Submitted to Analytical Methods

Supplementary Information

A Wi-Fi-enabled, automated device for pathogen detection via LAMP and paper-based colorimetric assay

Yi Ting Lai [a], Nae Yoon Lee [b], and Jyh Jian Chen [a*]

[a] Department of Biomechatronics Engineering,

National Pingtung University of Science and Technology

1, Shuefu Road, Neipu, Pingtung, 91201, Taiwan (email: yorkpcy@yahoo.com.tw, YTL)

[b] Department of BioNano Technology,

Gachon University

1342, Seongnam-daero, Sujeong-gu, Seongnam-si, Gyeonggi-do, 13120, South Korea (email:

nylee@gachon.ac.kr, NYL)

*Corresponding author. Tel.: +886 8 7703202 ext. 7029/7839; Fax: +886 8 7740420.

E-mail address: chaucer@mail.npust.edu.tw (JJC)

Unlike previously reported manually operated slider-based microfluidic systems that often require off-chip sample preparation and external detection modules, the present platform integrates automated DNA extraction, thermally regulated LAMP amplification, and enclosed colorimetric readout within a single device. This higher level of workflow integration, combined with wireless monitoring capability, improves operational consistency and suitability for decentralized point-of-care testing. A detailed comparison with previously reported portable nucleic acid detection platforms is provided in Table S1.

Table S1. A detailed comparison with Seok et al. (2022) Slider Cassette.

Feature	Seok et al. (2022) Slider Cassette	Typical Portable LAMP Devices	This Work
Fluid actuation	Manual sliding	Manual pipetting or capillary flow	Mechanically guided + automated control
Sample preparation	Off-chip	Often off-chip	On-chip DNA extraction (sealed)
Reaction isolation	Partially enclosed	Tube-based	Wax-encapsulated closed chamber
Amplification control	External heater	External heater	Integrated temperature control module
Detection method	Fluorescence or external reader	Fluorescence or visual dyes	Integrated paper-based colorimetric readout (fuchsin)
Optical components required	Yes (in many formats)	Often yes	No optical excitation/detector needed
User intervention	Multiple manual steps	Moderate	Fully automated workflow
Contamination risk	Moderate (open handling)	Moderate	Reduced via sealed fluidic path
Connectivity	Not reported	Rare	Wi-Fi remote monitoring
Intended use	POCT molecular testing	POCT	Automated POCT + field surveillance

A comprehensive table of reagents and hardware is presented in Table S2 to facilitate independent replication of our study.

Table S2. Reagents and hardware

Category	Item	Source/Manufacturer
Biologicals	*Vibrio vulnificus* (Strains)	Department of Veterinary Medicine at National Pingtung University of Science and Technology, Pingtung, Taiwan
	QIAamp DNA Mini QIAcube Kit	QIAGEN N.V., Hilden, Germany
Reagents	*Bst* DNA polymerase (8 U/μL)	Eiken Chemical Co., Tokyo, Japan
	2× LAMP Reaction Mix	Eiken Chemical Co., Tokyo, Japan
	Oligonucleotide Primers	Protech Technology, Taipei, Taiwan
Chemicals	Basic Fuchsin Dye	Sigma-Aldrich, St. Louis, MO, USA
	Sodium Sulfite (Na_2SO_3)	Sigma-Aldrich, St. Louis, MO, USA
	Hydrochloric Acid (HCl)	Sigma-Aldrich, St. Louis, MO, USA
	Paraffin Wax (m.p. 58-62°C)	Sigma-Aldrich, St. Louis, MO, USA
Hardware	NI myRIO-1900 Controller	National Instruments, Austin, TX, USA
	NI-9211 (temperature input module)	National Instruments, Austin, TX, USA
	cDAQ-9171 (USB CompactDAQ chassis)	National Instruments, Austin, TX, USA
	PMMA Sheets (2mm/3mm)	Dcacrylic, Kaohsiung, Taiwan
	TAS-G100EXD (Infrared thermal imaging analyzer)	Nippon Avionics Co., Ltd., Tokyo, Japan
	FTA Cards	GE Healthcare, Chicago, IL, USA
	Filter Paper (Whatman® qualitative filter paper, Grade 1, WHA1001090)	Sigma-Aldrich, Burlington, MA, USA
	Electrophoresis System (Mini-Sub Cell GT System)	Bio-Rad Laboratories, Inc., Hercules, CA, USA
	Certified Molecular Biology Agarose	Bio-Rad Laboratories, Inc., Hercules, CA, USA

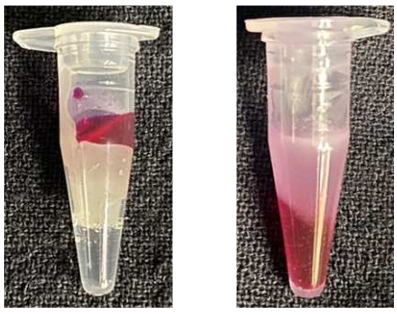
To statistically determine the limit of detection, concentrations near the detection threshold are tested in three independent replicates. The LoD is defined as the lowest concentration yielding positive results in ≥95% of reactions. Using this criterion, 1.5×10^2 CFU/mL is established as the LoD of the S-LAMP device.

