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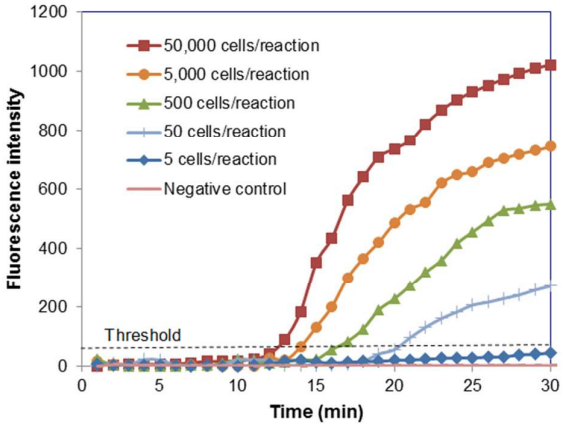

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Foodborne disease is a major public health threat worldwide. Salmonellosis, an infectious disease caused by *Salmonella* spp., is one of the most common foodborne diseases. Isolation and identification of *Salmonella* by conventional bacterial culture or molecular-based methods are time consuming and usually take a few hours to days to complete. In response to the demand for rapid on line or at site detection of pathogens, in this study, we describe for the first time an eight-chamber lab-on-a-chip (LOC) system with integrated magnetic beads-based sample preparation and loop-mediated isothermal amplification (LAMP) for rapid and quantitative detection of *Salmonella* spp. in food samples. The whole diagnostic procedures including DNA isolation, isothermal amplification, and real-time detection were accomplished in a single chamber. Up to eight samples could be handled simultaneously and the system was capable to detect *Salmonella* at concentration of 50 cells per test within 40 min. The simple design, together with high level of integration, isothermal amplification, and quantitative analysis of multiple samples in short time will greatly enhance the practical applicability of the LOC system for rapid on-site screening of *Salmonella* for applications in food safety control, environmental surveillance, and clinical diagnostics.

Introduction

Food safety remains a major public health threat in both developing and developed countries. Salmonellosis, an infectious disease caused by *Salmonella* spp., is one of the most common foodborne diseases worldwide. It is estimated that there are at least 100,000 salmonellosis cases per year in the EU countries[1], and approximately 1 million cases in the United States[2]. Syndromes of *Salmonella* infection may vary from gastroenteritis and diarrhea to severe life-threatening systemic diseases such as typhoid fever. Salmonellosis has an impact on society in term of morbidity, healthcare costs, and loss of productivities. As *Salmonella* is frequently found in large mammals, avian, reptiles and also in raw food products that come from animals, monitoring and surveillance control of *Salmonella* in food and food production lines are extremely important for food safety[3].

To date, the conventional bacterial culture method is still used as main workhorse and reference method for food safety control[4]. The methods involve several steps to obtain the final result: pre-enrichment, selective enrichment, isolation in selective agar, and serological and biochemical confirmation. The whole process usually takes 5 to 7 days to be completed. In the last few years, molecular diagnostic methods such as polymerase chain reaction (PCR) have been developed for the detection of *Salmonella* in much shorter time[5]. PCR allows for an accurate and unambiguous identification of target nucleic acid sequences. The detection limit of the method is 10 to 100 copies of purified genomic DNA and 10^3 to 10^4 CFU/mL for cultured samples[6]. Nevertheless, the use of PCR is limited by the thermal constrains. The PCR process needs an electrically powered thermal cycler with precise three stages temperature control and a fast transition between stages, which is usually accomplished by a bulky and power-intensive apparatus. Moreover, the whole procedures are costly, laborious, and prone to errors and contaminants[7].

Recently, an alternative approach the so called isothermal amplification has been developed to overcome the drawbacks of PCR[8]. The use of other polymerases allows nucleic acid amplification at constant and low temperature. There are a number of different isothermal amplification techniques, and among them, Loop-mediated isothermal amplification (LAMP) has attracted considerable interest due to its rapid amplification, simple operation, and high sensitivity and specificity[9]. LAMP is characterized by the use of four different primers, specifically designed to recognize six distinct regions on the target gene, which makes it highly specific for its target even in the presence of high concentration of non-target DNA. The process is performed at a constant temperature (60-65°C) using a strand displacement reaction, thus obviating the demand for sophisticated thermal control. Fast and efficient amplification can be achieved since there is no time required for temperature ramping during the LAMP process. Owing to these advantages, the LAMP

technology has been widely applied for rapid detection of different pathogens such as *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Staphylococcus aureus*, *etc.*[10]. A LAMP assay developed to detect *Salmonella* with sensitivity of 60 CFU/test has recently been reported [11].

