A microfluidic lab-on-a-disc integrated loop mediated isothermal amplification for foodborne pathogen detection

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Salmonellosis is one of the most common foodborne diseases caused by the genus Salmonella and is considered to be a significant global health concern. Therefore, the development of effective techniques for rapidly detecting Salmonella is fundamental in preventing foodborne outbreaks. This paper presents a lab-on-a-disc integrated loop mediated isothermal amplification (LAMP) on a microfluidic platform for Salmonella detection. We have developed a centrifugal microfluidic platform device in which the main steps in pathogen detection, reagent preparation, LAMP, and detection can be integrated onto a single microfluidic compact disc (CD). A forced convection heating source was used for wax valve actuation and temperature heating for the LAMP amplification, leading toward compactness and system miniaturization. The detection limit was $5 \times 10^{-3} \text{ ng/µL}$ DNA concentration when tested on tomatoes spiked with Salmonella. The whole procedure, from sample preparation (on the microfluidic CD) up to detection, was completed within 70 min using a fully automated process. The final detection step was performed via direct visual observation of the color change of the SYBR Green I dye. Our system offers a fast and automated molecular diagnostic platform, thereby reducing the need for skillful operators and expensive instrumentation. This developed portable device would have a wide range of potential biomedical applications, including foodborne pathogen detection and molecular diagnostics in developing countries.

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1. Introduction

Foodborne illnesses remain the major cause of morbidity and mortality worldwide [1]. The common etiologic agents of foodborne outbreaks are Escherichia coli O157:H7, Listeria monocytogenes, Campylobacter and Salmonella [2,3]. Salmonella enterica are Gram-negative facultative anaerobes that cause acute gastroenteritis and are responsible for nearly half of foodborne disease outbreaks worldwide [4]. Failure to detect these foodborne pathogens in contaminated food could cause significant losses in the food industry and health sectors [5]. Current conventional methods for detecting and identifying Salmonella are mostly based on cell culture and colony counting, which require a minimum of 3–4 days to obtain presumptive results and approximately 7 days to obtain definitive positive results [6]. To overcome these drawbacks, several methods for rapid detection have been introduced [7], such as, (1) Polymerase Chain Reaction (PCR) [8–10], (2) enzyme-linked immuno assay (ELISA) [11] and (3) microarray immunoassays [12–14]. However, immunoassay methods have limited usage because of their low specificity, particularly for Salmonella detection [15]. Furthermore, these methods are labor intensive, time consuming, costly for meeting food safety control standards and require bulky as well as sophisticated instruments. All these obstacles have limited the usage of these methods and make rapid detection difficult. Therefore, there is a crucial need for alternative methods that provide rapid, automated, easy and accurate detection of foodborne pathogens. Point-of-care (POC) devices are promising alternatives to rapidly identify pathogens in clinical and food samples, resulting in immediate and accurate diagnoses.

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Microfluidic devices, referred to as “lab-on-a-chip” (LOC), have played a significant role in the analysis and diagnosis of biological, clinical and chemical samples, including foodborne pathogens [16]. The automation of the operation (reduction of manual steps), shortened time-to-result, reduced reagent consumption, low cost, miniaturized size and precise volume control are the key factors that make LOC devices powerful tools in food forensics [17–19]. Therefore, rigorous research has been performed on foodborne pathogen detection using LOC devices [20,21]. The Xingyu Jiang Group has developed a lab-on-a-chip called µLAMP for the detection of Pseudorabies virus by integrating loop mediated isothermal amplification (LAMP) on an 8 channel microfluidic chip [22]. The Reddy Jr Group has introduced a silicon chip for the multiplexed detection of foodborne pathogens utilizing LAMP [23]. Another group has developed a microfluidic CD for the detection of Salmonella using recombinase isothermal amplification (RPA) [24]. Although the multiple functions for foodborne pathogen detection, such as DNA extraction, amplification and detection, can be performed on a single microfluidic chip, there is a need for microfluidic control, such as valving and mixing. Thus, lab-on-a-disc platforms are considered stronger contenders than lab-on-a-chip platforms because they eliminate the requirement for tubing and external pumping devices, as they require only a simple electric motor for fluid control [25,26]. Furthermore, the centrifugal force generated from the rotation of the microfluidic CD can be used for microfluidic propulsion [25,27], mixing [27–29], metering [27,30,31], decanting and calibration [27] of samples for diagnosis. Moreover, valving techniques are important for all microfluidic analysis platforms, as samples must be properly reserved until needed [17,27,30,32]. Many biological and biochemical assays requiring multistep processes, such as nucleic acid extraction, amplification and detection, also need valving techniques.

