A newly developed polydopamine-coated paper-based microchip for rapid and highly selectivity detection of foodborne pathogens

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Abstract

Background

In 2020, Covid-19 pneumonia has had a great impact on human health in although the countries around the world, it brings serious threaten to people's lives and resulted in serious economic losses. At the same time, a lot news about the detection of Covid-19 in food emerges endlessly, a rapid and high selectivity detection method or technology is in urgent need for its ability to help relevant departments effectively control the epidemic situation and ensuring people's lives and property safety. In recent years, loop-mediated isothermal amplification (LAMP) has been certified as a quick and highly selective technique to detect foodborne microorganisms.

Results

In this paper, a newly developed microchip with polydopamine-coated paper based on LAMP was fabricated. This microchip consists of nine chambers for sampling and reactions, the targeted nucleic acid of foodborne pathogens was labeled by calcein fluorescence rather than SYBR. The microchip is advantageous of lower cost of materials and simple pretreated methods, and is easy to operate without the need for complex controlled fluid flow. The LAMP procedure and fluorescence detection of pathogens can be carried on the chip without opening the lid, preventing aerosol contamination and reducing the probability of false positives. In experiments, the LAMP reaction conditions including the optimal reaction temperature and reaction time are thoroughly discussed and have been executed for various foodborne bacteria samples, including Escherichia coli O157:H7 (E. coli O157:H7), Salmonella spp., Staphylococcus aureus (S. aureus), and Vibrio parahaemolyticus (V. parahaemolyticus). Testing of E. coli O157:H7 proved to be highly selective and sensitive (as low as 0.0134 ng μL^{-1}). Additionally, experimental test of real milk sample was figured, the complete detection duration time was within 68 min, the limit of detection(LOD) for Salmonella spp. was determined to be lower than 12 CFU mL^{-1}.

Conclusion

In summary, a newly developed LAMP microchip with polydopamine-coated and calcein fluorescence labeling paper-based provides a lower cost, easy to use, highly selective, and multiplexable pathogen detection capability with great promise as a rapid, highly efficient, and economical solution for future foodborne pathogen testing.

Background

As pointed out by the World Health Organization (WHO), foodborne pathogens are the primary threat to food security and lead to local and regional foodborne diseases and food poisoning outbursts[1]. There are 200,000,000 foodborne diarrhea cases globally each year, including 65% or more of cases originating

from foodborne pathogens[2]. Annual global statistics show that 1.8 million people die of intestinal diseases each year[3]. Therefore, there is an urgent need for rapid screening of foodborne pathogens for clinical diagnosis[4]. Conventional microbial detection methods depend upon culturing organisms in selective media followed by microbial identification employed morphological, biochemical, or immunological characteristics[5]. However, these methods typically require three to five days to confirm the presence of pathogenic microorganisms[6]. This time frame does not meet the need for rapid clinical diagnosis or identification in field food security investigations, especially in the cases of acute infection. Therefore, it is essential to develop a rapid and sensitive method that can be broadly applied for detecting foodborne pathogenic microorganisms.

Recently, detection methods based on nucleic acid amplification have been introduced to identify highly sensitive and selective pathogens[7]. These methods mainly focused on polymerase chain reaction (PCR)[8][9], rolling circle amplification (RCA)[10], helicase-dependent amplification (HDA)[11], nucleic acid sequence-based amplification (NASBA)[12], recombinant enzyme polymerase amplification (RPA)[13][14], and LAMP[15][16]. LAMP is a particularly promising pathogen detection technology that relies on a set of oligonucleotide primers to identify the target of interest by the increment of Bst DNA polymerase displacement activity [17][18]. One advantage of LAMP is its ability to amplify nucleic acid sequences with high sensitivity and selectivity under isothermal conditions (60–65 ℃)[19].

LAMP has been demonstrated to be effective for detecting a variety of pathogens, including S. aureus[20], Salmonella spp.[21], and V. parahaemolyticus[22]. However, conventional LAMP reactions involve multiple wet-bench operations and the use of expensive equipment. Microfluidic technology can overcome this limitation of conventional LAMP schemes, and the most recent LAMP technologies provide significant improvements by reducing reagent consumption and reaction time[23][24].

Common methods for direct LAMP detection include gel electrophoresis using ethidium bromide (EtBr) or fluorescence detection with SYBR Green I[25][26]. EtBr and SYBR Green I are sensitive and can directly test LAMP amplicons. However, EtBr and SYBR Green I are known to be genotoxic and frameshift mutagens, they are not safe for users, therefore, prohibiting the opening of lids during the entire process is essentially complied, which is the most important and effective measure to prevent amplicon contamination. Pyrophosphate is a by-product of the reaction and can be used as an indicator of LAMP success. In order to monitor pyrophosphate production, methods including turbidity analyzer[27][28], naphthol blue chromometer[29] and calcein fluorometer[30] have been proposed. The biggest advantage of these indirect methods is they can be performed without opening the lid, as a result avoiding aerosol contamination. Because the slight turbidity of pyrophosphate is difficult to be distinguished with the naked eye, the dye test method like calcein in this paper is employed due to its simple protocol, convenience and high sensitivity.

The original microfluidic chip processing technology originated from micro-electromechanical systems (MEMS) processing technology. Microfluidics require the use of precision microprocessing equipment in a clean room. The design and processing cost of a microfluidic chip is very high, seriously hindering their

application in analytical chemistry and life sciences. Today, single microfluidic chips made of standardized glass or polymer materials and produced by microfluidic technology companies in Europe and the United States cost between tens of dollars to hundreds of dollars. In purpose of developing a lower-cost microchip device, we chose polycarbonate (PC) as the chip material and processed the chip by laser ablation. At the same time, the cost both of the fluorescent detection reagents (calcein) and the UV Analyzer are also very low.

In this study, we developed a microchip that integrates DNA extraction, LAMP amplification, and chip detection characters. In particular, a polydopamine-coated paper was used to purify the genomic DNA (gDNA) of *S. aureus* from the milk sample, and low-cost calcein was used for indirect detection of LAMP procedure. Rapid and parallel screening of multiple pathogens (E. coli O157:H7, Salmonella spp., S. aureus, and V. parahaemolyticus) was performed in a single assay. In addition, we analyzed the sensitivity and specificity of microchips and provided a optimized technology for the detection of foodborne pathogens.

Results

Optimizing LAMP reaction conditions

In order to optimize the conditions of the LAMP reaction, firstly, the reaction temperature was set to 60℃, 65℃ and 70℃, and the reaction time was set to 15 min, 20 min, 25 min, 30 min, and 40 min. Fig. 1a shows electrophoresis results for LAMP at different reaction temperatures. These results clearly show that there is no scalariform band at 70℃.However, there are scalariform bands at 60℃ and 65℃, and the latter is clearer and brighter than that of 60℃. Fig. 1b shows the electrophoresis results of LAMP at different reaction times, which indicates that there are no scalariform bands in the first three lanes, but the bands from fourth lane become clear and bright.