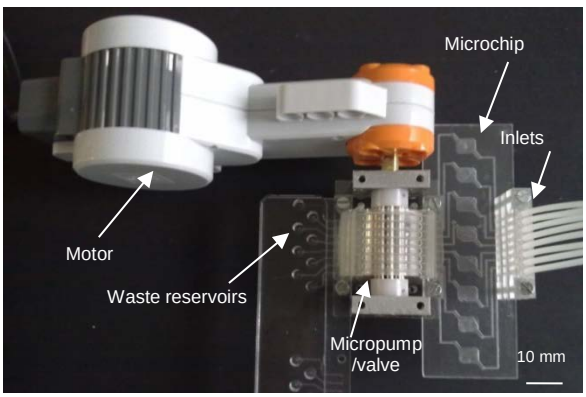
In order to comply with the demands from consumers for safe, pathogen-free food, there is an urgent need for development of a rapid and reliable method for on line or at site *Salmonella* detection. Adapting LAMP to Lab-on-a-chip (LOC) systems seems to be a very promising approach for monitoring pathogen at point-of-care (POC) settings[12, 13]. Tourlousse *et. al.* fabricated a chip containing 15 interconnected reaction wells with dehydrated primers for LAMP. The chip is designed to handle genomic DNA and could detect multiple pathogens, including *Salmonella, Campylobacter, Shigella* and *Vibrio choleae*, in less than 20 min[14]. A sensitivity of 10-100 gene copies/test was achieved by using real-time fluorescence detection. Fang *et. al.* reported another micro-LAMP system which adapted LAMP to a microfluidic chip for Pseudorabies virus analysis[15]. The readout was obtained via absorbance measurement by an optic sensor, giving a detection limit of 10 fg DNA/µl. The two systems demonstrated the feasibility of integrating LAMP on microfluidic chips for quantitative detection of pathogens. However, for these systems, both tedious sample preparation and nucleic acid purification processes were performed off-chip prior to the LAMP reaction. Wang *et. al.* reported a new diagnostic assay by combining nucleic acid extraction and LAMP in a magnetic bead-based microfluidic system for detection of *Staphylococcus aureus*[16]. The LAMP amplified products were analyzed by a spectrophotometer and the limit of detection was approximately 10 CFU/µl. Although in this work the sample preparation was integrated on chip, the complexity of system design and operation posed difficulties for the system to process more than one samples. The chip consisted of multi-layer PDMS structures and a glass substrate with metal electrodes, and required plasma treatment for bonding. The fabrication process was complicated and costly, therefore not suitable for mass production. Moreover, absorption measuring at end point was inadequate for accurate quantitative analysis.

Despite the fact that various LAMP-based LOC devices have been developed for rapid detection and identification of foodborne pathogens, portable platforms for on-site testing are still in the infancy stage[17]. Fully integrated LOC systems for multiple sample detection have seldom been reported. In this paper, we describe for the first time, a LOC system with integrated sample preparation and LAMP for rapid and real-time detection of *Salmonella* spp. in multiple samples. All of the steps from sample preparation, DNA purification to LAMP amplification were performed in one micro-chamber, which significantly simplified chip design and thus allowed for parallel analysis. By interfacing with an eight-channel peristaltic micropump, the developed microchip was capable of handling eight samples simultaneously. The real-time detection of LAMP products were achieved by using an appropriate fluorescence dye, which provided accurate quantification of *Salmonella* presented in different samples. The whole process took less than 40 min, and the detection limit of the system was 10 cells /µl. The microchip was made in low-cost polymeric material by injection molding which can be up-scaled for industrial production. The developed LOC system is suitable for on-site rapid detection of pathogens in food, environmental or clinical samples.