A recent method in nucleic acid detection is isothermal nucleic acid amplification, which generates a large number of target copies, thus significantly increasing assay sensitivity. Several isothermal nucleic acid amplification techniques, such as Nucleic Acid Sequence-Based Amplification (NASBA) [33], Strand Displacement Amplification (SDA) [34], Recombinase Polymerase Amplification (RPA) [35], and Rolling Circle Amplification (RCA) [36], have been used for pathogen detection. Another recent powerful analytical tool for pathogen detection is LAMP, which is the technique used in our study. LAMP is a nucleic acid amplification method that amplifies DNA/RNA under isothermal conditions (temperature between 60–65 °C) with high sensitivity and specificity using a set of six primers and a Bst DNA polymerase [37]. LAMP does not need a sophisticated heating block system that requires a high temperature (90–100 °C), as the amplification take place at a constant temperature of 63 °C for 60 min. LAMP method has sensitivity and specificity as the conventional FCR [22]. Some isothermal amplification methods use complicated and time consuming detection techniques, such as a strip lateral flow sensor, which needs to first absorb the liquids, followed by a period of waiting for the results to be confirmed as reported by Kim et al. [24], who developed a microfluidic CD platform using the RPA isothermal technique to detect Salmonella. In contrast, LAMP is considered to be a powerful and easy tool for detection process because it requires an easier and faster method when detecting the target. For example, monitoring the turbidity of LAMP reactions resulting from magnesium–pyrophosphate precipitation can be seen with the unaided eye as a white precipitate. For better visualization, SYBR Green I is added to the reaction to visualize the results by monitoring the color change which would give a more confirmed result. Liang et al. [38] developed a closed tube LAMP detection of using a Wax Sealed Fluorescent Intercalator to detect several viruses and pathogens (HBV, 2009H1N1), and SYBR Green I dye was added to this system to provide more definitive results.

In this paper, we present a microfluidic lab-on-a-disc integrated loop mediated isothermal amplification (LAMP) platform for automated foodborne pathogen amplification and detection. This proof-of-concept system demonstrates fully automated centrifugal functions, including pumping, mixing, metering and sealing integrated with LAMP to detect a foodborne pathogen target with higher sensitivity and specificity. The practical effectiveness of the system was validated through the detection of Salmonella, a major foodborne pathogen, in spiked tomato samples. Thus, reduction of manual steps and manpower, shortened time-to-result, low cost, portable and miniaturized size (miniaturize all laboratory equipment into a single microfluidic CD) will make our microfluidic CD more useful and practical.

2. Materials and methods

2.1. Cell culture and DNA preparation

A strain of confirmed Salmonella Enteritidis previously isolated from food samples was used for method development. The DNA was extracted using boiling method and a 2.5 × 10⁻³ ng/µL was used in each LAMP reaction (for more details about the extraction of the DNA, please refer to the “Supplementary material”).

To determine the specificity of the assay, the experiment was carried out with DNA from Salmonella and non-Salmonella strains (E. coli, Shigella sonnet, Listeria monocytogenes, Vibrio cholerae, Yersinia spp. and Acinetobacter baumanii). A 2.5 × 10⁻³ ng/µL DNA concentration from Salmonella and non-Salmonella strains was used for each LAMP reaction (pre-loaded to the amplification chambers).

