Supplementary Information

Integration of Paper-Based DNA Extraction with Digitized Image Analysis for Colorimetric LAMP-Based *V. parahaemolyticus* Detection

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Table S1. Primer sequences used for the detection of *Vibrio parahaemolyticus*.

Bacteria	Primers	Sequences	Lengths (bp)
V. parahaemolyticus	F3	ACGCCTCTGCTAATGAGGTA	20
	B3	GAGATTCCGCAGGGTTTGT	19
	FIP	GGCGCTTCTGGTTCAACGATTG-GAAACGATCGTAGAGCCGTC	42
	BIP	TGTGGCTTCTGCTGTGAATCCT-GCAGTACGCAAATCGGTAGT	42
	LF	CCACGCGTTATTTTATTTTTGGCAC	25

Table S2. Preparation of LAMP buffer.

Components	Volume (μL)
Warmstart® Colorimetric LAMP 2X master Mix	6.5
Primer mixture	1.0
Distilled water	5.0
Total	12.5

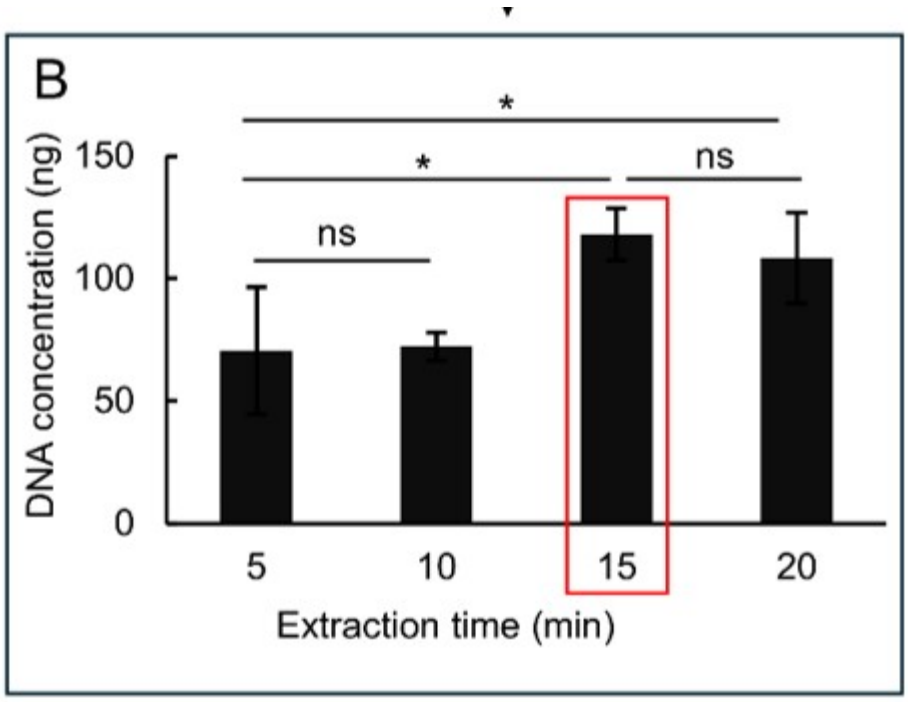
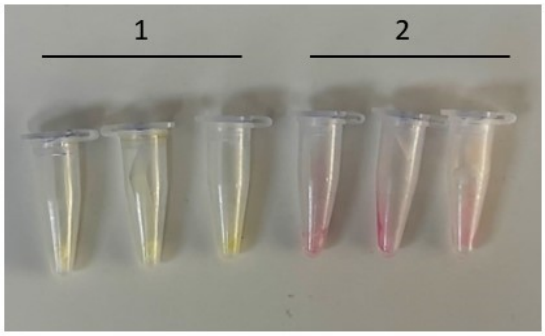


Fig. S1. (B) Quantitative comparison of DNA concentration obtained at different extraction times. Data are presented as mean ± SD (*$p < 0.05$; ns, not significant).

The DNA concentration extracted was measured using Nanodrop. Overall, the concentration of all treatments seems too high which can be resulted from the overestimation of DNA quantity when the purity is not high. Purity of the extracted DNA was also indicated by ratio A260/280 in Table S3. The ratios A260/280 of each extraction time treatments were all bellowed 1.8 which indicated the contamination of protein therefore interfered with the measured DNA concentration. Using the proposed method, DNA was successfully separated from lysis buffer which contained components that affected amplification reaction. However, non-selective binding of proteins also occurred which resulted in the low value of A260/280. The use of *Bst* polymerase with high tolerance with inhibitors like contaminated proteins can avoid the harmful effect of non-selective binding of proteins in paper strips, therefore, the amplification process can be successfully achieved.

Table S3. Purity of extracted DNA

Extraction time	**A260/280**
5	1.52
10	1.51
15	1.50
20	1.54



1: LAMP reaction targeting *tox*R gene with *V. parahaemolyticus*, *Salmonella*, and *E. faecalis* template
2: LAMP reaction targeting *tox*R gene with *Salmonella* and *E. faecalis* template

Fig. S2. Performance of the proposed methods in detecting target in the mixture with different types of bacteria.

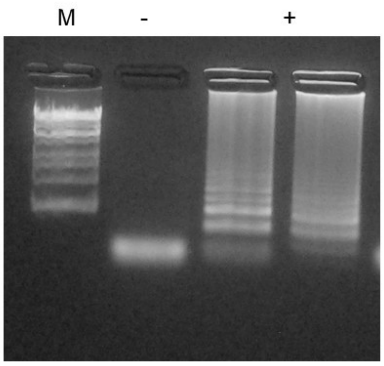


Fig. S3. Agarose gel electrophoresis of LAMP reaction targeting *tox*R gene of *V. parahaemolyticus*. M: Ladder; - : *Salmonella* template; + : *V. parahaemolyticus* template.

Table S4. Comparison with previous study for the detection of bacteria using isothermal amplification techniques

Target	**Features**	**Limit of detection**	**Analysis time**	**Ref**
Enterococcus faecium	Paper device integrated with LAMP and paper-based extraction	10^2 CFU/mL	60 min	19
Methicillin-resistant *Staphylococcus aureus*	Thermal lysis in combination with G-quadruplex based isothermal exponential amplification	0.1 fmol	120 min	20
Salmonella typhimurium	Thermal lysis in combination with recombinase -aided amplification	89 CFU/mL	40 min	21