Experimental

Design and fabrication of the LOC system

The LOC system consisted of a disposable microfluidic cyclic olefin copolymer (COC) chip with eight chambers, and a reusable actuation unit with magnets, an eight-channel pump, a heater for LAMP amplification, and a small ESE log detector for real-time fluorescence measurement. Fig. 1 shows the details of the device. The microchip consisted of eight chambers in a microscopy slide format and was in-house fabricated by injection molding (ENGEL, PA, USA). The master was made in aluminum using computer controlled milling (Folken Ind, Glendale, California, USA). Each chamber had dimensions of 5 mm (length) x 4 mm (width) x 0.5 mm (height), corresponding to a volume of 10 µL. Microfluidic channels with widths of 500 µm and depths of 200 µm led from the chambers and connected these to 0.8 mm diameter inlet and outlet through holes. The COC slide was lidded with a 200 µm-thin COC film using a bonding press (P/O/Weber, Remshalden, Germany) at 120 °C and 2 bars for 10 min.



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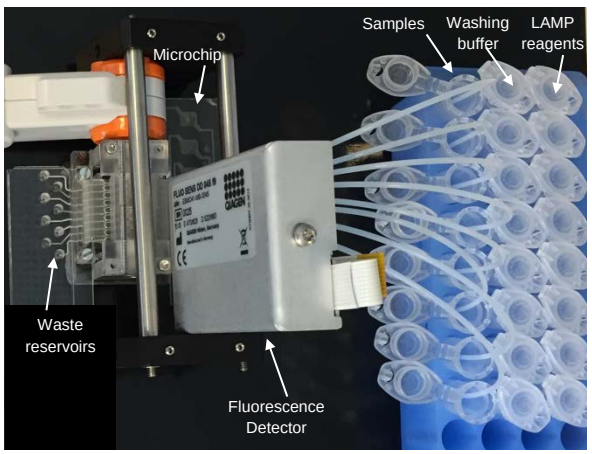


(b)

Fig. 1 (a) Integration of the COC microchip with the MainSTREAM micropump. (b) System overview. The LOC system consists of an eight-chamber microfluidic biochip made by injection molded on COC slide, an external heater element mounted under the microchip and a plastic frame containing eight magnetic elements was clamped on top of the chips for on chip sample preparation and LAMP amplification. An eight-channel peristaltic micropump was connected to the eight-chamber microfluidic chip. The samples and reagents were loaded to the micro-chambers through the plastic tubes connected at the inlets. The reservoirs at outlets were used to store waste. Mixture of BPW enriched pork meat sample and magnetic beads were pumped in to each chamber at flow rate of 50 µl per minute.

To handle multiple samples simultaneously, the COC microchip was interfaced to the eight-channel MainSTREAM micropump (Fig. 1a). The details of micropump fabrication were described elsewhere[18].

Briefly, the peristaltic micropump had three core components: a monolithic PDMS pumping inlay (PI) containing 8 integrated channels, a rotor bed (RB), and a multi-roller (MR). The PI was placed in the RB complementary in shape to the MR. The MR was driven by a stepper motor (Lego A/S, Denmark) which was originally designed for Lego Mindstorms robot. It comes with a programmable brick containing control software, USB communication port and rechargeable battery. As the MR rotated, it contacted and compressed the PI's channels and pushed fluid in the direction of rotation. The micropump could also function as microvalve when MR was kept static. The micropump/valve system was connected with the microfluidic chip through the PDMS inlay to address all the steps required for multiple-sample processing, including DNA purification and LAMP amplification. The other end of the PDMS inlay was coupled to waste reservoirs milled in a PMMA chip.

The system provided very easy world-to-chip interfaces (Fig. 1b). The samples, washing fluid and LAMP reaction mixture were simply placed in three rows of eight PCR tubes and loaded to the micro-chambers through the plastic tubes connected at the inlets of the chamber chip. During LAMP amplification, the plastic tubes were removed and the holes were sealed with PCR tape. In this system, only the eight-chamber microchip and the inlet plastic tubing were disposable, while the PDMS inlay and the chip containing waste reservoirs were re-usable.

Chemicals

All chemicals and reagents used in this study were of analytical grade and purchased from Pierce Inc., USA or Sigma-Aldrich, USA unless otherwise specified.