To determine the sensitivity of the assay, a 10-fold serial dilution of DNA extracted from a tomato spiked with Salmonella was used. A 2.5 × 10⁻³ ng/µL DNA concentration was also used in this method (pre-loaded to each amplification chamber, more details will be discussed in Section 3). The results obtained were compared with the conventional LAMP method and PCR assay. Each assay was repeated at least twice to ensure reproducibility. For more details about extraction of the DNA from Salmonella spiked tomato, please refer to “Supplementary material”.

2.2. Reagents and sample

A set of 6 primers was designed (unpublished work) using the LAMP Primer Explorer V4 software to specifically target Salmonella in the food sample. The primer set consists of 2 outer primers (F3 and B3), 2 inner primers (FIP and BIP) and 2 loop primers (LF and LB). All primers were commercially synthesized. One reaction of LAMP was performed in a total volume of 25 µL composed of 12.5 µL reaction mixture, 1 µL Bst DNA polymerase (Eiken Chemical Co., Ltd., Tokyo, Japan), 2 µL deionized water, 2.5 µL crude DNA extracted as discussed in previous section, 2 µL each of F3 and B3, 0.5 µL each of FIP and BIP and 1 µL each of LB and LF. The LAMP reaction was set at 63 °C for 60 min prior to the inactivation of Bst polymerase for 2 min at 80 °C.

2.3. Design and fabrication of the microfluidic CD

A microfluidic CD for automated Salmonella detection was designed using a computer aided design software (AutoCAD), as shown in Fig. 1. The microfluidic CD consists of top and bottom polymethyl methacrylate (PMMA) layers bonded with a pressure-sensitive adhesive (PSA) material, as shown in Fig. 1A. The microfluidic CD operations were engraved in the PMMA bottom layer using a Computer Numerical Control (CNC) machine as shown in Fig. 1B. The microfluidic CD operations and features in the PSA layer were cut using a cutter plotter machine. The microfluidic
chambers for mixing reagents, metering, isothermal amplification, and detection are engraved on the bottom layer of the CD (see Fig. 1B). The venting and loading holes for the LAMP assay, wax, and sealing material are located on the top layer of the microfluidic CD. When all three layers were completely fabricated, they were then aligned and pressed-bound together using a custom system to form a centrifugal microfluidic pathogen detection compact disc (CD).

2.4. Integrated microfluidic LAMP CD operation

After preparing the LAMP reagents as discussed in Section 2.2, they were loaded into the fabricated microfluidic CD in the loading chambers and the DNA was loaded to the amplification chamber as well. A customized CD spin test system was used for testing (see Fig. 1C), for more details about the component of the system and how it works, please refer to “Supplementary material”. The temperature for DNA amplification is produced by a cheap hot air gun on our system. Several experiments were conducted to optimize the required temperature from the hot air gun for isothermal amplification. This is done with the aid of a temp-plate sensor (Base Part No. 443, Palmer Wahl Instrumentation, Asheville, NC, USA) inserted near the amplification chambers to investigate the temperature difference between the surface of the microfluidic CD and inside the CD (LAMP assay temperature). The temp-plate sensor contains 3 calibrated temperatures of 60 °C, 65 °C and 82 °C. The color of a portion of the temp-plate sensor turns to black when its calibrated temperature is exceeded.

2.5. Reagent mixing and metering

As discussed in Section 2.3, the fluidic chambers for mixing and metering are located on the bottom layer of the microfluidic CD. The mixing chamber is located after the loading chambers to ensure that the LAMP reagents mix well. The first loading chamber contains the primers, Bst polymerase and deionized water (40 μL), whereas the other chamber contains the reaction mix (50 μL). The metering process consists of 4 chambers located after the mixing chamber. Amplification chambers are located after the metering chambers, as shown in Fig. 1B.

