

Electronic Supplementary Information (ESI)

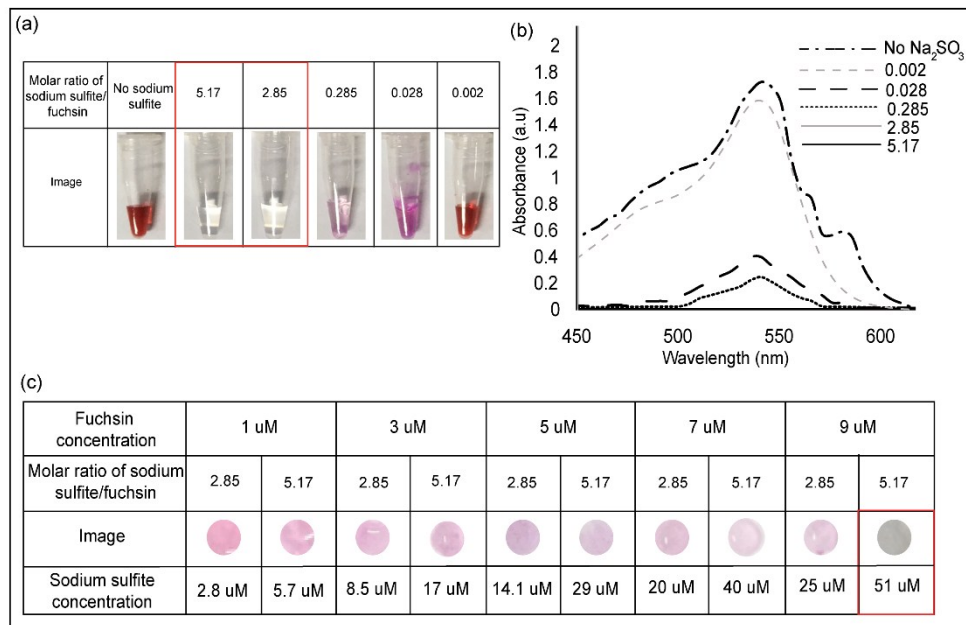
**A foldable isothermal amplification microdevice for fuchsin-based
colorimetric detection of multiple foodborne pathogens**

Thi Ngoc Diep Trinh and Nae Yoon Lee*

**Department of BioNano Technology, Gachon University, 1342 Seongnam-daero, Sujeong-gu, Seongnam-si, Gyeonggi-do, 13120, Korea.*

Table S1 The primer sequences used for loop-mediated isothermal amplification of three microorganisms

Target genes	Primer name	Primer sequences (5'-3')
<i>invA</i> gene (<i>Salmonella</i> spp.)	F3	CCAACAATCCATCAGCAAG
	B3	AGCATATGTTTTGTTTCCTGAA
	FIP	AACACATAGCCAAGCTCCCGCAGTCAGTATTTCTGGGTAAC
	BIP	GAACGCGCTTGATGAGCTTTTCGAAATATTCATTGACGTTGC
	LF	GAGTTTCTCCCCCTTTCATGC
	LB	ACCACTGTCTGGCGGTGA
<i>nuc</i> gene (<i>Staphylococcus aureus</i>)	F3	AACAGTATATAGTGCAACTTCAA
	B3	CTTTGTCAAACCTCGACTTCAA
	FIP	ATGTCATTGGTTGACCTTTGTACATAAATTACATAAAGAACCTGCGA
	BIP	GTTGATACACCTGAAACAAAGCATCATTTTTTTCGTAATGCACTTGC
	LF	CCGTATCACCATCAATCGCTTT
	LB	AGGTGTAGAGAAATATGGTCCTGA
<i>eaeA</i> gene (<i>E.coli</i> O157:H7)	F3	TCTGATGCCAATGACGAAT
	B3	GGTAGCATCATCGAGAGG
	FIP	CATTCAAATCTCAAGAGCGAAGTTGATTTATGGTGTCTGTGGGA
	BIP	CAAATTATGTTTTGCAGAGAATGGAAACGTAACGCTAACACACAG
	LF	AGAGTTATCTGTCGGGAATT
	LB	TGGCCCATATCTGTGCTA

Optimization of fuchsin and sodium sulfite concentration**Figure S1.** Decolorization of fuchsin by sodium sulfite. (a) The effect of molar ratio on the color in solution state, (b) UV-vis absorption spectra of the solutions, and (c) decolorization of fuchsin on paper

To choose the suitable concentration of fuchsin and sodium sulfite for discriminating negative and positive LAMP product, we examined the influence of sodium sulfite on the color of fuchsin solution.

First, the molar ratio between sodium sulfite and fuchsin solution was adjusted within the ranges from 0.002 to 5.714 to form colorless leucofuchsin. These solutions were allowed to react at room temperature and then the absorbance was measured.

After choosing the suitable molar ratio which could completely make fuchsin colorless, we evaluated the color performance of the mixture on paper. The concentration of fuchsin was varied within the range of 1 to 9 μ M.

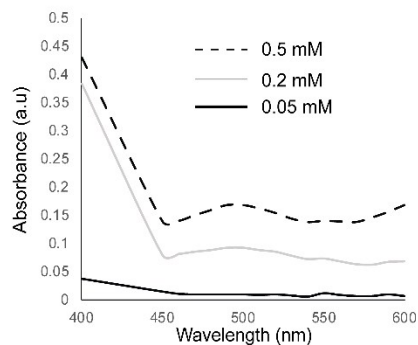
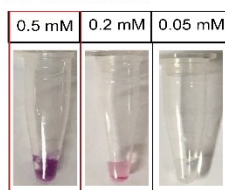
Based on the molar ratio chosen in the previous experiment, the concentration of sodium sulfite was calculated.