Table S3. Replicate analysis for LoD determination

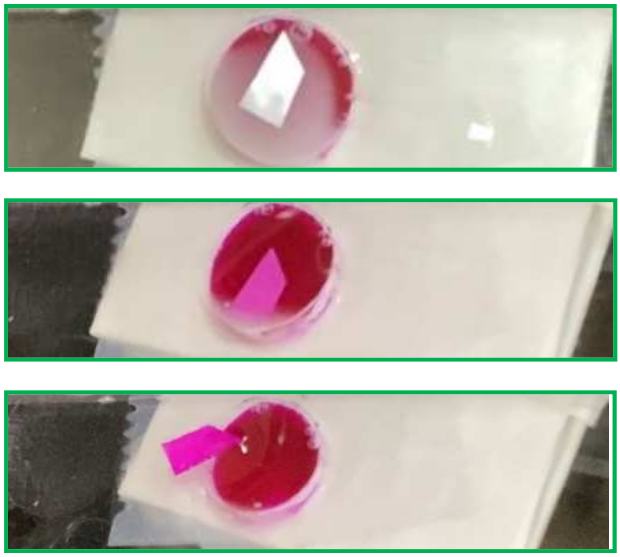
Concentration (CFU/mL)	Positive / 3 Replicates	Detection Rate
1.5×10^3	3 / 3	100%
1.5×10^2	3 / 3	100%
1.5×10^1	1 / 3	33%
Negative control	0 / 3	0%

1. The isolated process by wax

After adding the bacterial solution to the FTA card, an extraction step is carried out for 30 minutes. During the extraction, the wax layer acts as a barrier between the DNA pretreatment zone and the water for dilution. After extraction, the wax is heated and then melted, allowing the FTA card and reagent filter paper to come into contact with water, which initiates the amplification process. The isolated process by wax is tested in the PCR tube. First, 25 μL of water is added to the test tube, and the wax is lit to form a temporary layer that prevents the water from mixing with the material above the wax. When the wax cools and solidifies to form an insulation layer, 3 μL of dye is added. The test tube is sealed at room temperature for 30 minutes and then at 65 °C for 1 hour. Figure S1(a) shows that the dye and the water are separated by the insulation layer before the wax melts, and are mixed after the wax melts. The wax insulation process is also verified in the PMMA reaction chamber with a diameter of 5.8 mm and a depth of 4.5 mm. 22 μL of water and 3 μL of dye are added into the chamber and sealed with wax. Filter paper is put onto the wax barrier. After keeping the room temperature for 30 minutes, the reaction chip is heated at 65 °C for 1 hour using a TEC. Figure S1(b) presents the wax insulation, the filter paper falling into the dye solution, and the dyeing filter paper immersed in the solution. Thus, the wax layer serves as an effective temporary barrier, isolating the filter paper from water during the extraction process. Upon heating, the wax melts, allowing contact between the FTA card, reagent paper, and water, which initiates the amplification process, as demonstrated in both PCR tubes and PMMA reaction chambers.



(a)

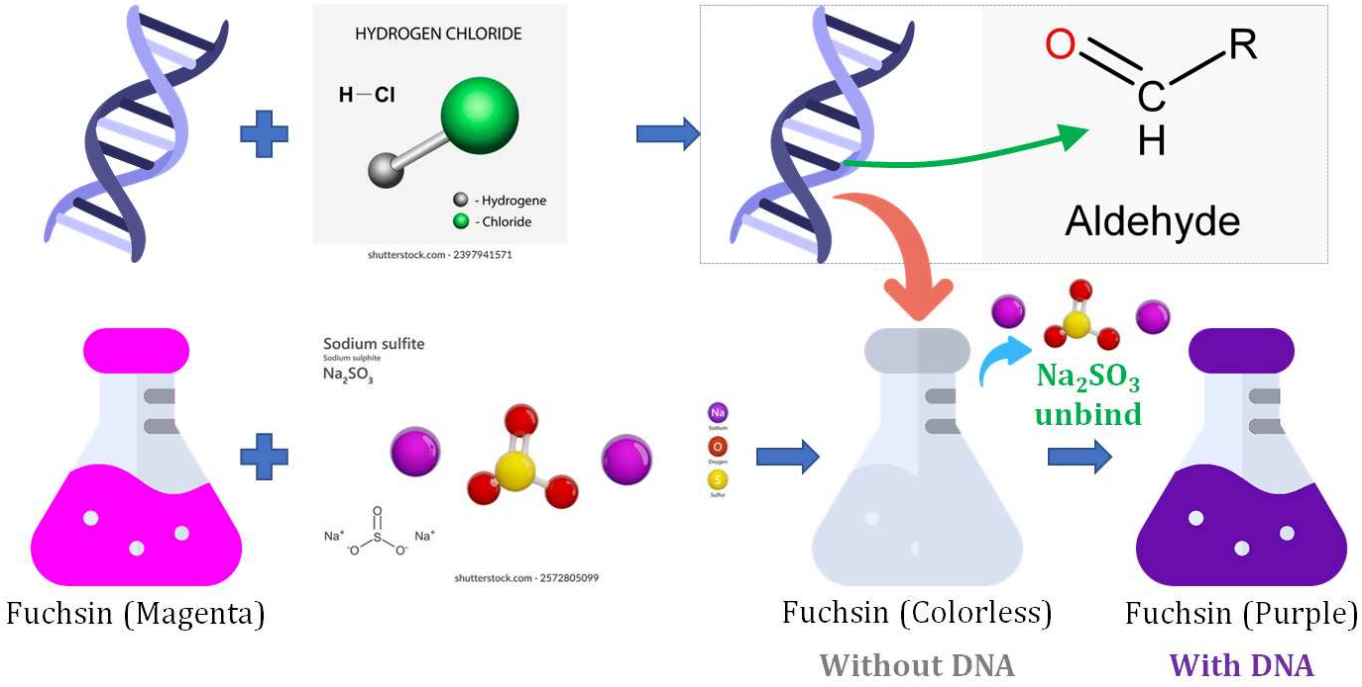


(b)

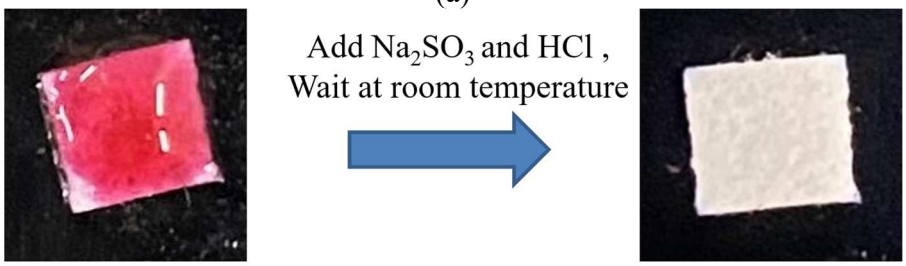
Figure S1. The tests of the isolated process by wax (a) inside the PCR tube and (b) inside the microfluidic chip, respectively.