The microfluidic process starts after loading the LAMP reagents (50 μL of reaction mix and 40 μL of primers and Bst DNA polymerase) into the loading chambers and loading 2.5 μL Salmonella DNA to amplification chambers 1–3 while the DNA in chamber number 4 was loaded with deionized water as negative control. Then, the LAMP reagents are pumped through a zigzag channel that is purposely designed for mixing until they fill the mixing chamber. After that, they flow to the metering chambers, but do not yet enter the amplification chambers because the metering chambers and amplification chambers are connected through a narrow channel (pneumatic valve) that requires a high rpm speed for the LAMP reagents to flow to the amplification chambers. Subsequently, by increasing the rpm speed of the microfluidic CD to 1500 rpm, the LAMP reagents are transferred to the amplification chambers. Then,
the connection channel between the metering and amplification chambers is sealed (refer to Section 2.6) to prevent evaporation during the isothermal amplification.

2.6. System sealing process

The complete sealing of the microfluidic system is accomplished by the application of epoxy resins, such as Norland UV curable adhesives (NOA 61, NOA 75, NOA 85V, NOA 89, NOA 1315, NEA 121 and UVS 91; Norland Products Inc., Cranbury, New Jersey, USA). To prevent liquid loss evaporation during the isothermal amplification, the microfluidic CD must be sealed effectively. The sealing agent was pre-loaded into a sealing chamber when all LAMP reagents and DNA was loaded (see Fig. 2A). The UV curable adhesives are controlled by a portable UV light source (Opticure LED 200, Norland Products Inc., Cranbury, New Jersey, USA). Once the LAMP reagents were transferred to the amplification chambers, the sealing step is initiated. The sealing steps start with melting the wax to release the UV curable adhesives to flow to the sealing channels (see Fig. 2B). After that, the UV light source was applied on the UV curable adhesives to solidify them in the connection channels (see Fig. 2C). Several experiments were conducted to ensure the accuracy of sealing process before applying the method. The sealing process occurs when the UV curable adhesive in its liquid form is transformed into a solid form by exposure to UV light of 365 nm.

2.7. LAMP amplification

Once the microfluidic CD was completely sealed and secured, the LAMP amplification processes began. The automated process of this system starts with pumping of the LAMP reagents, mixing, metering, sealing, LAMP amplification and detection of the Salmonella. The hot air gun was powered ON, set at 170 °C during amplification and was raised to 250 °C to stop the amplification. The isothermal amplification took 60 min at 63 °C, and then the temperature was raised to 80 °C to stop the amplification. To validate the system sensitivity, a 10-fold serial dilution of the homogenate from a tomato spiked with Salmonella was conducted and compared with the conventional LAMP method and PCR. A specificity test was performed by introducing six different foodborne pathogens with one negative control and one DNA sample from a tomato spiked with Salmonella.

3. Results and discussion

The results are presented according to the sequence of experiments described in Section 2. The first section includes microfluidic CD operations, LAMP reagent mixing and metering, and the temperature profile, whereas the LAMP reagent amplification and sensitivity and specificity test will be discussed in the next two sections.

3.1. Microfluidic CD operations

3.1.1. LAMP reagent mixing and metering

Fig. 3 shows the sequence of the microfluidic CD operations, mixing and metering processes. The figure contains the real process images and schematics. The schematics shown are the same as real process images. In images it is hard to see the microfluidic processes due to the fact the LAMP reagents are transparent. As shown in Fig. 3A, the LAMP reagents were loaded into the loading chambers and pumped from there through the zigzag channel into the mixing chamber. The reagents converged at the mixing chamber and remained there for a few seconds to ensure that all the reagents were mixed thoroughly to avoid any failure of the DNA amplification (see Fig. 3B). Then, the LAMP reagents were pumped and filled the first two metering chambers (see Fig. 3C). Next, chambers 3 and 4 were sequentially filled (see Fig. 3D and E). The LAMP reagents did not yet enter the amplification chambers because of the connection channel (pneumatic valve) that was designed (less than 0.1 mm in depth and 0.2 in width) to keep the LAMP reagents in the metering chamber until it is full. Then, increasing the rpm speed caused the connection channel (pneumatic valve) to open, and the LAMP reagents flowed into the amplification chambers (see Fig. 3F). After all metering chambers were filled, no extra liquid flowed into the waste chamber because of the use of a precise metering technique. After the LAMP reagents were in transit to the amplification chambers, a sealing process was applied to the con-
connection channel to avoid loss and evaporation of the LAMP reagents during amplification. We pre-loaded Norland UV curable adhesives into the sealing chambers and controlled the process using a wax valve. Once the LAMP reagents were in the amplification chambers, we opened the wax valve by heating the surface of the microfluidic CD up to 60°C to melt the wax, enabling the UV curable adhesive to flow to the connection channel to seal the amplification chambers. Then, a handheld UV light source was powered on to cure the UV adhesives and solidify it in the connection channels (see Fig. 3F).