Bacterial strains and culture conditions

Salmonella enterica reference strain CCUG - 32352 originated from Culture Collection, University of Göteborg Sweden was provided by National Food Institute, Technical University of Denmark (DTU-Food). The strain was resuscitated and selected on Xylose Lysine Desoxycholate Agar (XLD) and grown overnight on Blood Agar (BA, 40 g/l of blood agar base no.2 (CM271, Oxoid, Basingstoke, UK) supplemented with 5% calf blood at 37 °C before use.

DNA preparation

The chromosomal DNA from *S. enterica* used for sensitivity study was isolated using DNeasy Blood and Tissue kit (Qiagen, Germany). The DNA concentration was determined by Nano drop (Thermo Scientific, USA). Two ng/µl of *S. enterica* genomic DNA was prepared to test the performance of LAMP on bench-top thermal cycler.

Spiked BPW enriched pork meat samples

Pig meat samples (1:10) enriched in Buffered Peptone Water (BPW) were collected from slaughters. At the slaughter, the samples were tested for the presence of *Salmonella* using both conventional culture and molecular methods as described in literature.[19] On arrival, the *salmonella* status of the BPW enriched pork meat samples was confirmed by TaqMan real-time PCR.

Briefly, 1 ml from the top phase of the BPW was collected and centrifuged at 3,000 ×g for 5 minutes and the supernatant was discarded. Next, the pellet was re-suspended in 200 µl of 1 x Tris-EDTA buffer and was heated at 96 ˚C for 10 minutes for DNA extraction. TaqMan real-time PCR targeting at *ttrRSBCA* locus was used to detect *Salmonella* spp.. All the negative samples were collected and stored at 4 °C for later use.

To prepare Salmonella-spiked samples, a serial 10-fold dilution of *Salmonella* in 1 ml of saline water (0.9% NaCl) was prepared from a *S. enterica* CCUG 32352 stock (OD_{600} = 0.8, corresponding to 10^8 cells/ml). Ten µl of *Salmonella* of each dilution was added to 90 µl of the enriched pork meat juice to give a final concentration of *Salmonella* ranging from 10^0 to 10^4 cells/µl. The *Salmonella*-spiked BPW enriched pork meat samples were used to test and evaluate the performance of the LOC system. A non-spiked sample was also included the experiment as a negative control.

LAMP on conventional thermal cycler

In-tube LAMP reactions were carried out on a conventional benchtop real-time PCR thermal cycler (ThermoFisher Scientific, MA, USA) at constant temperature of 65 °C for 30 minutes. The reactions were terminated by heating up to 80 °C for 2 minutes. Loopamp *Salmonella* Detection Kit from Eiken Chemical Co. Ltd (Tokyo, Japan) was used for the experiments. The kit consists of a set of specially primers for *Salmonella* invasion gene *invA* (Eiken Chemical Co. Ltd, Tokyo, Japan).

The LAMP master mixture contained 9.5 µl of Reaction Mix. Sal and 0.5 µl of *Bst* DNA polymerase. One µl of *Salmonella* DNA (2ng/µl) was mixed with the LAMP master mixture. In initial experiments, different DNA intercalating dyes, such as Eva green, SYBR Green I, SYTO-26, SYTO-62 and SYTO-82, were added to LAMP reactions to test the possibility to use the dyes for real-time LAMP detection. All dyes were purchased from Invitrogen (CA, USA). One µl of fluorescent dye (5 µM) was added to the LAMP mixture and fluorescent signals were recorded with 1 min intervals. End-point analysis of LAMP products was done by using 2% agarose gel electrophoresis with 1X TAE buffer.

Study of the inhibitory effect of magnetic beads on LAMP

A total volume of 20 µl, 50 µl, 80 µl, 100 µl, 150 µl and 200 µl magnetic beads solution were taken from Dynabeads® DNA DIRECT™ Universal kit (d = 2.8 µm, approximately 2×10^9 beads/ml, ThermoFisher Scientific, MA, USA) and transferred to PCR tubes. The respective tubes were centrifuged at 5000 ×g for 5 min and the lysis solution was discarded. Beads in all tubes were washed three times with sterile water. After washing, 10 µl of LAMP master mixture and 1 µl of 2 ng/µl *Salmonella* DNA were added into the tubes and LAMP was performed at above mentioned condition. The inhibition effect of the magnetic beads was assessed by gel electrophoresis.