2. The colorimetric consequence of the filter paper without target DNA in the microfluidic chip

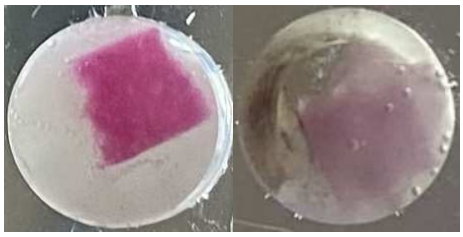
The study utilizes cellulose filter paper to store all components for the LAMP and DNA detection, including reagents, primers, dyes, and reducing agents. The average pore size of the filter paper is about 11 μm (Whatman® qualitative filter paper, Grade 1, WHA1001090, Sigma-Aldrich, Burlington, Massachusetts, USA). The size of the paper is set to 3 mm × 3 mm, allowing for easy fitting into a reaction chamber with a diameter of 5.8mm. The fuchsin dye is used to verify the presence of DNA in a sample solution, illustrated in Figure S2(a). When Na_2SO_3 and HCl are added to a solution with fuchsin, the fuchsin leucosulfonic acid forms, and the red color from the fuchsin becomes colorless. However, if the solution contains the DNA sample, the HCl triggers the acid hydrolysis. This process changes the DNA into molecules with aldehyde groups, which prevents Na_2SO_3 from removing the red color. As a result, the red color remains visible when DNA is present, but disappears when DNA is unavailable. This color change makes it easy to express whether a sample contains DNA. Figure S2(b) demonstrates the colorimetric result of the filter paper without target DNA. A piece of filter paper containing red fuchsin dye (3 μL of 1 mM solution) is placed in the chamber. Then, the paper changes color from red to white upon contact with another filter paper containing HCl and the reducing agent, Na_2SO_3 (2.6 μL of 6 mM). This study utilizes wax to separate the fuchsin and Na2SO3 papers, and then the two papers come into contact with each other when the wax melts, resulting in a color change. A 3 mm × 3 mm paper containing fuchsin dye is placed in the reaction chamber, and 12.5 μL of water is added. A wax layer is placed over the water, and the immersed paper is used as a barrier. After the Na_2SO_3 paper is put on the wax layer, the TEC under the reaction chip is powered. Figure S2(c) demonstrates the wax melts, causing the Na_2SO_3 paper to fall into the reaction chamber and come into contact with the fuchsin paper and ultrapure water. The Na_2SO_3 paper changes color due to the chemical reaction that occurs in the solution. The above experiments show that in the absence of DNA, the filter paper changes color from red to white due to a chemical reaction between fuchsin, Na_2SO_3, and HCl.



(a)



(b)



Before and after the wax melts

(c)

Figure S2. (a) The DNA detection process schematic diagram with the fuchsin colorimetric change. (b) The colorimetric result of the filter paper without target DNA. (c) The colorimetric consequence of the filter paper without target DNA in the microfluidic chip.

The following experiments involve adding blank filter paper (3 mm × 3 mm) to a PCR tube to conduct a colorimetric reaction using Na_2SO_3 and fuchsin dye solution. Different molar ratios of the two substances are prepared to observe the color changes on the test paper. Figure S3 indicates that the paper appears dark red without the Na_2SO_3 solution. When the molar ratio of Na_2SO_3 to fuchsin in the test tube solution exceeds 2, the solution becomes transparent.

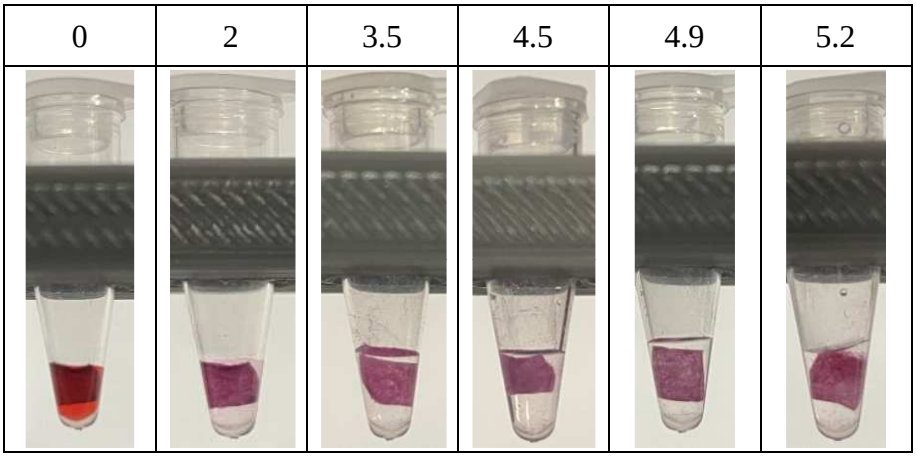


Figure S3. The colorimetric configurations of the various molar ratios of sodium sulfite/fuchsin.

Next, a small filter paper is immersed in a 33.3 mM Na_2SO_3 solution. After washing, the papers are dried at room temperature for oxidation at different time periods: 0, 15, 30, 45, and 60 minutes. Each dried paper is placed in a PCR tube, and 25 μL of ultrapure water is added to it. Different amounts of a fuchsin dye solution are added: 0, 4, or 8 μL (with a concentration of 5.56 mM). Figure S4 indicates that the solution stays clear without dye, and the paper remains white. At 4 μL, a noticeable color change appears on the paper. With 8 μL, the paper becomes darker. These color changes are similar, regardless of the paper's drying time (from 0 to 60 minutes). Since the color difference became apparent at 4 μL and above, 4 μL of dye solution was used to test the results of a 60-minute LAMP amplification experiment.