We present a microfluidic metering process that has several advantages when compared with the metering method performed by Mark et al. [39]. Their method required a high spinning frequency to create the turbulence at the air–liquid contact point for the liquid to flow into the destination chambers. Another advantage is that we
designed the metering chambers to include precise metering that is independent of the volume of the pre-loaded LAMP reagents and to have a maximum number of chambers. A further advantage of our metering method is that it can be accomplished at lower spinning frequency (less than 500 rpm). Moreover, the amplification chambers are connected to the metering chambers on the microfluidic CD, which adds an advantage by implementing a pneumatic valve at appropriate points. We used a pneumatic valve with a geometry of less than 0.1 mm depth and 0.2 mm width and a burst frequency between 600 and 1500 rpm, which are lower values than those used by Mark et al. [39].

We also introduced a sealing process by using Norland UV curable adhesive that solidifies in seconds and is extremely stable when not exposed to ultraviolet light. UV curable adhesives are sensitive to the whole range of UV light from 320 to 380 nm with peak sensitivity at approximately 365 nm, which is the peak sensitivity used in this study. The cure time depends on the light intensity and the thickness of adhesive layer. We used a handheld UV light source that is safe and easy to use with a peak intensity of 356 nm. It took approximately 10–20 s for the UV curable adhesive to solidify in the connection channel when the UV light source was held at a distance of 3 cm above the connection channel. Channels designed with a depth of 0.1 mm and a width of 0.2 mm are considered to be the best match to minimize the exposure time of the UV adhesive relative to a previous study [40]. We used this UV curable adhesive because it undergoes no viscosity increase during the amplification process because it can withstand temperatures of 150 °C, and we only used 63 °C in our amplification method. Furthermore, it is non-reactive with liquid crystal chemistry, even though we designed the sealing channels bearing in mind that the minimum dimensions of the channel could contain a thinner layer of UV adhesive, which leads to reduced exposure time. Through all the experiments, we detected no direct contact between the LAMP reagents and the sealing materials in the amplification chambers, as the amplification was successful.

3.1.2. Temperature profile

After the system was completely sealed, the power source (hot air gun) was turned ON and positioned exactly above the amplification chamber. The amplification process took approximately 60 min at 63 °C, and then the temperature was increased to 80 °C for 2 min to stop the amplification. The hot air gun was positioned 1 cm above the amplification chambers with a fixed temperature of 170 °C. The LAMP primers were designed to ensure that the primer melting temperatures ranged from 60 °C to 65 °C, thereby increasing the efficiency of the LAMP assay. Therefore, temperature was a critical parameter when performing the LAMP assay on the microfluidic CD, because we wanted to maintain the temperature between the range 60 °C to 65 °C inside the microfluidic CD so that the DNA amplification could be performed within the range. Although we can determine the surface temperature of the microfluidic CD continuously by a non-contact infrared thermometer, the temperature inside the microfluidic CD chamber would be lower than its surface temperature. Hence, we designed an experiment to determine the correlation of the microfluidic CD surface temperature and the temperature of LAMP assay inside the amplification chambers. In the experiment, a temp-plate sensor of three calibrated temperature was placed inside the microfluidic CD and heating was performed by the hot air gun.