On-chip detection of Salmonella using spiked samples

The feasibility of the LOC system for rapid and parallel detection of *Salmonella* was demonstrated using the serial dilutions of the *Salmonella* spiked BPW enriched pork meat samples. By integrating sample preparation and real-time LAMP (rtLAMP) amplification in one chamber, the LOC was able to handle eight samples at one time. The procedures were as following. Five µl of spiked samples were mixed with 20 µl magnetic beads suspended in lysis buffer (Dynabeads® DNA DIRECT™ Universal kit, ThermoFisher Scientific, MA, USA). The mixtures were transferred to the sample reservoirs on the microchip and pumped through the microchambers using the eight-channel micropump with flow rate of 50 µl/min. The DNA bound magnetic beads were then captured by the magnets

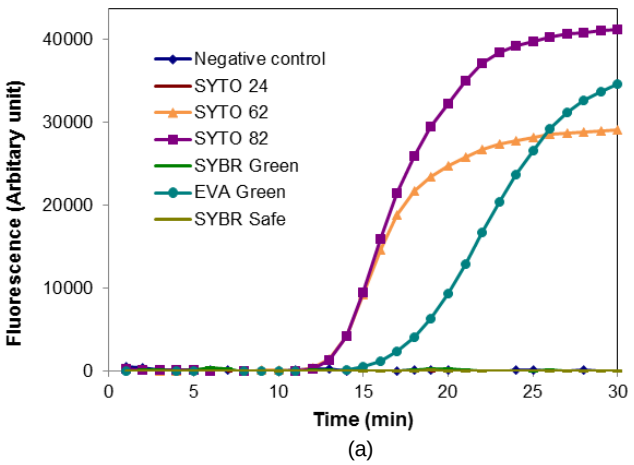
situated below the microchip. Right after, 200 µl washing buffer was pumped through each microchamber to remove any residual contaminants and potential rtLAMP inhibitors in the BPW enriched pork meat samples. Next the micro-chambers were dried by pumping air in for 1 min. The chambers were then filled up with LAMP reaction mixture (10 µl LAMP master mixture and 1 µl SYTO-62), and LAMP amplification was carried out by heating up the microchambers to 65°C for 40 min. Real-time monitoring of eight samples was accomplished by sequentially moving the ESE log detector (Qiagen, Germany) onto each microchamber. Fluorescent signals at 670 nm were recorded with 1 min intervals. For quantification, a concept of threshold time (T_t), which is similar to the threshold cycle (C_t) in rtPCR, was applied. The T_t was defined as the interpolated time at which the baseline-subtracted signal was equal to 10 times the standard deviations of the baseline signal, which is equivalent to a signal-to-noise ratio (SNR) of ten[14]. Standard curves were plotted for the dilution series.

Results and Discussion

Comparison of multiple DNA intercalating dyes for real-time LAMP amplification

Several studies have reported the use of the LAMP, and various detection methods have been developed to identify the amplified LAMP products. In most cases, LAMP was applied for pathogen screening, and a "yes-or-no" answer was adequate. For such applications, the simplest method for judging a positive or negative LAMP reaction is to access the turbidity of the solution by the naked eye[20]. For better visibility, a dye such as SYBR green[21] is added to the solution after the reaction is completed, and a colour change is observed under UV light for a positive LAMP reaction. These methods are very cost-effective, however, they are limited by the low sensitivity. In order to accurately quantify the amount of the pathogen, the reaction should be followed in real-time. This was possible by using a real-time turbidimeter[22] or by measuring the signals from DNA produced via fluorescent intercalating dyes[21, 23]. Fluorescent detection is by far the most sensitive method, however, in a previous study, the intercalating dyes were shown to partially or completely inhibit PCR-based amplification, which compromised the advantages of using fluorescence measurement[24]. To our best knowledge, only a limited number of intercalating dyes such as SYTO 9[21], SYTO 82[14] or SYBR Green[21] have been used rtLAMP. However, in all these studies, the inhibitory effects of the dyes on LAMP have not been evaluated. In order to select suitable dye candidates for rtLAMP detection, we screened six DNA intercalating dyes from different dye families and with a wide range of optical properties.