As mentioned before, calcein has the ability to detect the products of LAMP reaction, for what we chose calcein as the detection reagent in this study. In order to test its reliability, we had figured the following experiments. Fig. 2a shows the reaction principle using calcein to test LAMP amplicons. Fig. 2b shows the results of the calcein method for LAMP testing with heating at 65℃ for 30 min. In the reaction mixture, strong fluorescence can be observed in the presence of the DNA template. However, fluorescence signal is invisible without the DNA template.

Multiple channel integrated inspection

Fig. 3a shows the results of a multi-channel microchip test with E. coli O157:H7, Salmonella spp., S. aureus, and V. parahaemolyticus respectively added in reaction chambers from #1 to #4 in turn, where positive results are indicated by green fluorescence, as compare, the other four chambers without primers are used as negative controls. Obviously, the controls have no green fluorescent light emitted. In order to certify the existing of foodborne pathogens, gel electrophoresis results shown in Fig. 3b, samples in the

chambers #1~#4 unfolded bright scalariform bands, while the control group chambers are very clean, which further displays the consistent results.

Investigation of sensitivity and specificity

Taking in consideration of the qualitative and quantitative analysis of the microchip ability, we conduct LAMP amplification on E. coli 0157:H7 DNA at different concentrations, and then read the result by fluorescent light and gel bands. Fig. 4a shows that the micro-device can detect E. coli 0157:H7 DNA with concentrations as low as 0.0134 , it is more sensitive than previous report of paper-based LAMP[31] marketed with other dye. At the same time, the result by Agarose Gel Electrophoresis (AGE) is shown in Fig. 4b. In chambers #1~#6, E. coli 0157:H7 DNA concentration reduces from 134 to 0.00134 , and scalariform bands are clearly detected in chambers #1~#5.

To study the specificity of the device, a sample of target template (E. coli 0157:H7) with a concentration of 0.0134 is injected for LAMP reaction. As shown in Fig. 5a, no fluorescence signal is detected in the reaction chamber containing primers for S. aureus, Salmonella spp., and V. parahaemolyticus. Fluorescence is only detected in the chamber containing E. coli 0157:H7 primers and the template. AGE was used to confirm the specificity of the LAMP test with 3 of solution from each reaction chamber. As expected, only the chamber containing E. coli 0157:H7 primers and DNA template shows scalariform bands as shown in Fig. 5b.

Experimental application of real milk sample

Finishing the basic test of microchip, we successfully detected Salmonella spp. in real food sample. As shown in Fig.6a, after adding Salmonella spp. and diluting to different concentrations, milk samples are figured in below concentrations: 1.2×10^4, 1.2×10^3, 1.2×10^2, 1.2×10^1, and 1.2×10^0 CFU mL^{-1}. The sample with bacteria was injected in the first two chambers for every microchip, the other six chambers are of control groups. The next all sample undergone the same LAMP reaction and calcein marking, the results shown that the fluorescence intensity of chambers #1 and #2 is the same for every chip, but reduces rapidly as bacterial concentration reduces. The negative controls in chambers #3~#8 show negligible fluorescence signals. The minimum of concentration determined from the chips is approximately 12 CFU mL^{-1}. In the practical application of pathogenic bacteria detection when food poisoning occurs, the content of pathogenic bacteria in general samples is above 10^3 CFU mL^{-1} as the national standard required. The detection limit of our microchip is able to meet the requirements of lowest limit of detection.

Fig.6b shows the AGE test result, as the Salmonella concentration is reduced, the brightness of the resulting scalariform bands are accordingly reduced. The minimum detection concentration is consistent with the results on the microchip. The total duration time from sample extraction to fluorescent detect is also illustrated respectively in table 1. There are approximately 68 min in total, which is much comparable to previous study[32] with microchip LAMP, who used a centrifugal device rather than paper based method.

Table1 Time required for each operation step when performed using the introduced microchip

Procedure	Extraction	Amplification	Detection	Total analysis
Time	35min	30min	3min	68min

Discussion

Optimizing LAMP reaction conditions

As Fig. 1a shows, the blank lane at 70℃ signifies that LAMP reaction does not occur at 70℃. According to the bands clearer and brighter at 65℃ than 60℃, 65℃is deemed to be the optimal temperature for the reaction. Similarly, the brightest bands occur at 30min lane in Fig. 1b indicating that it is the optimize reaction time.

Thermal denaturation of the DNA template is avoided at initial stage of LAMP reactions, thus shortening the reaction start time. Because the LAMP amplification template has a dumbbell-shaped structure and contains multiple amplification start points, the amplification process can be conducted simultaneously to improve efficiency. While PCR needs at least 90 min for a complete amplification reaction, LAMP only needs 30 min to complete the reaction, significantly reducing testing time[33].

Fluorescence detecting of LAMP reaction by calcein

According to a previous report[34], if magnesium ion is added to calcein before LAMP reaction, the green fluorescence of calcein will be quenched and the dye will become orange. After LAMP amplification, the pyrophosphate and manganese ions generated by the reaction combine and deposit, the magnesium ion will have the opportunity to combine with the calcein and affect the fluorescence signal of calcein. In such a case, the color of a positive detector tube is observed as green fluorescence, as opposed to the initial orange red color, and a negative detector tube will remain orange red. Altogether, the final result will be strong green fluorescence in a positive reaction and weak green fluorescence in a negative reaction when stimulated by 365 nm blue light as shown in Fig. 2a. As Fig. 2b shows, the results acquired from the paper is consistent with the results from the tube, there is also fluorescence signal on the paper containing the DNA template. Additionally, when acquiring the fluorescence spectrum of the amplified solution as a reference, there is a significant difference between the tubes before and after LAMP reaction.

Multi-channel parallel inspection

A single test was able to detect four types of food-borne bacterial pathogens via our paper based LAMP microchips as Fig. 3 shows, providing a simple and effective substitution test for harmful microbes. These results verify that the microchip is an ideal tool for rapid mixture of samples and amplification reagents without the need of complicated valves. Additionally, the LAMP test on the microchip provides a simpler and more accurate diagnosis tool for detecting of multiple pathogens than routine PCR-based

methods. Given the experimental properties of LAMP, a single-temperature heater can be used rather than complicated heating equipment. Additionally, compared with direct detection and tests on LAMP amplicons using SYBR Green I and Fisetin[35], this method greatly reduces the probability of false positives through the use of calcein. Calcein overcomes the limitations of direct tests on LAMP amplicons, and we have known that SYBR Green I and Fisetin require reaction tubes to be opened after the LAMP reaction which is complete, introducing the possibility for aerosol contamination. Moreover, SYBR Green I is a latent human mutagen.

