Supplementary material

1. Material and methods

1.1 Fabrication of SPC chip

The mold was prepared using two layers soft lithography technique. The patterns of channels and chambers of SPC chip were designed by CorelDRAW X8 and printed on two transparency films as masks, respectively. First, negative photoresist (#SU-8 3050, MicroChem, USA) was coated onto a clean, dry silicon wafer at a speed of 2700 rpm for 30 s using spin-coater (Chemat, USA) as the first layer. After soft baking at 65 ℃ for 1 min and then 95 ℃ for 15 min, the channels pattern was exposed by the one-side stepper (Nanguang Vacuum Technology, China) under the mask. The exposed wafer was baked at 65 ℃ for 1 min and at 95 ℃ for 5 min. Then, the fabrication of second layer was similar to the first layer. SU-8 3050 was coated onto the wafer at a speed of 1300 rpm for 30 s, and then the wafer was soft baked (65 ℃ for 1 min and then 95 ℃ for 40 min). Before exposure, the mask of chambers pattern must be aligned with the first layer. After exposing for 25 s, the wafer was post exposure baked as mentioned. Finally, the treated wafer was developed in SU-8 Developer (MicroChem, USA) until the mold was defined. In order to make the mold have better mechanical properties, baking at 300 ℃ for 20 min was conducted. Then, the mold was ready to replicate PDMS

chips.

Before each use of the mold, it was treated by trimethylchlorosilane (#104814, Aladdin, China) to prevent PDMS adhesion. First, a thin PDMS layer containing component A and B (#RTV 615, Momentive, USA), at a ratio of 5: 1, was spun on the mold at 1500 rpm for 30s, which formed a reaction layer. To avoid solution evaporation in the reaction, after baking at 85 ℃ for 5 min, fluorinated polymers Novec EGC-1720 (3M, USA) was spun onto the reaction layer at 1000rpm for 30 s followed another 85 ℃ baking for 5 min. And then, a PDMS mixture (A: B= 10: 1) was poured onto the chip. Through 85 ℃ baking for 90 min, the solidified supporting layer generated. The multilayer chip was peeled off from the mold to punch inlet and outlet. Finally, the PDMS device was bonded on a coverslip pre-treated by oxygen plasma followed another 85 ℃ baking for 90 min. The top of chip was covered by transparent adhesive tape for better degasification. Overall, the SPC microfluidic chip was ready-to-use.

1.2 Off-chip conventional LAMP and PCR reaction

The commercial LAMP assay kit (# 310005, Loopamp, Japan) and PCR kit (# B532081, Sangon, China) were applied to the reactions. The primer sets and reaction condition of conventional LAMP were the same as the MD-LAMP. The F3 and B3 were used as primers of the PCR

reaction and 1.5, 2.5, 3.5, 4.5 mmol/ L $MgCl_2$ were used to optimize the condition. The PCR amplification procedure was as follows: First, started with a 4 min pre-duration step at 94 ℃. And then, 35 thermal cycles of at 94 ℃ for 30 s, 55 ℃ for 30 s and 72 ℃ for 30 s were run. Finally, post-extended for 10 min at 72 ℃. Nine replicates of each concentrations of VP template were tested. The result of the conventional LAMP reaction were read by adding Fluorescent Detection Reagent (# 310007, Loopamp, Japan) and PCR reaction were read by 2% agarose gel electrophoresis marked by SYBR Green I.

2. Supplemented tables and figures

2.1 Supplemented tables

Table S-1. List of used primers and the cloned gene sequence.

Description	Sequence (5'-3')
The cloned part sequence of *Vibrio parahemolyticus tlh* gene	CCAGCTACTCGAAAGATGATCCAGCGACCGATTGGGAATGGGCA AAAAACGAAGATGGTAGCTACTTCACCATTGACGGCTACTGGTGG AGCTCCGTTTCATTTAAAAACATGTTCTACACCAACACGTCGCAAA ACGTTATCCGTCAGCGTTGTGAAGCAACATTAGATTTGGCGAACG AGAACGCAGACATTACGTTCTTCGCCGCTGACAATCGCTTCTCAT ACAACCACACGATCT
F3-*tlh*	AGCTACTCGAAAGATGATCC
B3-*tlh*	GGTTGTATGAGAAGCGATTG
LF-*tlh*	ACCAGTAGCCGTCAATG
LB-*tlh*	TTAGATTTGGCGAACGAGA
FIP-*tlh*	ATGTTTTTAAATGAAACGGAGCTCCGGCAAAAAACGAAGATGGT
BIP-*tlh*	ACGTCGCAAAACGTTATCCGGCGAAGAACGTAATGTCTG

Table S-2. The advantages of the CDM-LAMP compared with the conventional methods.

	CMD-LAMP	Conventional LAMP	Conventional PCR
Pre-enrichment	No	Yes	Yes
Detection Time	2 hours	> 10 hours	> 12 hours
Limit of detection	1×10^3 CFU/ mL	~10^5 CFU/ mL	~ 10^5 CFU/ mL
Quantification	Yes	No	No
Key instrument	Cheap heating plate (Total < $ 100)	Culture-related device; Cheap heating plate (Total > $ 9000)	Culture-related device; Thermal cycler; Electrophoresis apparatus (Total > $ 12000)
Readout device	Handheld light source	Handheld light source	Gel Imaging System
On-site operation	Easily	Normally	Difficultly

Table S-3. The comparison among the CDM-LAMP and the other LAMP methods for VP detection.