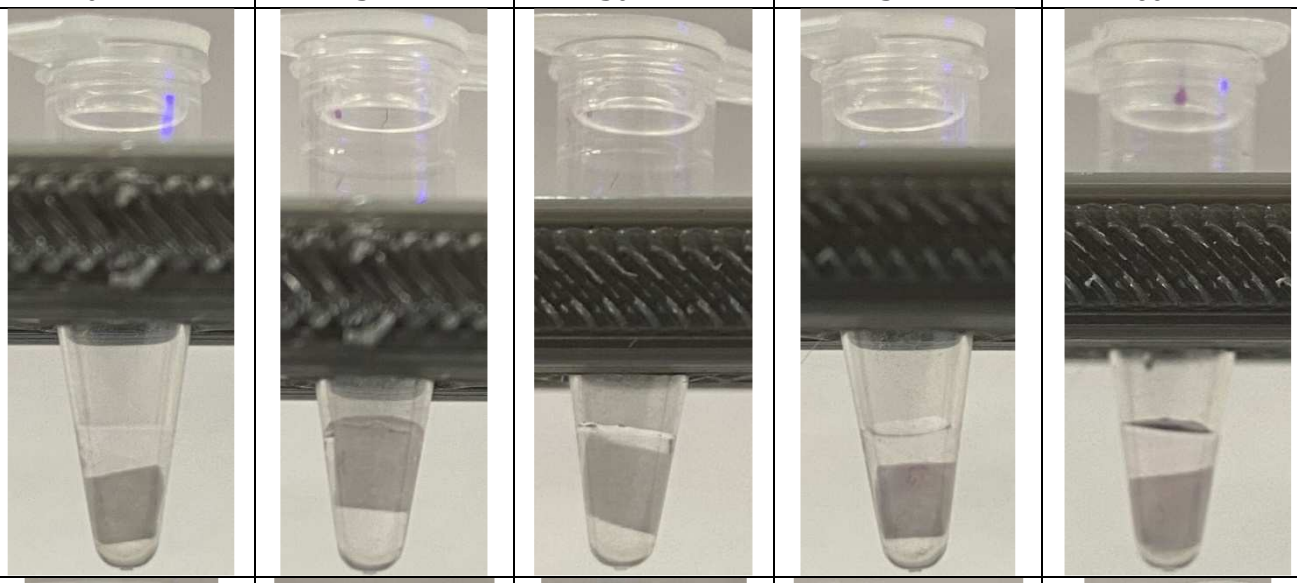
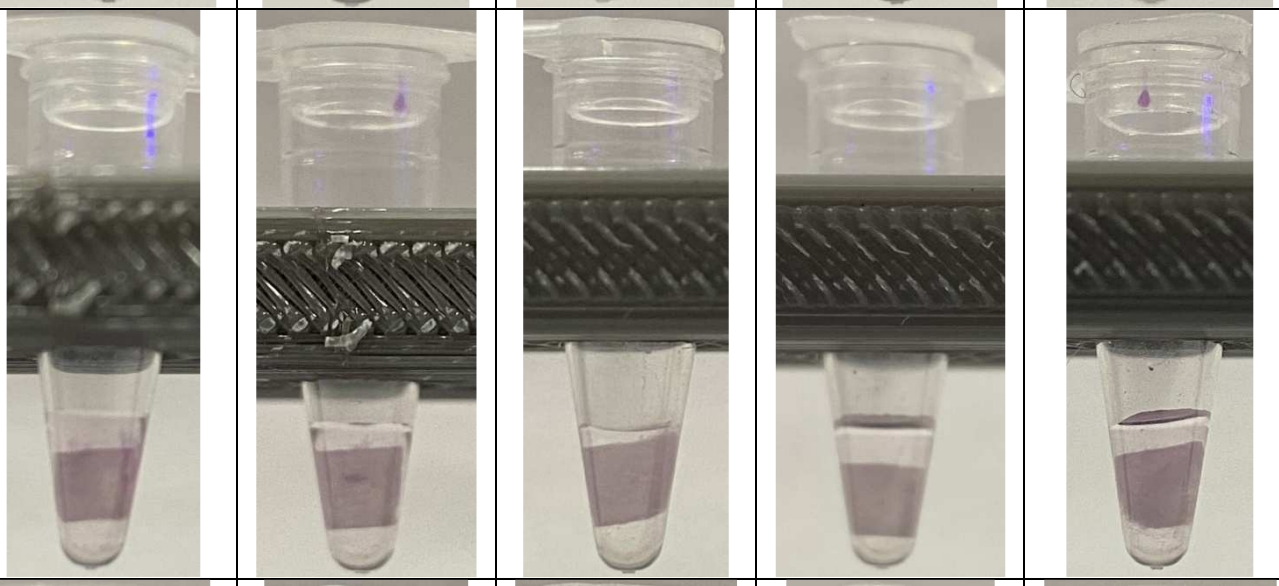
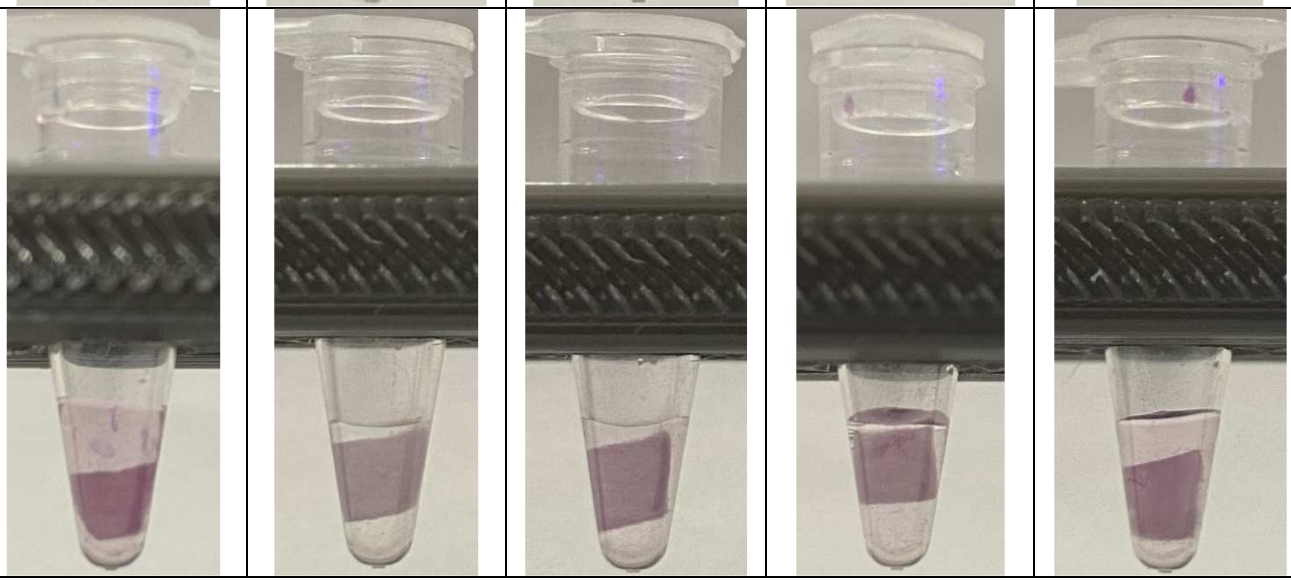
µL	0 min	15 min	30 min	45 min	60 min
0					
4					
8					