High sensitivity and specificity

The first method is to use the fluorescence detection to evaluate the sensitivity of the microchip. After generating the LAMP products and when irradiated by UV light, the pyrophosphoric acid ions in the LAMP solution and Mn^{2+} combine and release calcein, causing the fluorescence to turn from colorless to green. As the concentration of DNA template is reduced, the pyrophosphate ions are also reduced, leading to lower fluorescence intensity from positive reactions which is the principle of the phenomenon in Fig. 4a. AGE is the other method for sensitive testing, the brightness of the band depends on the concentration of DNA, so as the concentration of DNA template reduces, the brightness of band reduces accordingly which is consisted with Fig. 4b, and the minimum detection result is the same as the on-microchip detection result. In reaction chambers #6, #7, and #8, there are no obvious scalariform bands and the visible band is a primer dimer, which is common in LAMP analysis by AGE[36].

Compared with the traditional PCR method, four primers in the LAMP system must respectively match with six or eight specific areas of the target gene to produce a reaction, however the PCR system needs only one primer in the upstream and downstream regions to match with the target gene. For that reason, the LAMP reaction for a sample with E. coli O157:H7 can only occur in the reaction chamber who contains E. coli O157:H7 primers as Fig. 5 present. This indicates that LAMP has higher specificity[37].

Experimental application of real milk sample

Dopamine (DA) is a functional biomolecule found muscle adhesion proteins that can polymerize under alkaline conditions to form polydopamine (PDA), which can readily adhere to many organic and inorganic materials[38][39]. What's more, the PDA material shows excellent hydrophilicity and biocompatibility, because it has many functional groups (catechol hydroxyl, amino, imine, quinone) that can react with many molecules. DNA extraction would be advantageous[40].

Salmonella spp. DNA purified from milk by polydopamine has the following characteristics: the quinone gene group in the polydopamine-coated paper reacts with the milk and calcium ions in the solution via a schiff base reaction and chelation reaction. When the microchip rotates, the purified DNA is dispersed to each reaction chamber while the rest of the milk components remain in the sample chamber, as verified by the appearance of scalariform bands indicating LAMP amplicons. That is to say, the paper with polydopamine has the function to remove the inhibitor of LAMP reaction. And the results in Fig.6a, Fig.6b

certifying our microchip has the ability to detect the real sample to insure there is or is not foodborne pathogens.

Conclusions

In summary, our microchip can be used to detect foodborne pathogens with high sensitivity and selectivity. This method has several advantages. First, utilizing the hydrophilic virtue of regenerated cellulose paper, all components except DNA can be loaded onto the paper sheet without the need for complicated pumps or valves of microfluidic system. Second, the microchip can be reused simply by replacing the paper parts and sterilizing with UV irradiation. Third, the microchip can simultaneously detect multiple samples with high sensitivity and selectivity. For bacterial samples of E. coli O157:H7, the sensitivity is 0.0134 ng μL^{-1}. For the detection of Salmonella spp. in milk samples, the sensitivity is 12 CFU mL^{-1}. Fourth, it provides direct detection of successful target amplifications, enabling faster identification of pathogens (approximately 68 min) than was previously possible. Therefore, the newly developed microchip provides a promising platform to detect multiple targets simultaneously. With appropriate modifications to the reagents, the microchip can be used for nucleic acid analysis in other fields, such as single nucleotide polymorphism identification, genetic diagnosis of clinical samples, and infectious disease monitoring. In addition, due to the convenient design and manufacturing of PC microchips by computer engraving technology, more microchannels and reaction units can be integrated into one chip to detect a large number of DNA targets simultaneously. The LAMP reaction can generate a visible signal based on an increase in calcein fluorescence added before the reaction during the amplification process. In the future, by integrating microfluidic modules (especially DNA extraction modules and optical imaging modules) on a small instrument, the reported method will be generally useful in the fields of foodborne pathogen detection.

Materials

Materials

A laser engraver (KB-4060) was bought from Liaocheng Keba Laser Equipment Co., Ltd. (Shandong, China). Fluorescence spectrum was acquired using a NanoDrop 3300 fluorescence spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Thin polycarbonate (PC) sheets (thickness: 1 mm) were purchased from Shanghai Chenchuang Plastic & Rubber Technology Co., Ltd. (Shanghai, China), sealing membranes were purchased from Rongxin Packaging Material Co., Ltd. (Shenzhen, China). Regenerated cellulose membrane filters with 0.2 μm bore diameter were purchased from CHMLAB group (Barcelona, Spain). The heater (XH-RP5050) was purchased from Jiangsu Xinghe Electronics Co., Ltd. (Jiangsu, China), andthe rotator from Jiangsu Xinkang Medical Equipment Co., Ltd. (Jiangsu, China). The UV Analyzer (ZF-7A) was purchased from Shanghai Qinke Analytical Instrument Co., Ltd. (Shanghai, China). Polydopamine was purchased from Sigma Aldrich (St. Louis□USA). Aseptic paraffin oil was purchased from Hengkang Medical (Hebei, China). LAMP kits containing detection reagent (Bst DNA polymerase and primer), reconstitution fluid (10x isothermal amplification buffer solution, dNTP mixture, 100mM

MgSO4), colorimetric indicator (calcein, including Mn^{2+}), a positive control (target bacteria DNA), and a negative control (non-objected bacteria DNA) were purchased from Guangdong Huankai Microbial Sci. & Tech. Co., Ltd. (Guangdong, China). Biowest agarose and loading buffer were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). A fully-automatic gel-imaging analysis system (Shanghai, China) was used to test the target band. 100bp DNA marker and genomic DNA extraction kit were purchased from Takara (Shiga, Japan).

Bacteria preparation and DNA extraction

E.coli O157:H7, Salmonella spp., S. aureus, and V. parahaemolyticus were obtained from the Institute of Hygiene and Environmental Medicine (Tianjin, China). E. coli O157:H7, Salmonella spp., S. aureus, and V. parahaemolyticus were cultured overnight in 5 mL Lysogeny Broth (LB) (37℃, 200 rpm oscillation). gDNA was extracted from 1 mL of culture solution using the DNA purification kit. gDNA concentration and mass was determined by UV-visible spectrophotometer and NanoDropTM spectrophotometer. gDNA was then stored at -20℃ for future use.

Optimizing LAMP reaction conditions

To optimize the reaction temperature and time of LAMP on the microchip, the reaction temperature was set to 60℃, 65℃ and 70℃. The effect of temperature on the reaction was then determined by the resulting fluorescence intensity. This approach was used to determine the optimal reaction temperature. Optimal reaction time was determined in a similar manner. The reaction time was set to 15 min, 20 min, 25 min, 30 min, and 40 min, and optimal reaction time was also determined based on fluorescence intensity.

