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Development of a Portable Loop-mediated Isothermal Amplification Device for DNA Detection and Determination Using Absorbance Measurement

Zhuo Huang,[1] Shoji Yamamoto,[1*] Yifan Liao,[1]
Kazuhiro Morioka,[2] and Hizuru Nakajima[1**]

[1]Department of Applied Chemistry, Graduate School of Urban Environmental Sciences, Tokyo Metropolitan University, 1-1 Minami-osawa, Hachioji, Tokyo 192-0397, Japan
[2]Department of Biomedical Analysis, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

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A portable multisample absorbance detection system for loop-mediated isothermal amplification using a positive temperature coefficient (PTC) heater was developed in this study. The system can simultaneously measure up to four samples and consists of four LEDs, four photodiodes, two PTC heaters, a sample holder, and a short-pass filter. The system successfully differentiated samples with and without human genomic DNA with photodiodes measuring absorbance in real time. We have also demonstrated that the quantitative analysis of human genomic DNA is possible at initial DNA concentrations ranging from 2.11×10^2 to 2.11×10^4 ng/μL. This device has potential use in on-site genetic testing owing to its compact size, portability, and capability to provide rapid results.

1. Introduction

The outbreak of the new coronavirus has led to a rapid increase in the number of infected individuals in recent years.(1) As a result, medical institutions and the economy have been severely impacted, making rapid and decentralized diagnostic testing critical.(2) Polymerase chain reaction (PCR)(3) is the most well-known specific DNA amplification method. In PCR, DNA is amplified by repeated cyclic reactions of denaturation, annealing, and extension at different temperatures. The PCR method is a powerful tool for detecting viruses by amplifying specific genes. However, it has the drawback of requiring expensive equipment and a long detection time.(4–6)

Alternatively, the loop-mediated isothermal amplification (LAMP)(7) method enables specific DNA amplification at a constant temperature of approximately 65 °C. This method does not necessitate costly equipment, offers shorter detection times, and maintains high specificity, making it suitable for low-resource settings and real-time detection. Compared with the

*Corresponding author: e-mail: shoji-yamamoto@tmu.ac.jp
**Corresponding author: e-mail: nakajima-hizuru@tmu.ac.jp
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traditional PCR method, LAMP is more user-friendly in terms of both equipment and operation, enabling rapid screening and results acquisition. A significant advantage of LAMP is its isothermal operation, which reduces equipment costs and eliminates the need for complex temperature control systems. LAMP exhibits high specificity and sensitivity toward the target DNA sequence, which is particularly beneficial in the early detection of pathogens.[8] Additionally, the amplification process can be completed in a single step, simplifying automation and large-scale processing. Consequently, the LAMP method is cost-effective and particularly advantageous in resource-constrained environments, and it contributes to the reduction in size and cost of genetic testing equipment.[9]

In the LAMP method, a significant number of protons are generated during the gene amplification reaction, resulting in a reduction in the pH of the solution. This pH change can be visually confirmed using indicators such as phenol red to detect the presence or absence of target-gene amplification.[10,11] In fluorescence and colorimetric detection, it is common to add a buffer to the sample, which prevents the observation of pH decrease due to proton generation. Nevertheless, by adjusting the amount of buffer in the sample solution, it is possible to induce a pH decrease associated with the LAMP reaction. Many researchers have developed pH sensors and indicators to detect isothermal gene amplification reactions based on pH changes.[12–14] Visual changes permit the rapid and intuitive detection of target DNA without the need for special equipment. However, detection can be challenging when DNA concentrations are low or color changes are subtle, requiring detectors for accurate judgment. Absorbance measurements are expected to be accurate because of their high sensitivity and user-friendly owing to their low cost.[15]

Herein, we have developed a measurement system for the LAMP method that uses absorbance detection. Furthermore, we have developed a portable gene amplification test device capable of performing simultaneous multiple-sample detection. Furthermore, the capability for quantitative analysis was newly demonstrated through calibration curves, which correlated the initial sample DNA concentrations with the logarithms of reaction times measured by the developed device. The performance of this system was rigorously validated through the LAMP quantitative analysis of human genomic DNA, following assessments of its absorbance detection and heating efficiency.