With increasing amounts of sodium sulfite, the color of the solution was reduced. When the molar ratio of sodium sulfite to fuchsin were 5.71 and 2.85, the solutions were almost bleached into colorless (Fig. S1a). The absorbance graph also displayed the decolorization ability at these ratios (Fig. S1b). Based on these results, the molar ratios of 5.71 and 2.85 were chosen for the subsequent experiment performed using paper (Fig. S1c).

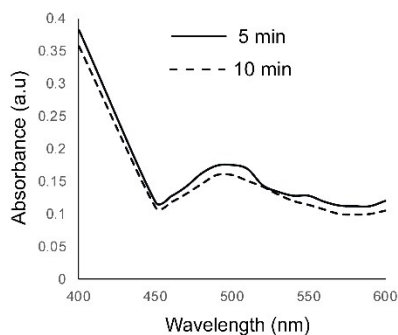
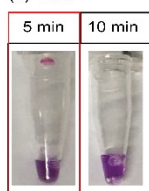
Next, the concentration of fuchsin was varied from 1 to 9 μM . The concentration of sodium sulfite was calculated based on the molar ratios for 5.71 and 2.85. Sodium sulfite with concentration of 51 μM and fuchsin at a concentration of 9 μM completely decolorized on paper. Therefore, these concentrations were finally chosen for further experiment for colorimetric detection of LAMP amplicons.

Acid hydrolysis condition

(a) HCl concentration



(b) Time



(c) Temperature

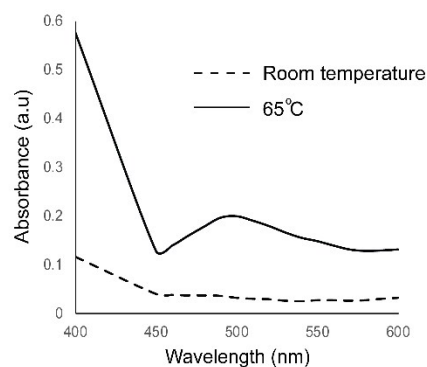
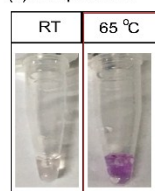


Figure S2. The effect of (a) HCl concentration, (b) reaction time, and (c) temperature on the acid hydrolysis process.

We evaluated the optimum condition for acid hydrolysis. Acid hydrolysis is a process that produces aldehyde group from DNA molecule under acidic condition. HCl with three concentrations (0.5, 0.2, and 0.05 mM) were tested. Afterward, fuchsin and sodium sulfite were added. The color change was observed and absorbance was analyzed. Without the formation of aldehyde group, sodium sulfite will decolorize fuchsin. However, when aldehyde is formed, solution will display purple color. In addition, reaction time of 5 and 10 min were tested. Reactions were also compared by conducting at room temperature and at 65 °C. The results in Fig. S2 showed that HCl with 0.5 mM, reaction time of 5 min, and reaction temperature of 65 °C were optimum conditions for acid hydrolysis.

Selectivity test

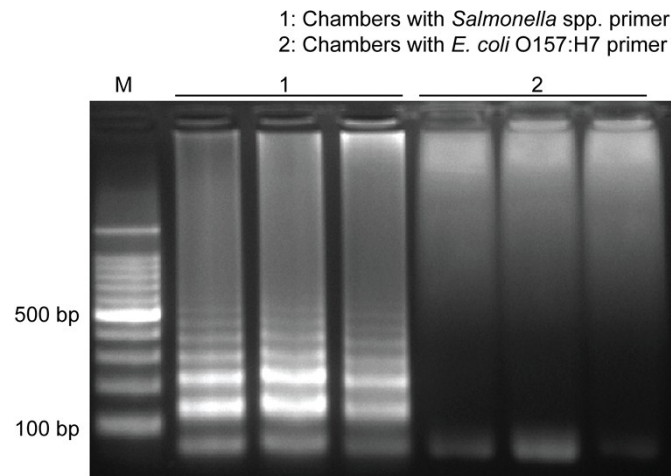


Figure S3. Result of agarose gel electrophoresis showing selectivity test performed using the microdevice when milk spiked with *Salmonella* spp. was used.

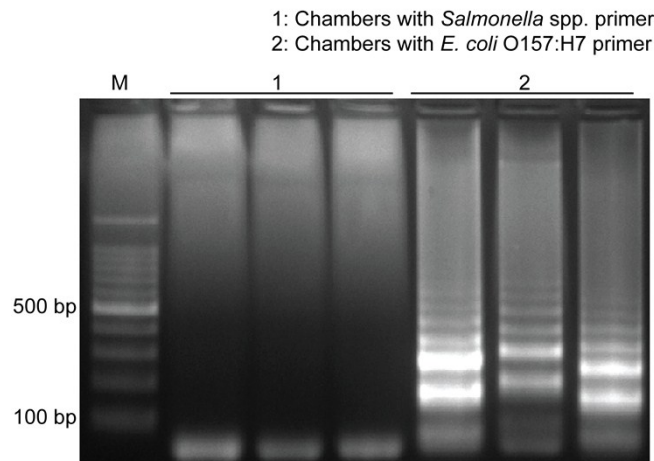


Figure S4. Result of agarose gel electrophoresis showing selectivity test performed using the microdevice when milk spiked with *E. coli* O157:H7 was used.