Testing calcein fluorescence

As previously reported[41] mix 25 umol L^{-1} calcein with 300 umol L^{-1} manganese chloride to quench the fluorescence of calcein. Add the quenched calcein to the LAMP reagent, then shake the solution and observe it under UV radiation. Next, remove the solution and place it in a 65℃ water bath for 30 min, then observe the solution under UV radiation again. To test the fluorescence of calcein on paper, soak the paper in the quenched calcein solution and let it dry at room temperature. After drying, check the paper under UV radiation. Last, use the paper dipped in calcein to test the LAMP byproduct.

Microchip fabrication

The portable microfluidics chip used for multichannel LAMP testing is made in two layers. The 40×40×1 mm PC board is composed of eight reaction chambers, each having a radius of 2.5 mm. Reaction chambers are connected to the center chamber, which has a radius of 8mm, by a 5 mm micro-channel that is 0.5 mm deep. The total volume of each reaction chamber and sample chamber is 10 μL and 100 μL, respectively. The hole at the cavity position is made by a computer-aided direct current engraving machine and the upper part of the PC plate is sealed by the sealing membrane. First, cut the cavity and

the channel on the PC plate shown in Fig. 7a. Second, embed the regenerated cellulose paper plate with 0.2 μm pores with LAMP reagent stored in the reaction chamber in Fig. 7b. Third, attach the upper-part of the sealing membrane to the top of the PC plate as Fig. 7c. The device is then ready for amplifying various DNA templates from the sample as Fig. 7d.

Micro-device manufacturing

Use the LAMP kit from Guangdong Huankai Microbial Sci. & Tech. Co., Ltd. which contains primers for the target bacteria. Because there is no need for self-designed primers, the experimental workflow can be further simplified. To amplify and test multiple DNA templates with the device, soak each kind of paper in the reaction chamber with different primers, keep each reaction chamber containing the dry LAMP reagent. Then amplify the paper plate containing different target DNA primers and calcein. Inject the mixed solution containing template DNA of E. coli O157:H7, S. aureus, Salmonella spp., and V. parahaemolyticus into the sample chamber via the inlet on the upper layer of the sealing membrane. Then position the device and set the rotator's velocity to 4000 rpm to uniformly push the sample solution into the reaction chamber via centrifugal force. After completing the rotation step, bring the sample solution to 10 μL in each reaction chamber, then place the device on the portable heater and perform the LAMP reaction at the optimal reaction temperature and time. Store the reagent with the paper plate before placing it in the cavity, doing so eliminates the need for steps involving sample and reagent injection, which is different from the complicated design of other technologies that rely on different rotating speeds.

On-microchip LAMP test

Prior to starting the reaction, place the paper plate containing quenched calcein into the reaction chamber. After the reaction is complete, the pyrophosphate ions and manganese ions combine to show the fluorescence signal of calcein under UV radiation. To verify the test, LAMP amplicons were subjected to AGE for 30 min, and then photographed under transparent UV radiation using the Bio-Rad Molecular Imager Gel Chemi Doc XR imaging system.

Sensitivity and Specificity testing

Test the sensitivity by performing a continuous dilution 10 times of the initial concentration of pathogen gDNA to determine the sensitivity of the microfluidic device's visual inspection of LAMP amplicons. Use the UV-visible spectrophotometer to measure gDNA concentration by the following equation: DNA concentration = F×A260×molar absorption coefficient (ng μL^{-1}), where F is the dilution ratio of the original DNA solution before measurement and A260 is the absorbency reading at 260 nm. The molar absorption coefficient of double-stranded DNA is 50 ng μL^{-1}. Use only E. coli O157:H7 gDNA to evaluate the sensitivity of the device and verify the results by AGE for 30 min.

Use the microfluidic device to test the specificity of the LAMP test for gDNA at the lowest detectable concentration based on the sensitivity experiment. Use only the E. coli O157:H7 gDNA to evaluate the

specificity of the device. Place the primers for E. coli 0157:H7, Salmonella spp., S. aureus, and V. parahaemolyticus in chambers 1-4, respectively. Inject the template DNA of E. coli O157:H7 into the central sample chamber and use chambers 5-8 as negative control chambers. Transfer the solution in the central sample chamber to the reaction chambers via centrifugal force, then heat the device for reaction on the heater at 65℃ for 30 minutes. Take 3 uL of the reaction solution to verify amplification by AGE after the reaction is complete.

Using real samples for on-chip analysis

Insert the paper coated with polydopamine into the central sample chamber to purify DNA from the degenerative milk solution. First, add the Salmonella spp. bacteria solution into the milk and incubate at 37℃ for 12 hours. Then heat the degenerative milk at 90℃ for 5 min to destroy bacterial cell walls. Next, incubate the solution at room temperature for 30 min to prepare the bacterial sample and polydopamine-coated paper for sufficient reaction. Lastly, apply centrifugal force to distribute the purified DNA solution to each reaction chamber, after loading the sample, conduct the on-chip LAMP reaction and the follow-up fluorescence detection. See Fig. 8 for the entire operation workflow.

Abbreviations

LAMP: Loop-mediated isothermal amplification; E. coli O157:H7: Escherichia coli O157:H7; S. aureus: Staphylococcus aureus; V. parahaemolyticus: Vibrio parahaemolyticus; WHO: World Health Organization; PCR: polymerase chain reaction; RCA: rolling circle amplification; HAD: helicase-dependent amplification; NASBA: nucleic acid sequence-based amplification; RPA: recombinant enzyme polymerase amplification; EtBr: ethidium bromide; MEMS: micro-electromechanical systems; PC: polycarbonate; UV: ultraviolet; DNA: Deoxyribonucleic acid; AGE: Agarose Gel Electrophoresis; CFU: Colony-Forming units; LOD: limit of detection; DA: Dopamine; PDA: polydopamine; gDNA: Genomic DNA.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All the data required is included in the manuscript.

Competing interests

The author declare that they have no competing interests.

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Authors' contributions

JL and MZ carried out the sample preparation and experiments. YZ and YF gave experiment guidance and carried out the study design. ZS and YL help sample preparation and data analysis. YS and LN drafted the manuscript. JL and MZ wrote the manuscript. All authors read and approved the final manuscript.