The inhibition effects of the respective dyes were investigated by carrying out LAMP on a conventional real-time PCR machine. Fig. 2 compares the performance of various intercalating dyes. From the real-time amplification curves (Fig. 2a), the T_t value was determined and used as an indicator of inhibitory effects on LAMP efficiency, since significant decreases in efficiency would result in increased T_t value. The dyes could be divided into three classes according to the degree of inhibition. SYTO-62 and SYTO-82 had very similar T_t value of 13 min, indicating that the dyes had the least effect on LAMP efficiency. Eva Green showed an increased Tt value of 16 min, suggesting that it partially inhibited LAMP reaction. In contrast, SYTO-24, SYBR Green I and SYBR Safe showed the clear negative effects on LAMP as no T_t could be determined. The effects of the respective dyes on LAMP were further confirmed by gel electrophoresis (Fig. 2b). Compared to the positive control, SYTO-62 demonstrated no change in the LAMP product, whereas SYTO-82 showed very high background noise, suggesting lower dye concentration should be used. Eva Green showed a large decrease in the amount of LAMP product, while for other dyes with high inhibition effect (SYTO-24, SYBR Green and SYBR Safe), no amplified product was observed. An appropriate intercalating dye is a fairly important component of real-time LAMP assay, as it could increase the sensitivity and reduce the false positive and false negative rates for pathogen detection. Our results strongly suggest that Eva Green, SYTO-24, SYBR Green and SYBR Safe are not suitable for use in rtLAMP. SYTO-62 and SYTO-82 are the good candidates, but care must be taken for the high background noise of SYTO-82. SYTO-62 was therefore used for rtLAMP for the rest of study in this paper.



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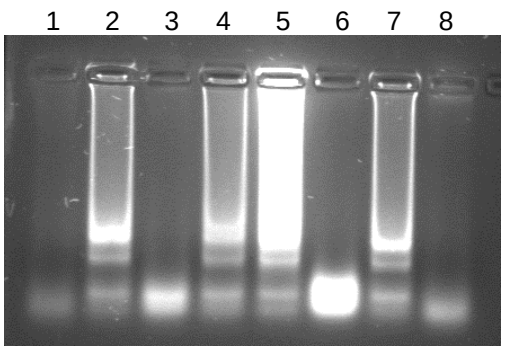

(b)

Fig. 2 Comparison of the effect of DNA intercalating dyes on LAMP efficiency. The reaction mixture consisted of 10 µl of LAMP master mixture, 1 µl of *Salmonella* DNA template (2ng/µl) and 1 µl of each fluorescence dye (5 µM). LAMP was performed on a conventional real-time PCR machine at 65 °C for 40 min. (a) Amplification curves in real time. (b) End point analysis of LAMP products using 2% agarose gel electrophoresis at 100V for 45 min. Lane 1: negative control; lane 2: positive control (without addition of dye to the LAMP reaction media); lane 3: SYTO 24; lane 4: SYTO 62; lane 5: SYTO 82; lane 6: SYBR Green; lane 7: Eva Green; lane 8: SYBR Safe.

Investigation of the inhibitory effect of magnetic beads on LAMP

Recently, a few LOCs have been developed for pathogen detection and some involved complex operations including total sample preparation processes, amplification, and finally detection[25]. However, to address the multiple steps involved in nucleic acid extraction and to avoid possible contamination to the downstream amplification, most of the published systems tended to perform DNA purification and amplification in two separate chambers[26, 27]. As a consequence, multiple pumps and valves were required to precisely control fluid transfer, which greatly increased the complexity of system design and operation, and hence limited the practical applicability of the LOC systems. Moreover, such two-chamber system also poses difficulties in processing more than one sample due to the constraints of the size and cost of the control elements. One efficient way to avoid these limitations is to combine sample preparation and LAMP amplification in a single chamber, and this requires a simple and LAMP-friendly DNA extraction method. Magnetic beads have proven to be effective in obtaining high sensitivity and selectivity when used to extract DNA from biological samples[28], however their potential inhibitory effect on LAMP amplification has not been investigated. In order to adapt the magnetic bead-based sample preparation method to our LOC system, we tested the efficiency of LAMP in presence of magnetic beads.