2. Materials and Methods

2.1 Reagents and materials

Sterile water was acquired from Fujifilm Corporation (Tokyo, Japan). WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA) was obtained from New England Biolabs Japan Inc. (Tokyo, Japan). The target DNA, specifically human genomic DNA (female), was purchased from Eurofins Genomics Co., Ltd. (Tokyo, Japan). Human genomic DNA was used to prepare DNA-positive samples, whereas for DNA-negative samples, sterile water was used instead of human genomic DNA. The volume of each sample was 25 μL. The colorimetric LAMP Assay Kit Components are shown in Table 1. Standard oligos including FIP, BIP, F3, B3,

Table 1
Colorimetric LAMP assay kit components.

Reagents	Negative (μL)	Positive (μL)
LAMP Master Mix	12.5	12.5
LAMP Primer Mix	2.5	2.5
Human genome DNA	—	5.0
Nuclease-free water	10.0	5.0

LOOP F, and LOOP B were purchased from Eurofins Genomics Co., Ltd. (Tokyo, Japan). A primer mix was prepared using these six types of primer and sterile water. The primer mixcomponents used for 40 reactions are shown in Table 2.

2.2 Visual detection

We confirmed the change in color during the experiment using the following protocol. The total reaction volume of 25 μL was prepared by mixing 12.5 μL of LAMP 2X Master Mix, 2.5 μL of the primer mix (total 1.6 μM for each primer), 5 μL of template DNA for the DNA-positive control (PC), and nuclease-free water (as detailed in Table 1). The mixture was then incubated isothermally at 65 ℃ for 15–40 min in a hot water bath, high-temperature water tank, or heat block. The incubation temperature was closely monitored and maintained at 65 ℃. The color change was observed visually by comparing the reaction tube with a control tube. A positive reaction, indicating the presence of target-gene amplification, resulted in a color change from red to yellow, while a negative reaction resulted in no color change.

2.3 Construction of absorbance detection system

The detection system is shown in Fig. 1. The entire detection system comprised a power supply (SPPS-C3010W, IKococater), a white LED (OSW54K5B61A, OptoSupply), two optical fibers (FT600EMT, THORLABS), a sample holder fabricated using a 3D printer (Adventurer 4, FLASHFORGE), a palm-sized spectrometer (HR2000, OceanInsight), and a PC. The light emitted from the LED passes through the tube in the sample holder, and the transmitted light is guided by an optical fiber to a spectrometer, which measures the light intensity. As a result, changes in absorbance according to the characteristics of the sample can be detected.

2.4 Development of a compact LAMP device for simultaneous multiple detection measurements

We developed a compact absorbance detector capable of measuring multiple samples simultaneously. This device measures four samples simultaneously, and the detection section includes four white LEDs (OSW54K5B61A, OptoSupply), four photodiodes (PDs) (G6262, Hamamatsu Photonics K.K.),[16,17] two positive temperature coefficient (PTC) heaters (B07NL8YFH4, Zerodis), a sample holder, and a short-pass filter (SV0490, Asahi Spectroscopy Co., Ltd.) (Fig. 2). The optical properties of the PDs are most sensitive at 470 nm, and their

Table 2
Primer mix kit components.

LAMP Primer Mix	Reaction 40 times (μL)
FIP	16
BIP	16
F3	2
B3	2
LOOP F	4
LOOP B	4
Nuclease-free water	56

F3: TCCTTGAACTTTGGTCTCC
B3: CAGTTCATAAAGGAATTGATAGC
FIP: ATCCCCAGTCTGTGAAATTGGGCAAAATGCTGGGATTATAGATGT
BIP: GCAGCAGAAAGATTATTAACTTGGGCAGTTGGTAAGTAAATGGAAGA
Loop F: AGAACCAGAGGCCAGGCGAG
Loop B: AGGCAGATAGGCTTAGACTCAA

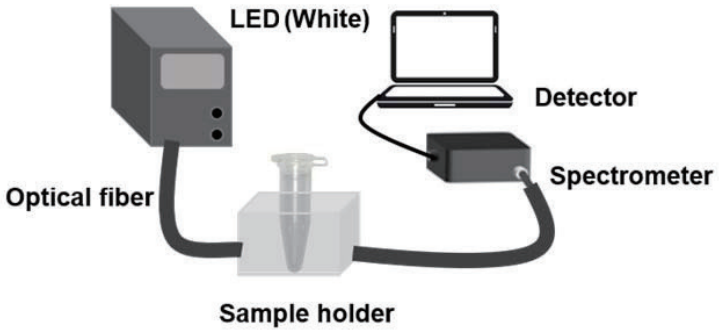

Fig. 1. Schematic of absorbance detection system.