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Figures

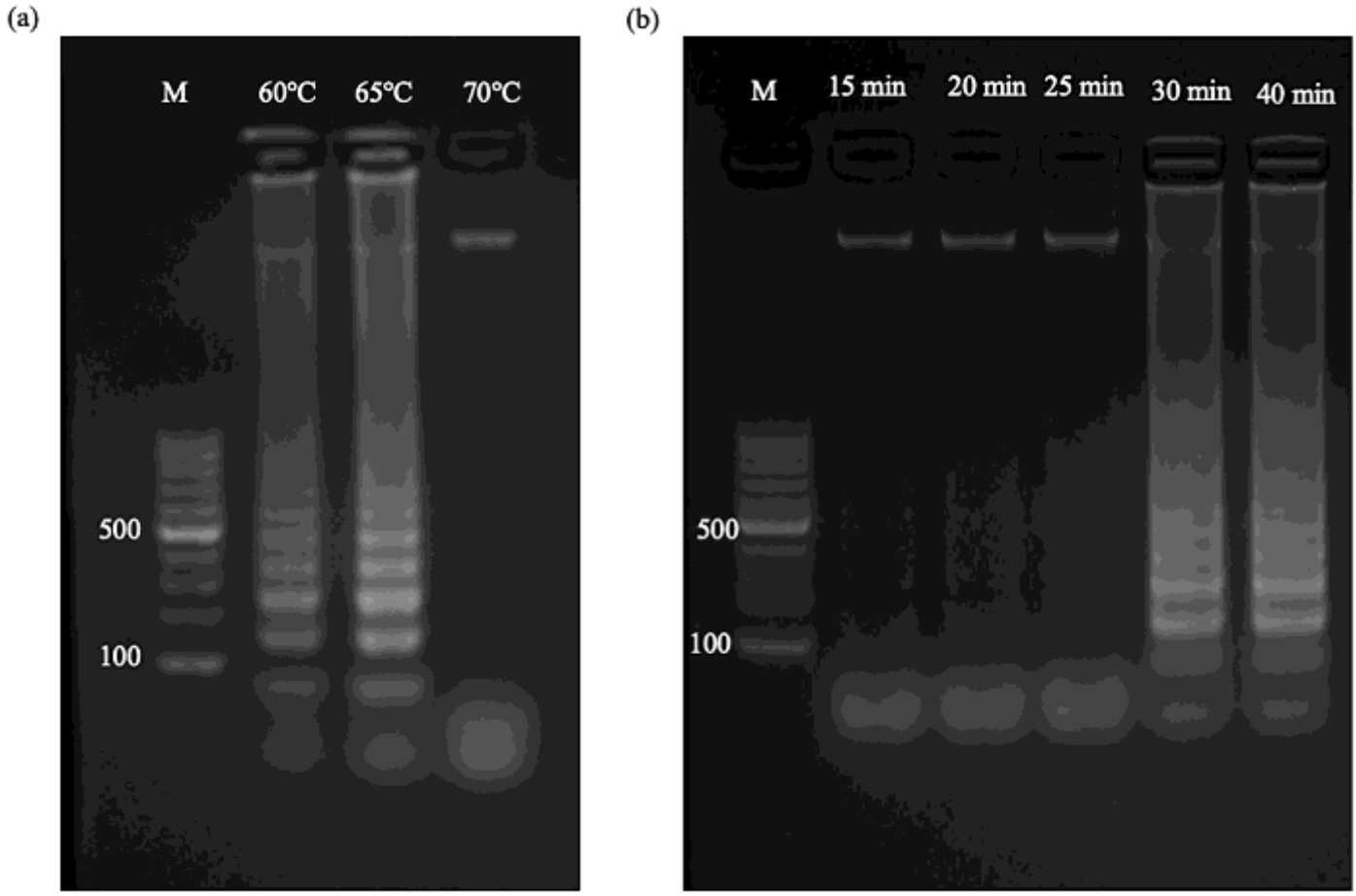

Figure 1

Results of optimizing LAMP reaction conditions (a) Optimization of reaction temperature: the lane 1~4 is respectively the electrophoresis result of market, or at 60℃, 65℃ and 70℃; (b) Optimization of reaction time: the lane 1~4 is respectively the electrophoresis result of market or at 15 min, 20 min, 25 min, 30 min and 40 min.

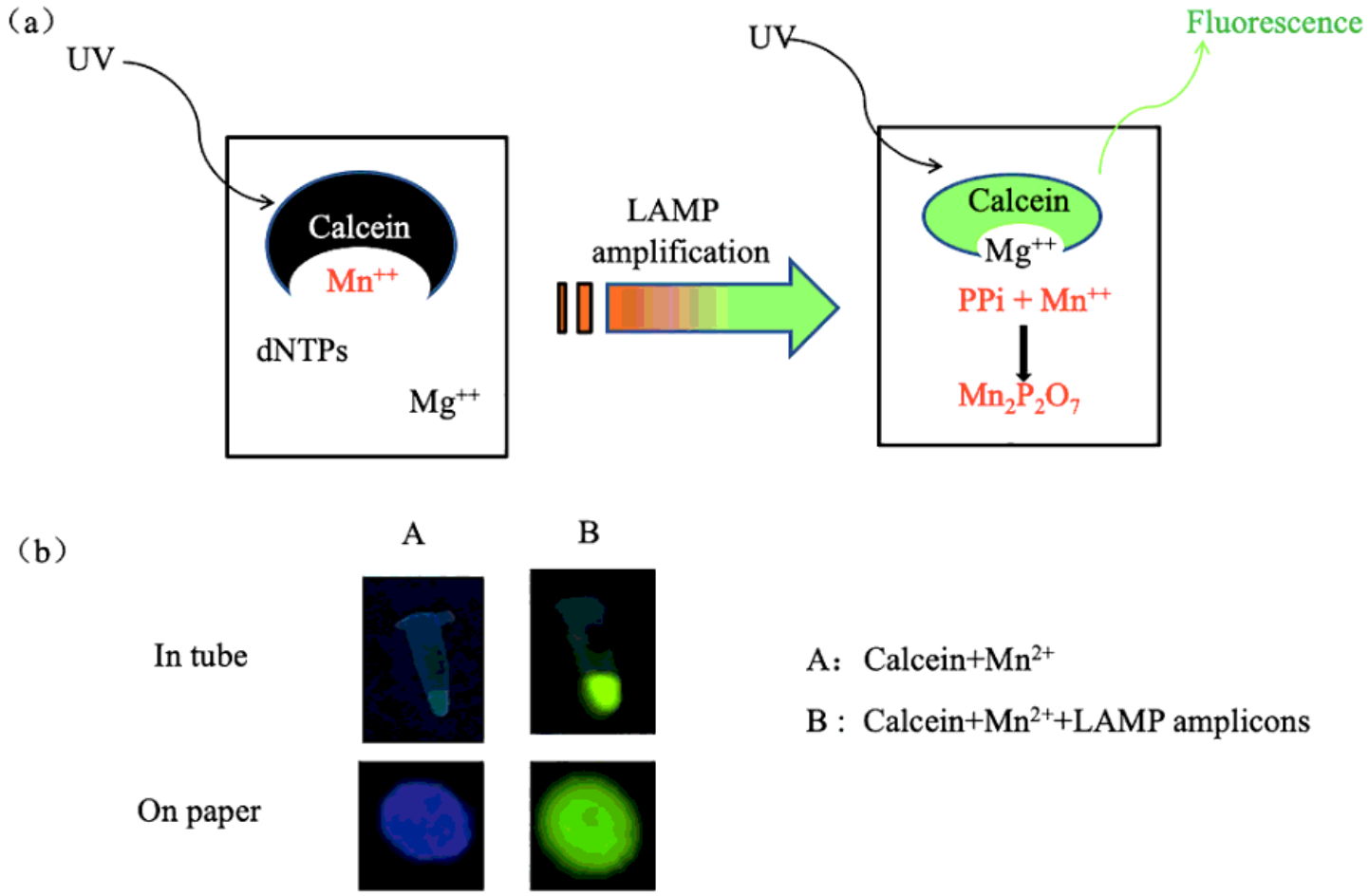

Figure 2

Testing of fluorescence signal. (a) Inspection mechanism of calcein; (b) Change in solution color when pyrophosphate ion exists after LAMP reaction.