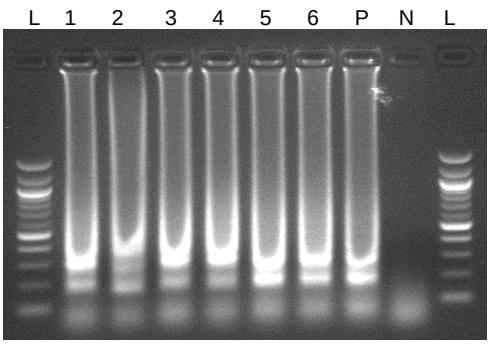

Fig. 3 Test of inhibitory effect of magnetic beads on LAMP reaction. 20 µl, 50 µl, 80 µl, 100 µl, 150 µl and 200 µl of Dynabeads® solution were centrifuged at 5000 ×g for 5 min and washed three times with sterile water. After washing, 10 µl of LAMP master mixture and 1 µl of 2 ng/µl *Salmonella* DNA were added into the tubes and LAMP was performed at 65 °C for 40 min and terminated the reaction at 80 °C for 2 min. L: ladder; lane 1: 200 µl; lane 2: 150 µl; lane 3: 100 µl; lane 4: 80 µl; lane 5: 50 µl; lane 6: 20 µl; P: positive control; N: negative control.

The LAMP amplification was carried out with the presence of magnetic beads at various concentrations. Fig. 3 shows the electrophoresis gel image of the LAMP products. The beads demonstrated no inhibitory effect at all on LAMP even at concentration of 4×10^{10} beads/ml (corresponding to 200 µl Dynabeads® solution). According to the manufacture's manual, 20 µl Dynabeads® can isolate at least 20 ng high quality genomic DNA, thus 20 µl beads should be sufficient to extract DNA from 5 µl spiked BPW enriched pork meat samples.

As magnetic beads are completely compatible with LAMP reaction, there is no need to elute the DNA and transfer it to a second chamber. After capturing DNA-magnetic bead complex and washing, the LAMP master mixture could be pumped directly into the chamber to perform the amplification. The integration of sample preparation and LAMP amplification in a single chamber greatly reduced the complexity of the microchip as well as the microfluidic control elements, which in turn increased the applicability of the system for on-line assay.