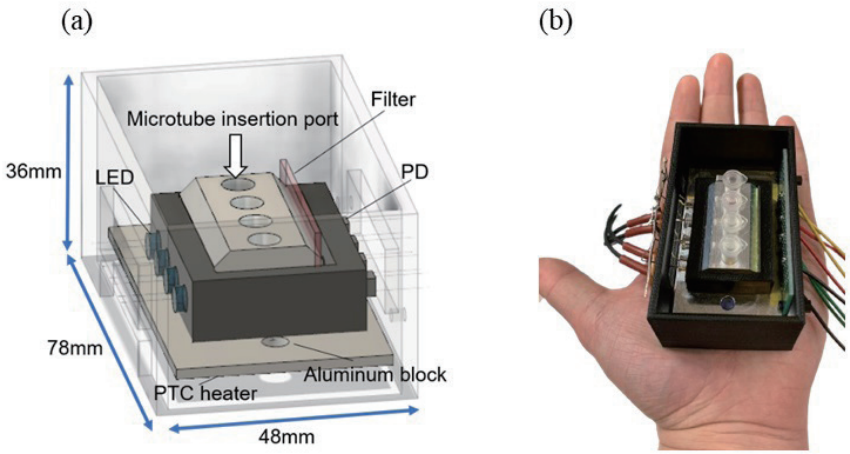

Fig. 2. (Color online) Design and photograph of palm-sized LAMP device for simultaneous multiple-sample detection measurements. (a) Schematic illustration of device components including four white LEDs, four PDs, two PTC heaters, a sample holder, and a short-pass filter. (b) Photograph of assembled LAMP device.

sensitivity increases with temperature. However, the optical properties of the short-pass filter allow light with wavelengths between 350 and 500 nm to pass through. On the basis of the results of experiments using the system described in Sect. 2.3 (the results are described later in Sect. 3.2), we found that the PC and negative control (NC) samples can be better distinguished at a wavelength of 454 nm. Considering the sensitivity characteristics of the PDs and the absorbance spectra of the samples, the PDs and the filter used in this experiment are suitable for detection. The light signal received by the PDs is converted into a voltage signal through a precision data logger (ADC-24, Pico Technology Ltd.). At around the wavelength of 454 nm, the voltage samples of PC detected as a signal gradually decreases as the LAMP reaction proceeds, while that of NC samples remains essentially unchanged. This detection principle allows the simultaneous and real-time measurement of absorbance changes in four samples at around 454 nm every 1 min for 40 min during the experiment. The absorbance was calculated using the Beer–Lambert law:

$$A = -\log(I/I_0). \tag{1}$$

Here, I represents the incident light, and I_0 represents the transmitted light from a blank solution, which in this case is nuclease-free water.

An aluminum block with excellent thermal conductivity was used as the sample holder. The aluminum block was designed using Autodesk Fusion 360 and manufactured by Meviy (Tokyo, Japan). To reduce the device cost, two small and easily controllable PTC[18] heaters were adopted. The PTC heaters were installed under the aluminum block, and a double-sided tape with a heat-conductive sheet (SILICONE-9X_qx, ADWITS) was used to firmly attach the aluminum block to the PTC heater, ensuring efficient and even heat transfer. To maintain the temperature at 65 °C, we used two PTC heaters with a designated temperature of 80 °C. Considering the room temperature of approximately 25 °C and the heat absorption by the holder, a PTC heater with a temperature higher than 65 °C is necessary. To shorten the heating time, we adopted two 80 °C PTC heaters and controlled the temperature by applying a constant voltage using a 12 V stabilized power supply. The microtube port shown in Fig. 2 has a diameter of 6.1 mm and a depth of 15.5 mm. This enabled precise and rapid temperature control, which is crucial for the LAMP method.

3. Results and Discussion

3.1 Results of visual detection

Comparison of NC and PC samples shows that the NC samples were not amplified and remained pink in color (Fig. 3). In contrast, the color of PC samples changed from pink to yellow owing to DNA amplification. The PC samples contained human genomic DNA at a concentration of 2.11×10^5 ng/μL. On the basis of these results, it can be concluded that discrimination is possible through absorbance.