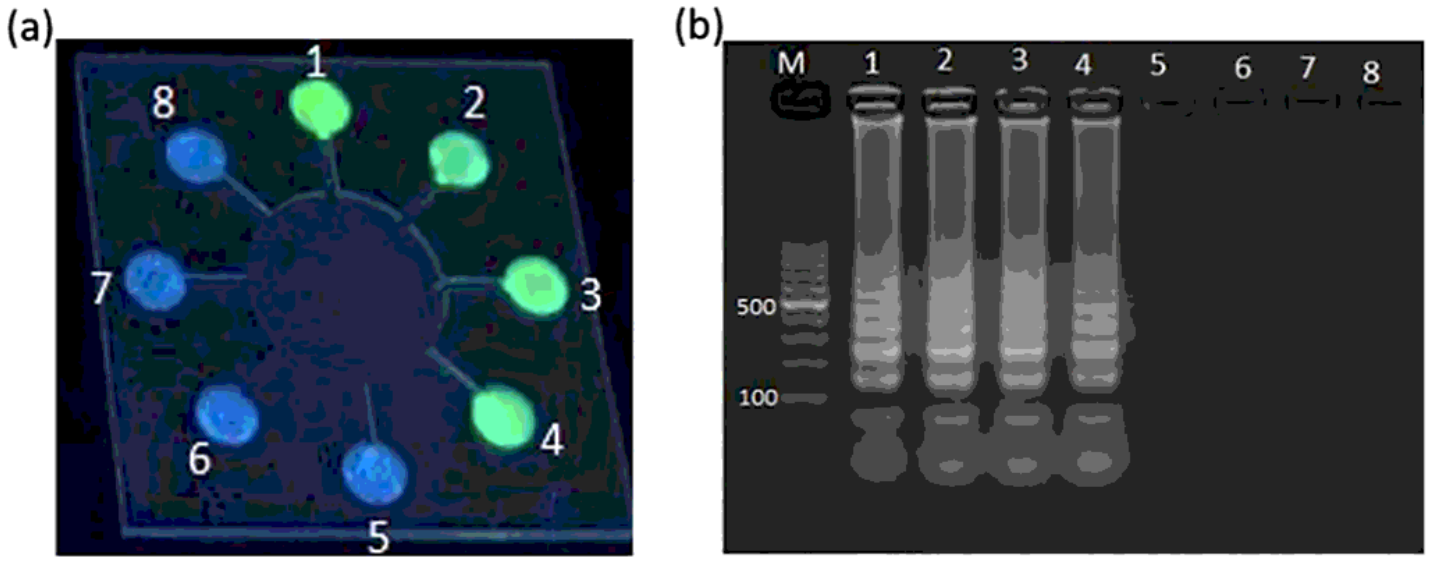

Figure 3

On-chip amplification of the four targets. (a) Microchip images of four food-borne pathogens detected simultaneously. The 1-4 chambers contained primer sets for E. coli 0157: H7, Salmonella, Staphylococcus aureus, and Vibrio parahaemolyticus, and the 5-8 chambers were negative controls. (b) Results of gel electrophoresis (the lane number corresponds to the chamber number).

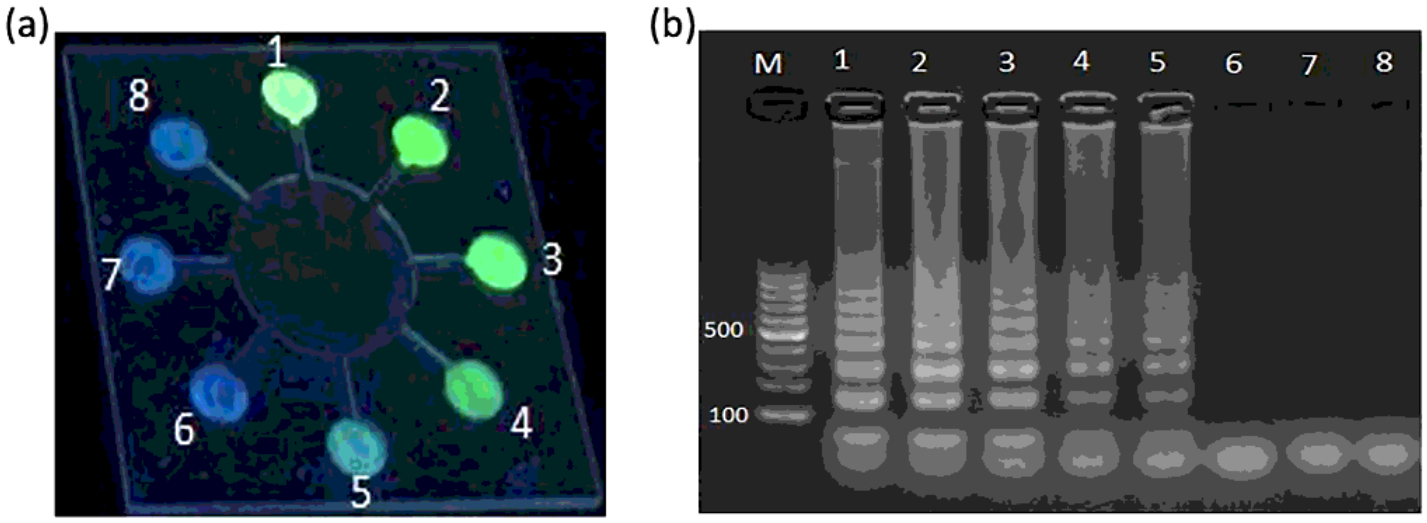

Figure 4

Sensitivity experiment achieved by testing initial serial 10-fold dilutions of the genomic DNA. (a) Image of microchip used for detecting E. coli 0157: H7 DNA in the UV light in the chambers 1~6, the DN concentration reduces from 134 ng μL-1 to 0.00134 ng μL-1. The chambers 7 and 8 do not contain the DNA template. (b) Results of gel electrophoresis (the lane number corresponds to the chamber number).