Figure S4. The colorimetric configuration of the Na_2SO_3 filter paper involves several steps. First, a small filter paper is immersed in a 33.3 mM Na_2SO_3 solution. After immersing, the papers are dried at room temperature for 0, 15, 30, 45, and 60 minutes. Each dried paper is then placed in a PCR tube, where 25 µL of ultrapure water is added. Following this, varying amounts of a fuchsin dye solution are added: 0, 4, or 8 µL, with a concentration of 5.56 mM.

In addition, the experiment adds 25 μL of ultrapure water and a piece of cellulose paper with basic fuchsin to a PCR tube. The volume of fuchsin, with a concentration of 5.56 mM, is increased from 1 μL to 3 μL. Additionally, a test paper immersed in a 33.3 mM Na_2SO_3 solution is dried at room temperature for 1 hour before being added to the tube. As shown in Figure S5, the solution with Na_2SO_3 paper undergoes a significant color change before and after the addition of the test paper, changing from dark red to pink.

Volume (μL)	1	1.5	1.8	2	2.5	3
No Na_2SO_3 paper						
With Na_2SO_3 paper						

Figure S5. The colorimetric configurations of the PCR tubes with 25 μL of ultrapure water and a piece of cellulose paper with basic fuchsin.

Furthermore, three cellulose papers are subsequently immersed in the 25-μL solution of fuchsin dye with a stock concentration of 5.56 mM at volumes of 1 μL, 2 μL, and 3 μL, respectively. These test papers are dried for one hour before proceeding with the following experiments. The 33.3 mM Na_2SO_3 cellulose papers are also prepared, and the Na_2SO_3 papers after one hour of drying are placed in three separate chambers, each containing 12.5 μL of ultrapure water. When the two types of test papers come into contact, the quinone compounds from the fuchsin react, causing a color change from the original red to a light pink, as illustrated in Figure S6.

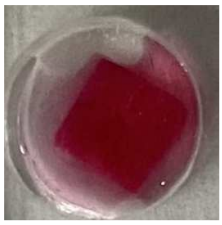
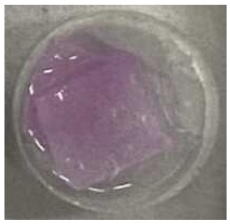
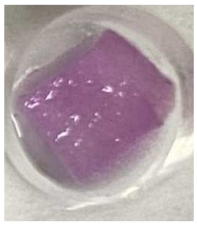
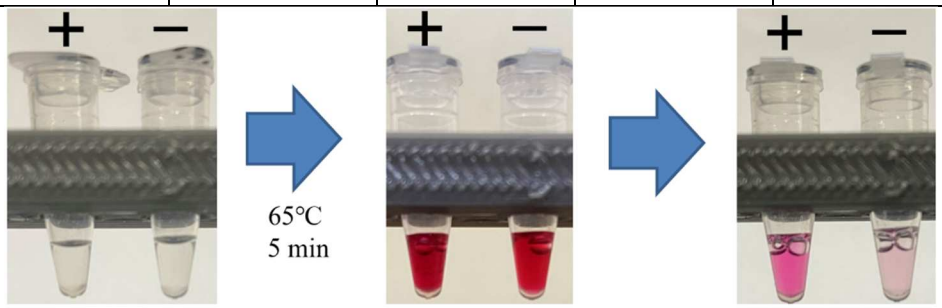
Fuchsin / Step	1 μL	2 μL	3 μL
Add fuchsin paper and water.			
Add Na_2SO_3 paper			
Wait			
Detect visually			

Figure S6. The colorimetric configurations of the reaction chambers with 12.5 μL of ultrapure water and a piece of cellulose paper with basic fuchsin.