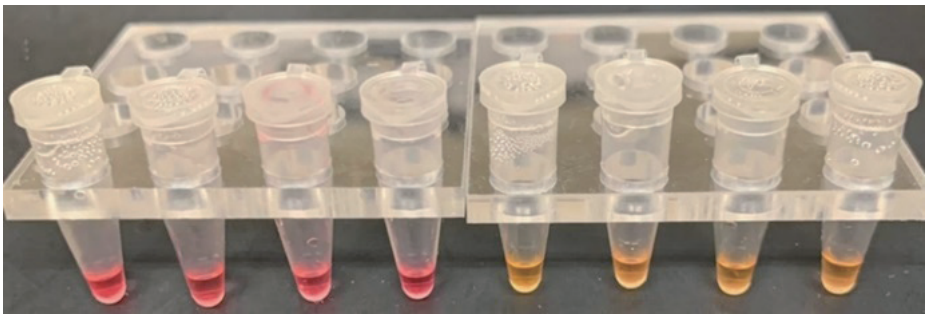

Fig. 3. (Color online) Visual detection of DNA amplification by color change. NCs remain pink, whereas PCs change from pink to yellow.

3.2 Evaluation of absorbance spectra

Using the absorbance detection system described in Sect. 2.3, we prepared samples containing human genomic DNA (PC) and samples without human genomic DNA (NC). The absorbance of these samples was measured before heating. Subsequently, the LAMP reaction was performed at 65 °C for 40 min, and the absorbance of the samples was measured after heating.

Before amplification, both the sample containing human genomic DNA before amplification (PC) and the sample without it (NC) appeared pink in color and exhibited almost identical absorbance levels. After amplification, the PC samples changed from pink to yellow, whereas the NC samples remained pink. The changes in absorbance before and after heating are shown in Figs. 4(a) and 4(b). Regarding absorbance, at the wavelength of 454 nm, a significant increase in absorbance was observed exclusively in the PC samples, whereas at the wavelength of 560 nm, the absorbance levels of both PC and NC samples showed minimal changes.

Because of the considerable increase in absorbance at 454 nm observed only in the PC samples, we focused on measuring the change in absorbance at this wavelength. The variation in the PC spectrum with time is shown in Fig. 4(c). We confirmed that the absorbance of the PC samples increased rapidly after 20 min. For each heating time in this experiment, a new sample was used.

From these observations, we determined that it is possible to distinguish between PC and NC samples by measuring the absorbance and following their changes in absorbance at the 454 nm wavelength over time.

3.3 PTC heater temperature control

Because the PTC heater is smaller than the aluminum block, heat conduction to the aluminum block is slow. To rapidly heat up the sample to 65 °C, two PTC heaters were employed. Temperature was measured directly in the liquid inside the microtubes. The temperature changes

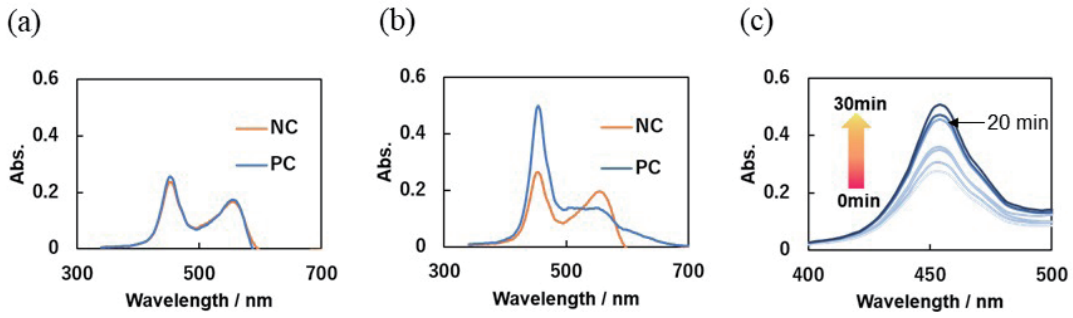

Fig. 4. (Color online) Evaluation of absorbance spectra for NC and PC samples: (a) before heating and (b) after heating. (c) Variation in PC spectrum with time.

