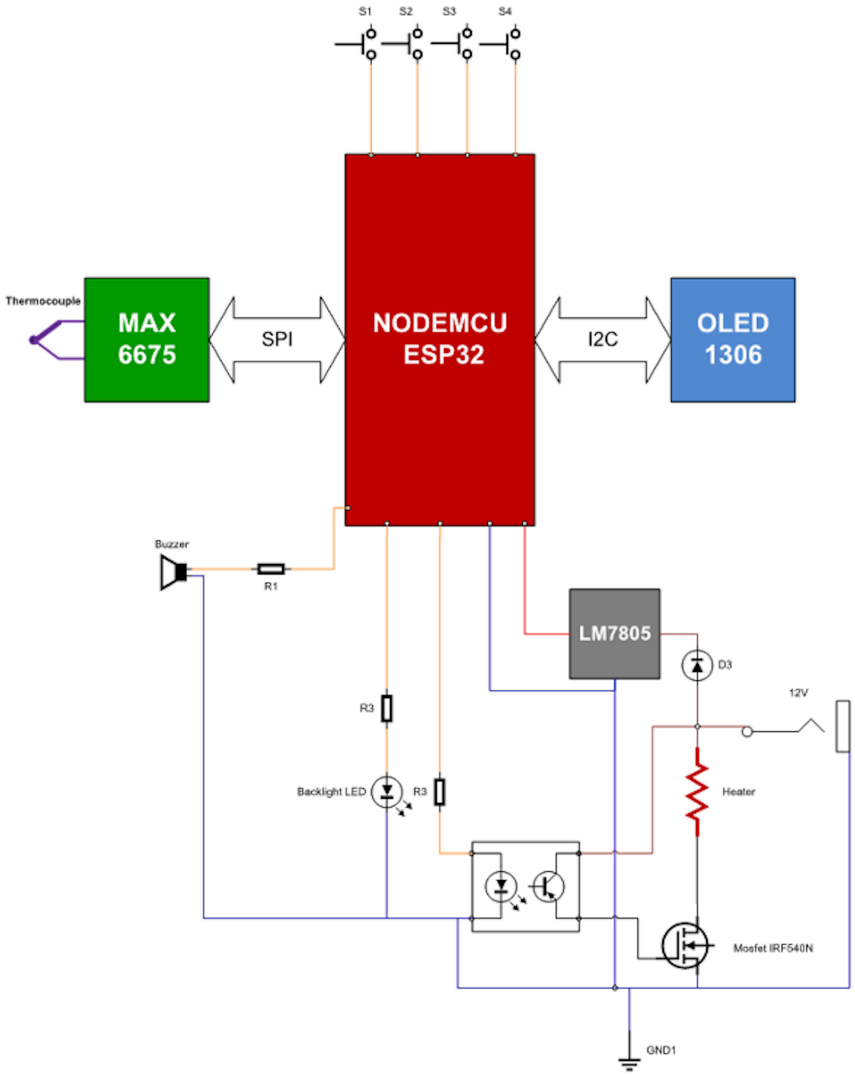
Figure 3 . Interface of the LAMP device through the server

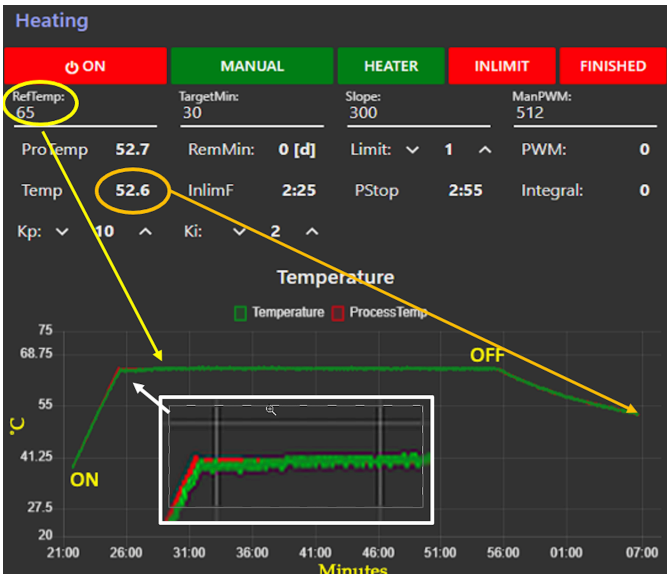
Figure 4 . Colony-LAMP assay for *E. coli* and *P. aeruginosa* colonies. **a)** Images of the colonies picked from*E. coli* plate. R: Colony diameter, *E. coli* : R_{min} [?] 1.437, R_{mean} [?] 1.744, R_{max} [?] 2.281 mm, *P. aeruginosa* : R_{min} [?] 1.537, R_{mean} [?] 1.853, R_{max} [?] 2.012 mm. **b)** Colorimetric Colony-LAMP reactions in the PDMS wells. Pink color indicates the negative, yellow color shows the positive results. Electrophoresis results for**c)** *E. coli,* **d)** *P. aeruginosa* colonies. L: DNA ladder, N: Negative control.

Figure 5 . Sensitivity and specificity of LAMP reactions using genomic DNA. **a)** Colorimetric LAMP reactions in the PDMS wells.*E. coli* cells: 1% = $5.68x10^{10}$, 50% = $2.87x10^{11}$, 100% = $5.34x10^{11}$ copy numbers. *P. aeruginosa* cells: 1% = $6.82x10^{10}$, 50% = $2.72x10^{11}$, 100% = $5.74x10^{11}$copy numbers. Pink color indicates the negative, yellow color shows the positive results. **b)** Electrophoresis results for *E. coli*and *P. aeruginosa* strains. L: DNA ladder, N: Negative control.

Figure 6 Sensitivity and specificity of Colony PCR reactions. L: DNA ladder, N: Negative sample, P: Positive sample, R: Colony diameter. *E. coli:* R_{min} [?] 1.5 mm, R_{mean} [?] 1.8 mm, R_{max} [?] 2.5 mm. *P. aeruginosa* : R [?] 2 mm.







Hosted file

`Figure 4_Colony LAMP assay.pdf` available at https://authorea.com/users/446123/articles/545452-a-low-cost-portable-and-practical-lamp-device-for-point-of-diagnosis-in-the-field