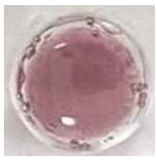
3. The colorimetric consequence of the filter paper with target DNA in the microfluidic chip

A *Vibrio vulnificus* sample is subjected to LAMP at 65 °C for 1 hour. Figure S7 shows the colorimetric comparisons of sample solutions containing DNA and those without DNA. No filter papers are utilized in the experiments. As shown in Figure S7(a), HCl is added after amplification, and the heating is maintained for 5 minutes. Additionally, fuchsin dye and Na_2SO_3 are added in sequence. The positive control tube, after amplification, contains a large amount of DNA. When fuchsin dye is added, it stains the DNA red, and this color remains unaffected by the Na_2SO_3 solution. In contrast, the negative control tube does not contain the target DNA, causing the color to revert to a light, transparent state after the addition of Na_2SO_3. In the chip experiments shown in Figure S7(b), a mixture containing DNA and one without DNA is added to a reaction chamber, along with 3 μL of fuchsin solution in the specified proportions. After LAMP, reducing agents (Na_2SO_3 and HCl) are added in sequence. The HCl solution initiates an acid hydrolysis reaction, preventing Na2SO3 from binding to the central carbon atom of fuchsin and thus successfully preserving its original color.

Reagent solution	DNA	Amplification (65 °C)	HCl (0.5 mM)	Heating (65 °C)	Fuchsin (11.25 mM)	Na_2SO_3 (22.5 mM)
Positive (23 μL)	2 μL	1 hour	0.5 μL	5 min	2 μL	2 μL
Negative (23 μL)	0	1 hour	0.5 μL	5 min	2 μL	2 μL



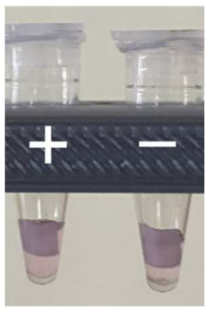
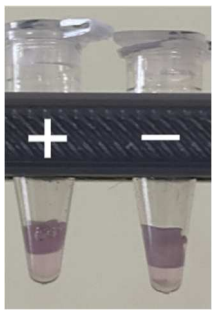
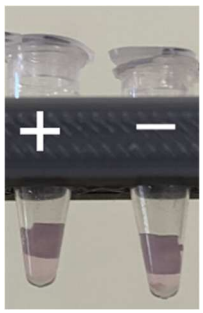
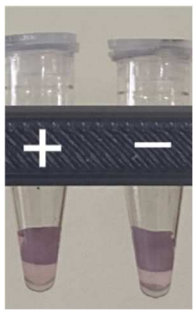
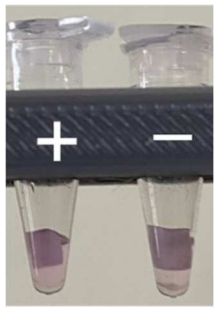
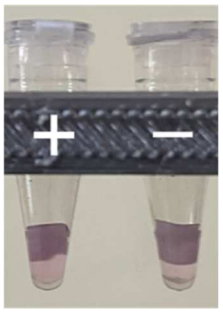
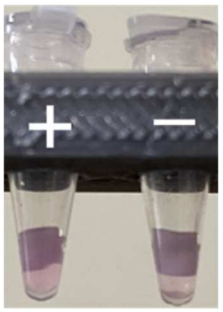
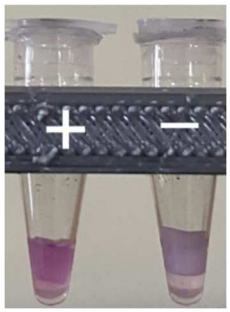
(a)

Reagent solution	DNA	Fuchsin (1 mM)	Amplification (65 ℃)	Na_2SO_3 (6 mM)	HCl (0.5 mM)	Heating (65 ℃)
Positive (23uL)	2 μL	3 μL	1 hour	1 μL	0.5 μL	5 min
						
Negative (25uL)	0	3 μL	1 hour	1 μL	0.5 μL	5 min
						

(b)

Figure S7. The colorimetric comparisons of sample solutions containing DNA and those without DNA. (a) After amplification in the PCR tube, HCl is added. Fuchsin and Na_2SO_3 are added in sequence after LAMP. (b) A mixture containing a DNA solution and fuchsin dye is added to a reaction chamber. After LAMP, reducing agents (Na2SO3 and HCl) are added in sequence.

This experiment involves DNA detection using test papers in a reaction chamber. Sample reagents, target DNA template, and blank papers are placed in a specific order. The reaction temperature is maintained at 65°C for 1 hour. After amplification, HCl is added to hydrolyze the target DNA. Fuchsin dye is then introduced for staining. Finally, Na_2SO_3 is added to observe color changes in the solution and the test papers. Figure S8(a) displays the colorimetric results of two tubes: one containing a positive control and the other with a negative control. After LAMP, fuchsin dye and Na_2SO_3 solution are added in a specific molar ratio. At a ratio of 3.4, the test paper shows no color difference in the tube; however, the solution has been reduced to a pink color. Fuchsin dye is gradually added to adjust the molar ratio and observe the color changes at different ratios. When the molar ratio reaches 2, a noticeable color difference between the positive and negative results is evident to the naked eye. Figure S8(b) demonstrates the reactions in which primers, target DNA, and LAMP reagent are added to the reaction chamber. After LAMP, the target DNA is hydrolyzed with HCl, stained with fuchsin dye, and subsequently treated with Na_2SO_3 to induce observable color changes in both the solution and the test papers. The solution of the positive and negative reactions is transparent and colorless. In contrast, the positive portion of the test paper remained pink, and the negative reaction appeared white.