When the hot air gun was initially turned ON, the microfluidic CD surface temperature increased dramatically within the first 8 min from room temperature of 25 °C to nearly 48 °C. The increase in temperature gradually slowed down after the first 10 min and approached a saturated asymptote within 15 min of heating the microfluidic CD at a peak of approximately 80 °C. The hot air gun was fixed at 170 °C for this experiment for a period of 60 min, and then it was increased to 250 °C for 2 min to stop the reaction. Likewise, when the hot air gun was turned OFF to cool down the microfluidic CD at a speed of 300 rpm, the surface of the microfluidic CD was found to have a rapid drop in temperature within the first minute. The difference between the microfluidic CD surface temperature and the inside temperature was analyzed according to the change in the temp-plate sensor temperature that was inserted inside the microfluidic CD. First, when the microfluidic CD surface temperature reached 75 °C, the temp-plate sensor color turned black, which means it exceeded 60 °C inside the microfluidic CD. Then, the microfluidic CD surface temperature range was between 75–82 °C when measured every 10 min. The inside microfluidic CD temperature range was observed to be maintained between 60 °C and 65 °C when the microfluidic CD surface temperature was measured every 10 min because there was no change in the color of the temp-plate. Finally, when the microfluidic CD surface temperature reached 100 °C the temp-plate sensor turned black, which means it exceeded 82 °C, and this was the temperature when we stopped the amplification. The data of all experiments and findings are presented in Fig. 4, in which the x-axis represents the time required for the LAMP assay, the y-axis represents the temperature, the blue line represents the microfluidic CD surface temperature measured with the IR thermometer, and the red line represents the calculated temperature inside the microfluidic CD (LAMP assay).

To ensure the repeatability of the heating profile for the system, we repeated the experiments three times. Fig. 4 shows the optimized temperature readings and the standard deviations for the three times that the experiments were repeated. The optimized average values of the amplification temperature on the microfluidic CD surface and inside the CD were found to be 82 °C and 64 °C, respectively. The standard deviation showed the same value as that indicated in the graph.

We used a forced convection heat source transfer as the heating block on our system because it offers distinctive advantages over the focused infrared (IR) lamp or laser light beam used in previous works [24,41–43]. Convection heat delivers heat only to the surface of the microfluidic CD, heating the amplification chambers underneath the top layer, whereas focused IR and laser light heating provide heat through all of the layers of the microfluidic CD, and causing unnecessary heating.

Through these experiments, we concluded that the temperature inside the microfluidic CD is equal to approximately 80% of the microfluidic CD’s surface temperature. It is also proven experimentally that the hot air gun source setting at 170 °C provides a temperature between 60 °C and 65 °C setting the hot air gun source at 170 °C provides the optimum balance between the LAMP assay completion without exposing the PMMA material to heat shock and causing shrinkage of the microfluidic CD.

3.2. LAMP assay amplification on microfluidic CD

As discussed in Section 3.1.2, the power source was turned ON and the LAMP assay reaction was initialized. First, the LAMP assay reaction took approximately 60 min at 63 °C, and then the temperature was increased to 80 °C for 2 min to stop the amplification. The CD spinning was stopped and left to cool down for 8 min. Then, the thermal sticker was removed from the surface of the amplification chambers to add 1 µL of SYBR Green I to each chamber for visual detection.

It can be seen from the results that the color of the SYBR Green I changed from orange to green in the first three chambers (as each chamber was pre-loaded with 2.5 × 10−3 ng/µL Salmonella DNA when all the LAMP reagents were pre-loaded to the CD), Chamber number 4 remained orange because was pre-loaded with 2.5 µL of deionized water (as negative control). This indicates that the three chambers with green color are positive results (Salmonella
detected), whereas the 4th chamber is a negative result (no *Salmonella* detected, see Fig. 5; is an expanded image of Fig. 3G).