Real-time detection of Salmonella using the integrated LOC system

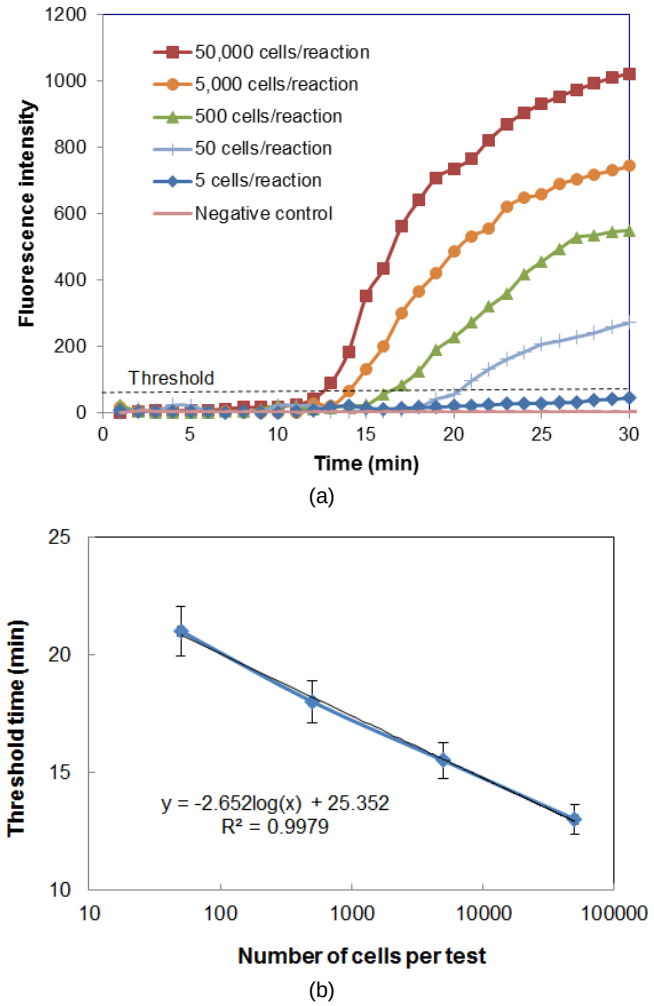

Fig. 4 Real-time detection of *Salmonella* on the integrated LOC system. Five spiked samples with concentration of *S. enterica* CCUG 32352 cells ranging from 10^0 to 10^4 cells/µl and one negative control were processed simultaneously on the eight-chamber microchip. (a) The real-time amplification curves for the six assays. The threshold was set at 10 times the standard deviation. The LOD was 50 cells/test. (b) The standard curve. It was determined based on the T_t values for different sample concentrations. The experiments were repeated three times.

The feasibility of the integrated LOC system was demonstrated by detecting *Salmonella* spp. in multiple *Salmonella* spiked BPW enriched pork meat samples. Five spiked samples with concentration of *S. enterica* CCUG 32352 cells ranging from 10^0 to 10^4 cells/µl and one negative control were processed simultaneously on the eight-chamber microchip. The real-time amplification curves for the six assays were shown in Fig. 4(a). The expected positive and negative signals were observed for all assays, implying that the chip was suitable for parallel analysis of several samples, without interference among assays. By setting the threshold at 10 times the standard deviation, the LOD system was able to detect as low as 50 cells/test, or 10 cells/ µl before sample preparation. Good linearity was observed between the T_t and logarithm of the amount of *Salmonella* cells per test (Fig. 4b), which showed that real-time fluorescence monitoring could be utilized for not only detection but also quantification of pathogens. The analytical sensitivity of the on-chip LAMP *Salmonella* assay was comparable to the conventional

PCR methods which can typically detect 1 to 10 cells/µl of cultured *Salmonella* cells[6]; however LAMP is superior to PCR in terms of simple thermal condition and short reaction time.

The successful detection of *Salmonella* spp. directly from the spiked BPW enriched pork meat samples using the eight-chamber LOC system were attributed to a number of factors. Firstly, the magnetic beads-based method was adopted for on-chip sample preparation. *Salmonella* nucleic acids were effectively captured by the magnetic beads; and with the freedom of particle manipulation by the magnetic fields, up to eight samples could be preceded at one time and downscaled to small sample volumes suitable for on-chip operation. Secondly, the magnetic beads were biocompatible with the LAMP polymerase. Since the beads showed no noticeable inhibition on LAMP, sample preparation and amplification were well integrated in a single chamber, which made it realistic for accomplishing parallel multi-sample analysis. Moreover, an efficient interacting dye SYTO-62 was selected for monitoring the LAMP amplification in real time. The low background and inhibition effects of the dye ensured high sensitivity of the on-chip LAMP assay.

Conclusions

In this study, we have developed a multichannel microfluidic system for parallel detection of *Salmonella* spp. in multiple samples. To our best knowledge, this was the first LOC system that integrated both sample preparation and LAMP for real-time detection of *Salmonella* spp. in food matrices. In contrast to conventional PCR assays which required 2-3 hours for DNA purification and amplification, the whole process using the LOC system described here could be accomplished within less than 40 min. The detection limit was 50 cells/test, comparable with the standard real-time PCR method. Of particular note, the stepper motor and the control unit from Lego were used for multi-channel pumping, which made the system small in size and easy to use. The simple design, low reaction temperature, quantitative analysis of multiple samples in a short time will eventually facilitate the use of this novel LOC system as a point-of-care device not only for fast screening of *Salmonella* or for other foodborne pathogens in food and animal production but also for clinical diagnostics and other applications in life sciences.

Acknowledgments

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Competing Interests Statement

The authors declare no competing financial interests.

Authors' Contributions Statement

Dang Duong Bang, Yi Sun, and Anders Wolff designed the research plan; Yi Sun, Than Linh Quyen, Tran Quang Hung and Wai Hoe Chin performed experiments; Yi Sun, Than Linh Quyen and Dang Duong Bang wrote the paper.

Notes and references

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