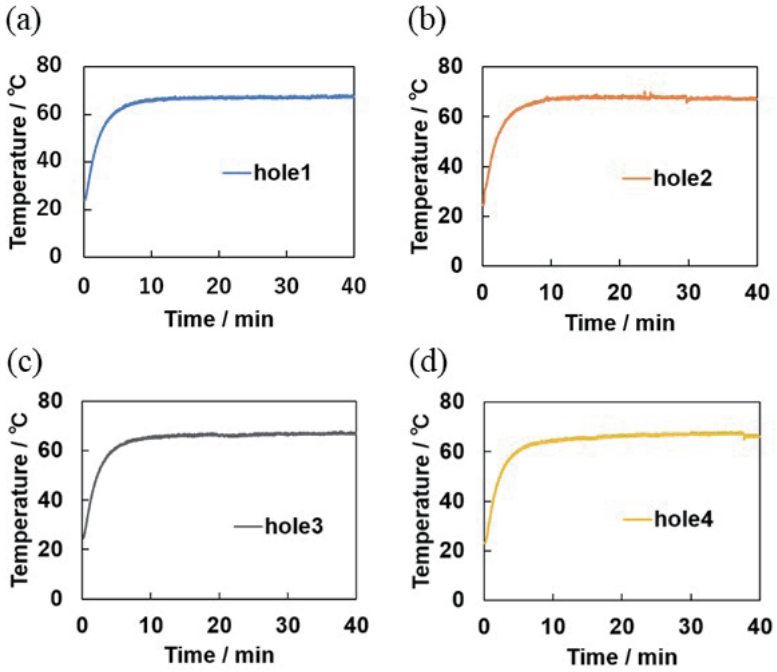

Fig. 5. (Color online) Temperature stability evaluation of heating unit across four sample holes. Temperature profiles of (a) first, (b) second, (c) third, and (d) fourth holes.

in each hole are shown in Fig. 5. Temperature was measured every 1 s using a thermistor (103JT-050, SEMITEC Co., Ltd). Consequently, the temperature reached 65 °C in about 5 min and stabilized at approximately ±0.35 °C. The temperature differences among the four holes were less than 1 °C, with temperatures ranging from 66.79 to 67.68 °C. These findings demonstrate that the temperature control performance is as stable and uniform as, or even superior to, that of conventional microplate readers (±2.0 °C)[19] and other LAMP analyzers (±1.0 °C).[20] It was confirmed that the system developed in this study possesses adequate performance for conducting the LAMP reaction.

3.4 Real-time measurement of LAMP reaction

Using the multisample absorbance detector described in Sect. 2.4, we measured the change in absorbance of human genomic DNA-positive samples in real time during the LAMP reaction. The PC samples contained human genomic DNA at a concentration of 2.11×10^5 ng/μL. The results are presented in Fig. 6. Experimental outcomes indicated that while the absorbance of the NC (n = 4) samples hardly changed, the absorbance of the PC (n = 4) samples increased considerably approximately 20 min after the start of amplification. Additionally, the starting time of the increase in absorbance for the PC samples at each detection site was nearly consistent, occurring between 20 and 22 min. This indicates that the heating temperature was uniformly maintained across all detection sites, with no disparity in heating temperature attributable to the site of detection. These results demonstrate that up to four samples can be measured simultaneously in real time during the LAMP reaction.

3.5 Performance evaluation of LAMP device

The system we developed was found to have sufficient heating performance for measuring absorbance during the LAMP reaction and maintain uniform and accurate temperatures at four measurement sites. This suggests that quantitative analysis using the LAMP reaction is also possible. To demonstrate this possibility, the LAMP reaction was carried out with the system developed using PC samples with various concentrations of human genomic DNA, and the absorbance was measured in real time. Figure 7(a) shows the measurement results at concentrations ranging from 2.11×10 to 10^5 ng/μL. In real-time absorbance measurement, the starting time of the increase in absorbance generally became longer as the initial concentration of human genomic DNA decreased. At a very high concentration of 2.11×10^5 ng/μL, there was no significant difference in the starting time of absorbance increase compared with 2.11×10^4 ng/μL. This suggests that the reaction might be inhibited at very high DNA concentrations. Additionally, we conducted experiments with a DNA concentration of 2.11×10 ng/μL, but there