Molar ratio of Na_2SO_3/fuchsin	3.4	3.2	3	2.8
				
Molar ratio of Na_2SO_3/fuchsin	2.6	2.4	2.2	2
				

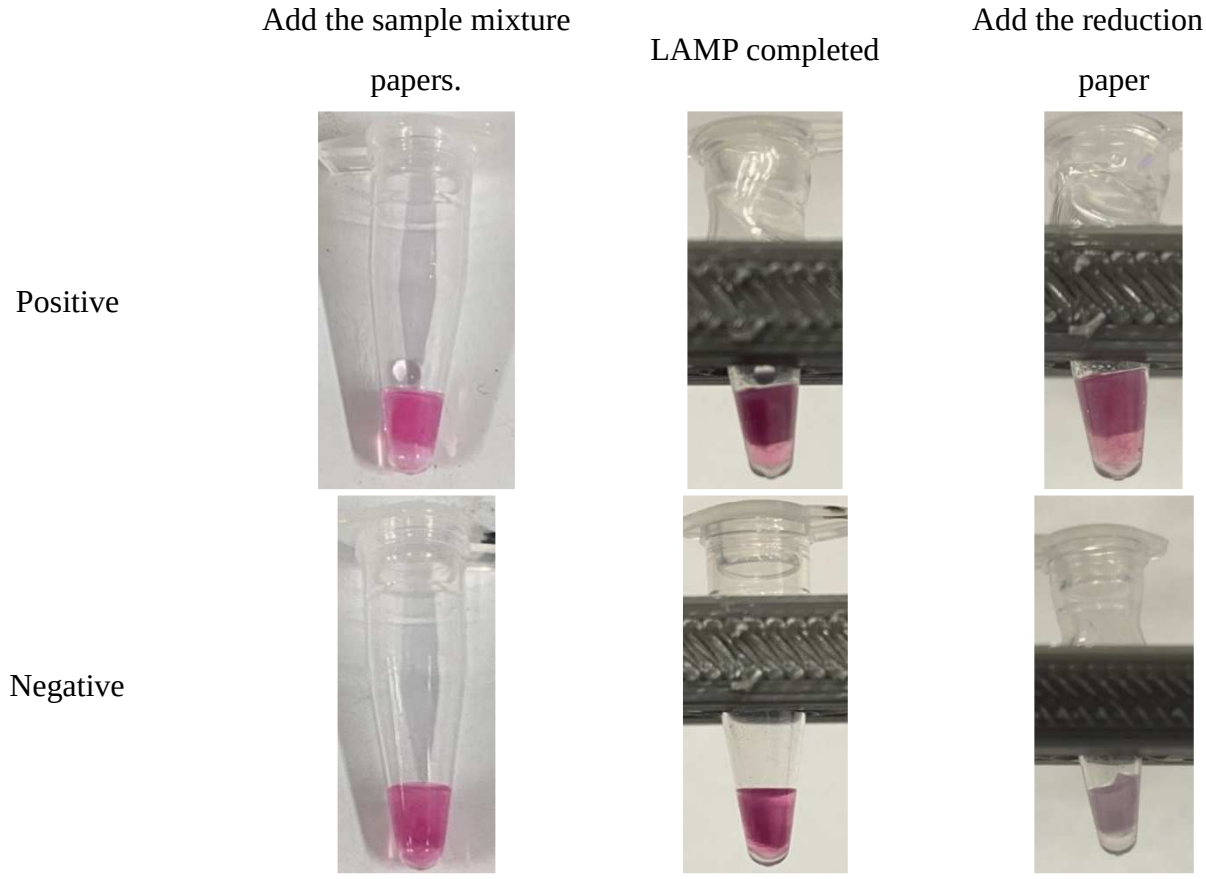
(a)

Reagent solution	DNA	Amplification (65 ℃)	HCl (0.5 mM)	Heating (65 ℃)	Fuchsin (5.56 mM)	Na_2SO_3 (33.3 mM)
Positive (23 μL)	2 μL	1 hour	0.5 μL	5 min	3 μL	1 μL
Negative (23 μL)	0	1 hour	0.5 μL	5 min	3 μL	1 μL

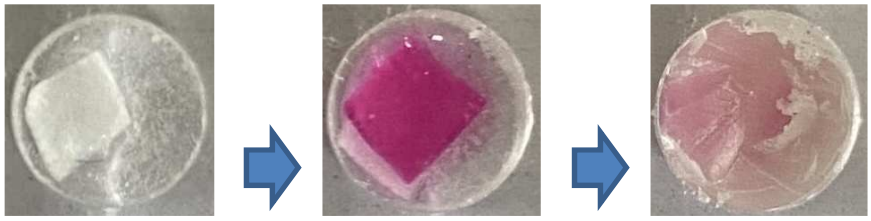
(b)

Figure S8. The colorimetric results of two sample solutions: one containing a positive control and the other with a negative control. (a) The influence of various molar ratios of Na_2SO_3/fuchsin on the color change of the test papers. (b) The reactions in which primers, target DNA, and dye are added to the reaction chamber, followed by LAMP. The target DNA is hydrolyzed with HCl, stained with fuchsin dye, and subsequently treated with Na_2SO_3 to induce observable color changes.

Figure S9 involves adding test papers immersed in a sample mixture and a reduced mixture to a PCR tube or a chamber chip, and then using the filter papers for LAMP. First, by leveraging the test papers' ability to store liquids, the sample mixture papers contain a DNA template and a primer set/fuchsin dye, which dyes the papers red, as shown in Figure S9(a). The papers are then added to 12.5 μL of ultrapure water and subjected to a LAMP process using a commercial thermocycler. Then, test papers containing Na_2SO_3 and HCl are added and heated at that constant temperature for 5 minutes. The color change of the papers is revealed. A positive control reaction contains 3 μL of target DNA template before amplification. After LAMP, the papers display a red color. A negative reaction with the DNA template results in the paper turning a light pink. In Figure S9(b), 12.5 μL of ultrapure water is added to the chip reaction chamber, and a test paper immersed in a DNA solution is then placed in the chamber. The functionalized filter papers are prepared by immersing 3 mm × 3 mm Whatman Grade 1 squares in either 5.56 mM basic fuchsin or a mixture of 33.3 mM Na_2SO_3 and 0.5 mM HCl for 30 minutes. A primer set/fuchsin dye paper is placed in sequence. The test paper comes into contact with the fuchsin dye, becoming stained as a result. Next, wax is dripped on the chamber to seal it. The chip is heated, and the wax is melted. The test paper is also moved to the amplification region. Once the amplification is finished, a test paper immersed in sodium sulfite and hydrochloric acid is added and heated at 65 °C for 5 minutes. The result shows that after amplification, the test paper in the reaction chamber with DNA retained its red color. To ensure long-term chemical stability and prevent the oxidation of the reducing agent, all strips were stored in vacuum-sealed amber vials at 4°C.