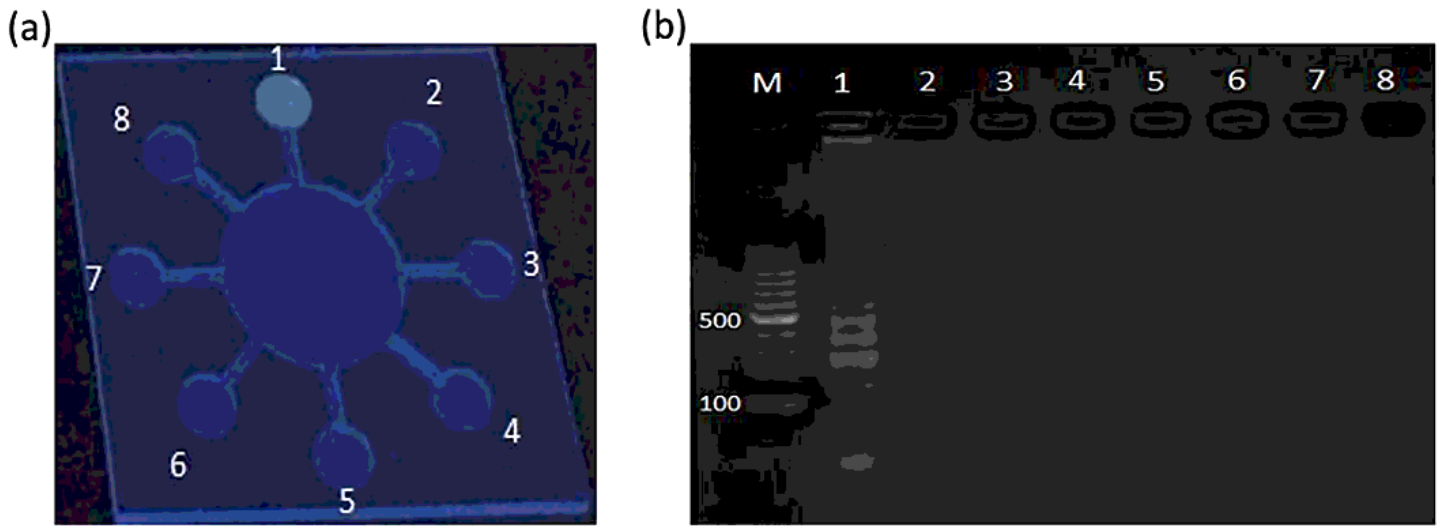

Figure 5

Selectivity of the microdevice for E. coli O157:H7 detection. (a) Image of microchip in the UV irradiation. The small chamber 1 containing E. coli 0157:H7 shows the positive reaction. The chambers 2~8 containing other bacterial primer group or containing no primer show the negative reaction. (b) Results of gel electrophoresis (the lane number corresponds to the chamber number).

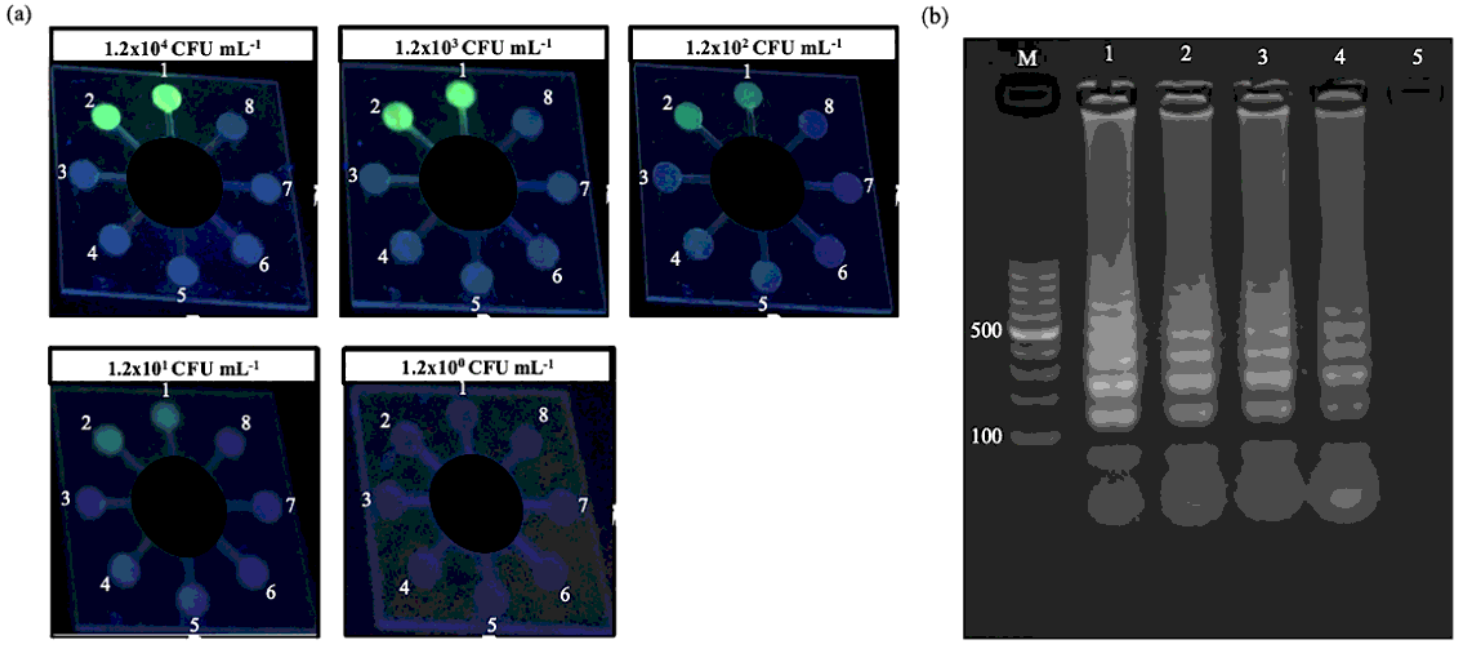

Figure 6

(a) Fluorescence image of on-chip Salmonella spp. detection using spiked milk solution. Limit of detection (LOD) test by changing the concentration of salmonella. The concentration of bacterium of salmonella of sheet 1~5 in turn is: 1.2×104 CFU mL-1, 1.2×103 CFU mL-1, 1.2×102 CFU mL-1, 1.2×101 CFU mL-1 and 1.2×100 CFU mL-1. The chambers 1 and 2 contain the paper plate injected by the

salmonella primer groups. The chambers 3 and 4 contain the paper plate loaded with the E. coli O157:H7 primer groups. The chambers 5 and 6 contain the paper plate loaded with the staphylococcus aureus primer groups. The chambers 7 and 8 contain the paper plate injected by the vibrio parahaemolyticus primer groups. (b) Results of gel electrophoresis (the lane number corresponds to the PC plate number). (c) Time required for each operation step when performed using the introduced microchip.