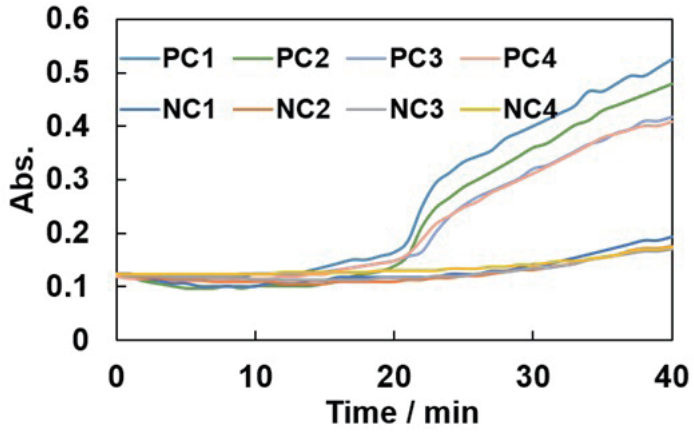

Fig. 6. (Color online) Time-series changes in absorbance of PC and NC samples at the four detection sites during LAMP reaction.

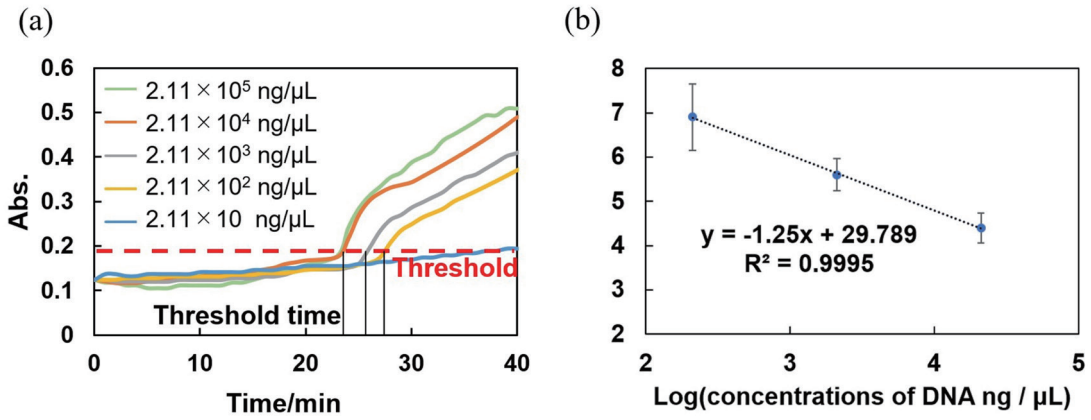

Fig. 7. (Color online) Performance evaluation of developed LAMP device. (a) Time-series changes in absorbance of samples with different concentrations of human genomic DNA. (b) Calibration curves for human genomic DNA.

was no significant change in absorbance, indicating that detection was not possible at this concentration. Figure 7(b) presents the calibration curve for human genomic DNA in the range from 2.11×10^2 to 2.11×10^4 ng/μL, which was prepared by plotting the threshold time against the concentration of human genomic DNA. The threshold time was defined as the time required to reach an absorbance level that is the average for the first 10 to 15 min of the experiment plus 30 times the standard deviation for the same period and was measured four times. This high threshold was chosen to ensure that the possibility of false positives is minimized, providing a stringent criterion for determining the presence of DNA amplification. When the average threshold time was plotted against the concentration of human genomic DNA and a calibration curve was created, a calibration curve showing good linearity ($R^2 = 0.9995$) was obtained. These results demonstrate that the quantitative analysis of human genomic DNA using this detection system is possible within the initial DNA concentration range from 2.11×10^2 to 2.11×10^4 ng/μL. While PCR generally offers higher sensitivity, these coefficients of determination are comparable to or exceed those reported for other fluorescence LAMP devices.[20–22] Our method, while maintaining similar sensitivity, provides a simpler and more cost-effective alternative to these traditional methods. However, the detection limit for human genomic DNA has not been thoroughly investigated and remains an area for future research. Furthermore, samples with low concentrations of DNA have shown substantial standard deviations, likely due to various experimental conditions such as the room temperature affecting the uniformity of heating times to reach 65 °C. Therefore, preheating to 65 °C beforehand or further improving the insulation of the heating unit might be required.

4. Conclusions

In this research, we developed a compact absorbance detector and constructed a measurement system on the basis of the principle of absorbance detection. Two PTC heaters were employed to ensure the temperature required for the LAMP reaction. By detecting absorbance, it was

suggested that DNA detection is not only possible at concentrations as low as those in conventional methods, but also that the original concentration can be estimated from the amplification start time. This device is small and easy to carry, allowing for quick test results, making it extremely useful for on-site medical testing.

Acknowledgments

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