Furthermore, the air bubbles (appearing) were only formed as the result of the removal of the thermal sticker (used to seal the loading holes of amplification chambers) in order to add SYBR Green I (using pipette) when detection process is performed. At this juncture, the air bubbles have no effect as reaction has completed. We have proven that loop mediated isothermal amplification (LAMP) can be integrated into a centrifugal microfluidic CD in a fully automated process from sample loading, isothermal amplification to detection. The whole microfluidic process operations were automatically performed on the microfluidic CD followed by isothermal amplification on the same setup (without moving, placing or stopping the CD Spin test system). Unlike the work done by Oh et al. [44] which has two disadvantages. First, when stopping the CD spin, this would cause the liquid to flow back to the channels and make the channel hydrophilic and that causes liquid loss. Second, when moving and placing the CD to another place (lab oven) this would raise the risk of contamination (to such sensitive assay). Furthermore, our system does not require any thermal cycler or lab oven for isothermal amplification, the cheap, integrated hot air gun in our system is enough to do the isothermal amplification on the same setup.

Our microfluidic CD was fabricated without the need for external pumps because the centrifugal force is sufficient to manipulate (automate the liquids by pumping, mixing and metering it) the liquids inside the microfluidic CD unlike other techniques [22,23]. The LAMP assay reaction and readout of results are simultaneously performed on the microfluidic CD. We used LAMP because it does not need complicated and expensive heating blocks. LAMP is only initiated once the temperature reaches 63 °C, unlike other isothermal amplification methods like RPA that function at lower temperatures (close to room temperature), which can cause a false reaction if the nucleic acid sample is premixed with the initiation reagents prior to compartmentalization [45]. We used an easy and fast detection technique because we used SYBR Green 1 which give results in seconds compared to other detection techniques that is complicated and time consuming, such as strip lateral flow sensor, which need at least 5 min to give confirmed results [24]. In our system, we avoided previously reported problems that can lead to the failure of the DNA amplification, such as cross contamination, and reagent evaporation, by completely sealing the connection channels with UV curable adhesive. This method prevents the commonly faced problems reported by other researchers designing DNA amplification channels [46]. The distinctive advantage of this microfluidic CD is that it does not need changes in the temperature profile that can introduce problems, such as bubble generation in the chambers. In this respect, the LAMP assay reaction is particularly compatible with the microfluidic CD (PMMA material). As an isothermal DNA amplification device, our microfluidic CD does not require a particular thermal cycling. A hot air gun alone is adequate for performing the LAMP assay reaction which is advantageous in resource-poor settings.

We have proven for first time that LAMP can be performed on the microfluidic CD for the detection of *Salmonella*. Because LAMP can be performed on the microfluidic CD, it can be further condi-

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Fig. 4. Microfluidic CD heating profile. Showing the temperature on the microfluidic CD surface and the LAMP assay amplification temperature inside the microfluidic CD (in the amplification chambers). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. LAMP amplification results. After the DNA amplification was stopped, thermal sticker that seal amplification chambers during amplification was removed and 1 μL of SYBR Green I added to each chamber. The green color demonstrates a positive result, whereas an orange color denotes a negative result. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
toned to be a platform for the detection of other organisms besides *Salmonella*.

### 3.3. Sensitivity and specificity

To further prove the successful operation and function of the microfluidic CD for *Salmonella* detection, we determined the detection limit of the LAMP assay using serial 10-fold dilution from $5 \times 10^{-1}$ to $5 \times 10^{-3}$ ng/µL of DNA extracted from a *Salmonella*-spiked tomato. The detection limit of the LAMP assay on the microfluidic CD was found to be $5 \times 10^{-3}$ ng/µL DNA, as shown in Fig. 6.

From the results in Fig. 6A, chambers 1–4 were found to be positive, as the green color indicates the positive results, and chambers 5–10 were found to be negative as indicated by the orange color, whereas chambers 11 and 12 were used as negative controls and produced the same orange color. Hence the detection limit was found to be in chamber 4, which has a DNA concentration of $5 \times 10^{-3}$ ng/µL.

We have compared the results obtained using our microfluidic CD with the conventional LAMP and PCR assay method. The sensitivity of conventional LAMP assay, PCR assay and LAMP assay on microfluidic CD were tested using 10-fold serial dilutions of DNA *Salmonella*-spiked tomato. We found that the same detection limit can be achieved by our microfluidic CD compared to conventional LAMP assay. The PCR assay can detect up to $3.4 \times 10^6$ CFU/mL *Salmonella*-spiked tomato while the detection limit of the conventional LAMP assay and LAMP assay on microfluidic CD on the same set of serially diluted spiked tomato was $3.4 \times 10^4$ CFU/mL which is 100 times more sensitive than conventional PCR. Each assay was repeated at least twice to ensure reproducibility.