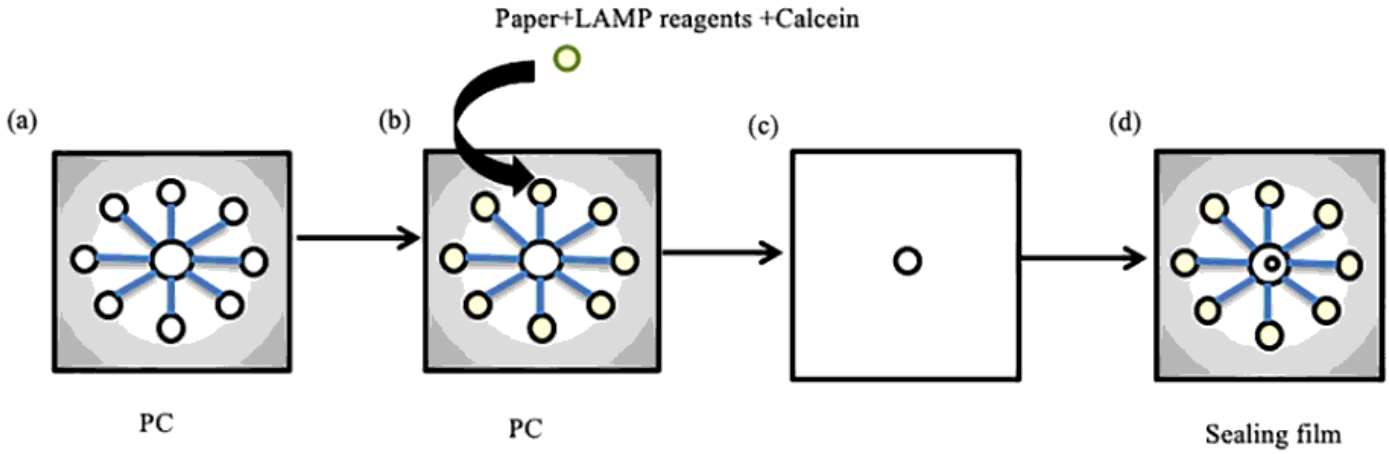

Figure 7

Making process of microchip. (a) Processed and ready PC sheet; (b) Insert the paper soaked by LAMP reagent and calcein into every reaction chamber; (c) Attach the sealing membrane; (d) Assembled microchip.

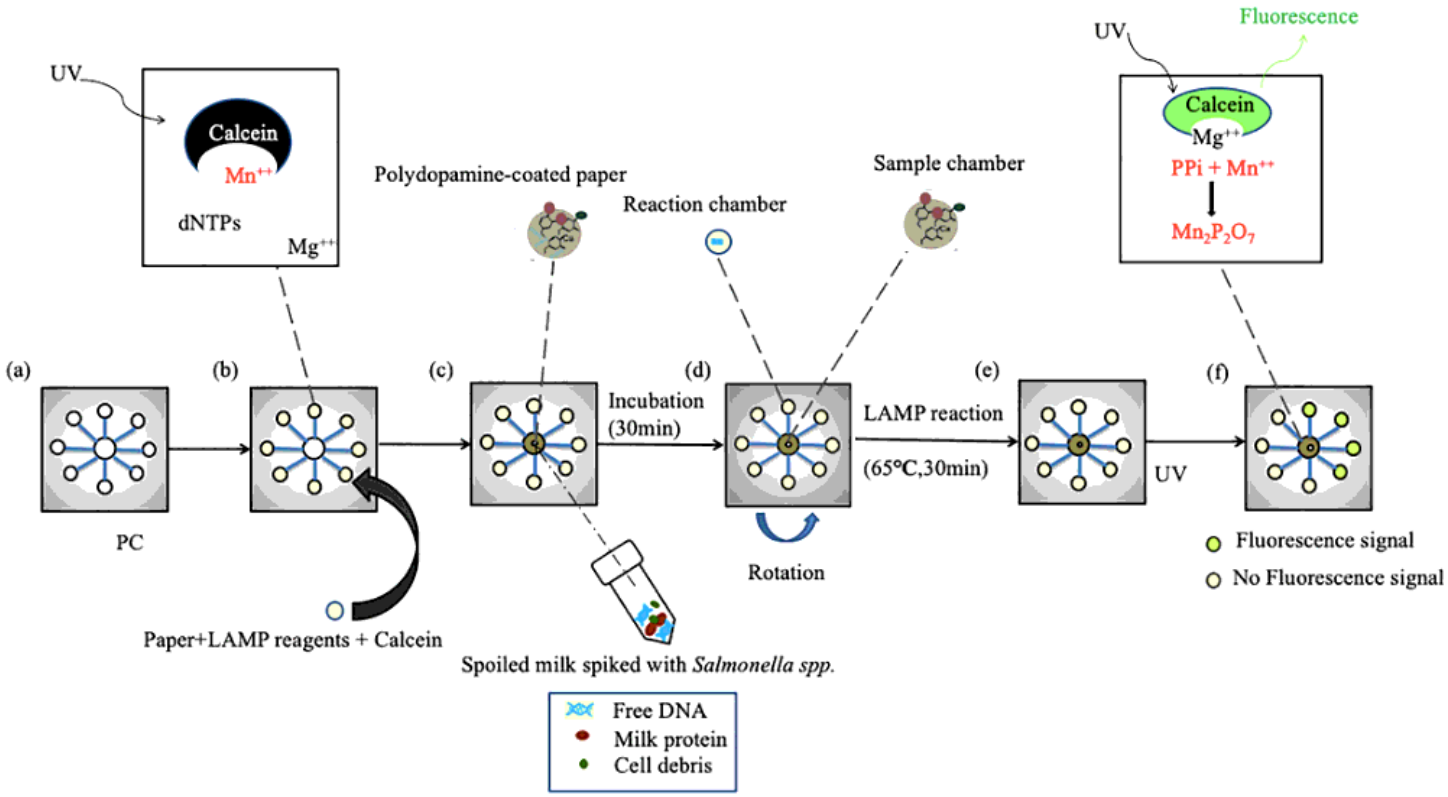

Figure 8

(a) Processed and ready PC sheet; (b) insert the paper soaked by LAMP reagent and calcein in every reaction chamber, where the manganese ion conducts the fluorescence quenching for calcein; (c) extract the DNA of salmonella from the marked milk by using the coated paper on the basis of thermal decomposiiton and polydopamine; (d) After centrifugation, the polydopamine-coated paper in the sample chamber will capture milk protein and cell debris, and the purified DNA is averagely thrown to the reaction chamber; (e) put the PC microchip on the heater to heat at 65℃ for heating for 30 minutes; (f) after the LAMP reaction is over, the pyrophosphate ion combins with the manganese ion to release the calcein and thereby the green fluorescence will be emitted in the UV radiation.