(a)



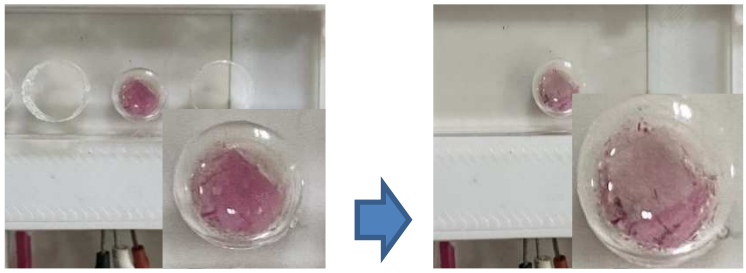


Figure S9. Adding test papers immersed in a sample mixture and a reduced mixture to a reaction, and then using the filter papers for LAMP. The colorimetric detection inside (a) the PCR tube and (b) the reaction chip.

4. The gel electrophoresis results of the filter paper with the target DNA extracted in the microfluidic chip and amplified in the thermal cycler

To provide a laboratory reference for comparison, conventional PCR was performed using the outer LAMP primers F3 and B3 targeting *Vibrio vulnificus* (F3: 5'-TCTTGAAGCCACTTATCGC-3'; B3: 5'-CAGCATCAACATCCAGTACA-3'). DNA extracted using the QIAamp DNA Mini QIAcube Kit served as the positive control. In parallel, bacterial suspensions are applied to FTA cards and processed using our on-chip compatible washing protocol (25 μL wash buffer followed by 25 μL TE buffer) to obtain purified nucleic acids. PCR reactions were carried out in a 50 μL mixture containing 25 μL of 2× *Taq* Plus Master Mix, 1.5 μL of each primer, 4 μL of DNA template, and ultrapure water to volume. Thermal cycling is conducted using the following program: initial denaturation at 94 °C for 4 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 40 s; followed by a final extension at 72 °C for 7 min. Negative controls are prepared by replacing the DNA template with ultrapure water. PCR products are analyzed by agarose gel electrophoresis and visualized under UV illumination.

The gel electrophoresis results are shown in Figure S10. Lane 1 represents the negative PCR control with no DNA template and shows no amplification band. Lane 2 corresponds to the positive control using purified genomic DNA, which produced a clear amplicon band. Lanes 3~14 show PCR products obtained from DNA extracted using FTA cards under different washing conditions. Samples washed once (lanes 3–6), twice (lanes 7–10), and three times (lanes 11–14) all yield visible amplification bands when target DNA is present, whereas negative control lanes (without template DNA) show no bands. These results indicate that even a single wash with 25 μL wash buffer followed by 25 μL TE buffer is sufficient to produce amplifiable DNA from the FTA card. PCR was used here solely as a laboratory reference method to verify the presence of the target sequence in prepared samples. In contrast, the proposed S-LAMP device is designed as a rapid, portable, and instrument-minimized alternative for point-of-care molecular detection.

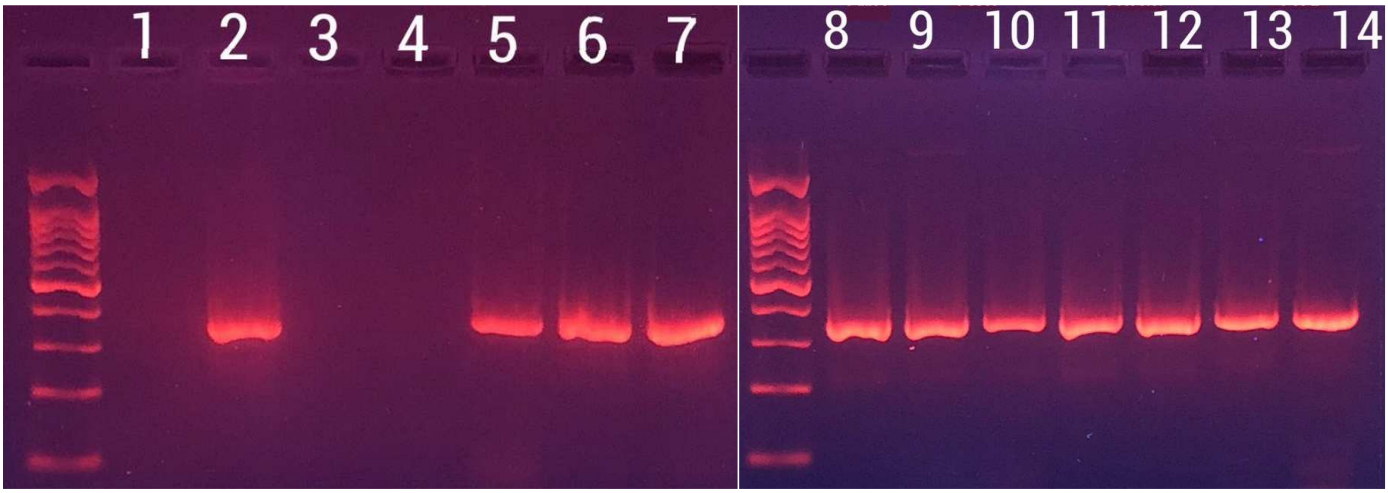


Figure S10. The results of gel electrophoresis analysis of the products. The first lane indicates the DNA ladder. Results from the traditional extraction kit show the products of negative and positive controls on lanes 1 and 2, respectively. Results from the FTA extraction show negative controls on lanes 3 and 4 and positive controls on lanes 5 and 6 for washing once, positive controls on lanes 7~10 for washing twice, and a positive control on lanes 11~14 for washing three times.