The detection limit of the LAMP assay in our system was more sensitive than that of the PCR assay used in this study and LAMP assay used to detect *Salmonella* from pork with detection limit of $10^6$ CFU/25 g as reported by Techathuvanan et al. [47].

In addition, the specificity of the system was evaluated with the tomato samples spiked with *Salmonella* and six different bacterial pathogens (*E. coli*, *S. sonnei*, *L. monocytogenes*, *V. cholerae*, *Y. enterocolitica*, *A. baumannii* and *V. parahaemolyticus*) as well as deionized water (DW) as a negative control. Fig. 7 shows the detection results of each pathogen target. As shown, a positive result was found to be in chamber number 8 which has *Salmonella*. The other pathogens showed no color change, which was the same as the negative control sample. This confirms that our proposed microfluidic DNA detection method is specific and definitive for *Salmonella* DNA detection.

### 4. Conclusions

In this study, we have successfully developed a lab-on-a-disc device for the specific detection of *Salmonella*. The whole process, including the sample preparation, mixing of LAMP reagents, metering of the LAMP reagents, sealing, and amplification to the final detection of the target, can be integrated into a single microfluidic CD in an automated process, which shows great potential as a point-of-care diagnostic tool. The whole process took approximately 70 min, which is much shorter than the conventional method that requires 3–4 h, and the final detection of results was simply performed by direct visual observation of the color change of SYBR Green I. The frugality and versatility of the designed system (microfluidic CD) was credited to the use of a microfluidic operation process and the hot air gun used for the wax valve actuation and as a heating source. We have shown a new technique of doing loop mediated isothermal amplification (LAMP on a microfluidic CD). By using this technique, we have eliminated the use of external pumps required to manipulate the LAMP assay when doing LAMP on other platforms such as lab-on-a-chip because the centrifugal force is sufficient to manipulate the liquid inside the microfluidic CD which
makes the system easier. We introduced several microfluidic operations to automate the LAMP on the microfluidic CD. For example, we automated the microfluidic mixing of all the LAMP reagents, which eliminates the use of laboratory equipment such as vortex, PCR cabinet, pipette, PCR tubes and other mixing equipment. Another advantage of the automation process is metering the LAMP mixture to precise and equal volumes which eliminates human error when using a pipette that could cause contamination when using conventional method. These points limit the use of expensive equipment and reduce manpower needs, as all processes from the DNA preparation to the final detection can be done on a single microfluidic CD. The introduction of a cheap hot air gun also contributes to the reduced cost and versatility of the system through the introduction of localized forced convection heat for DNA amplification; which eliminate the use of expensive heating block, and wax actuation. We conclude that the increase in the microfluidic CD surface temperature can be controlled by setting the temperature of the hot air gun until we obtain the desired temperature. Experiments have shown that setting the heat source at 170 °C produces a temperature within the range 60–65 °C, as indicated by the color change of the temperature sensor inserted near the amplification chambers. Furthermore, forced convection heating has several other advantages, such as the possibility of achieving a uniform heating temperature on the microfluidic CD surface, which enables the stable heating of the target solution as opposed to heating all microfluidic CD layers by using focused infrared (IR) lamp or laser light techniques. Using convection heating also helped avoid overheating of the liquid or the material of the microfluidic CD itself and enabled powerful cooling of the microfluidic CD because it can be spun during or between the heating steps, which enabled rapid cooling effect of the microfluidic CD body. In addition, we have introduced a successful sealing process by using a UV curable adhesive that solidifies when exposed to UV light in a period of less than 15 s. These advantages introduced in the microfluidic operations and heat source of our compact system will make this device accessible and affordable to personnel carrying out field studies in developing countries.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2015.10.116.

References


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