No.	Year	LOD* (CFU/ mL(g))	Target gene	Pre-enrichment	Quantification	Ref.
1		1000	*tlh*	No	Yes	This study
2	2017	10000	*toxR*	No	No	*1*
3	2016	18000	*ompA*	No	No	*2*
4	2016	100**	*pirA*	No	No	*3*
5	2016	9200	*toxR*	No	No	*4*
6	2014	1900	*tlh*	No	No	*5*
7	2013	1000**	*tlh*	No	No	*6*
8	2011	1800	*tlh*	No	No	*7*
9	2010	110000	*toxR*	No	No	*8*
10	2010	400	*tdh*	No	No	*9*
11	2010	10650	*trh1*	No	No	*9*
12	2010	2500	*trh2*	No	No	*9*
13	2008	530	*tlh*	No	No	*10*
14	2015	< 1	*tdh*	Yes	Yes	*11*
15	2015	2	*tlh/ldh*	Yes	No	*12*
16	2014	< 1	*tdh*	Yes	Yes	*13*
17	2014	0.19	*tlh*	Yes	No	*5*
18	2012	< 1	*tdh*	Yes	No	*14*
19	2011	1.3	*rpoD*	Yes	Yes	*15*

* LOD of contaminated samples.

** LOD of pure culture samples (LOD of contaminated samples wasn't shown).

2.2 Supplemented figures

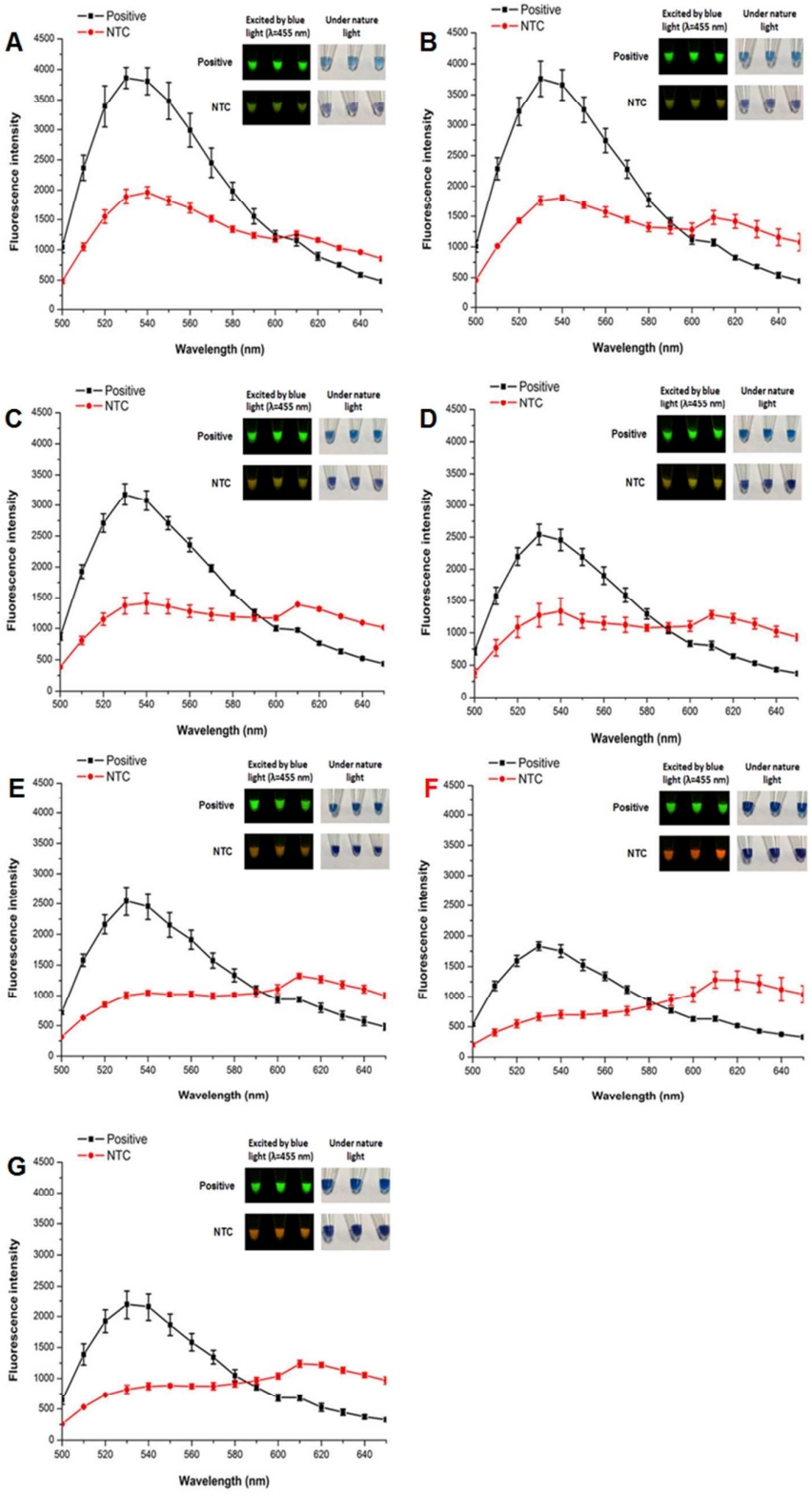


Figure S-1. Investigation on the optimal concentration of HNB for MD-LAMP according to color and fluorescence changes: (A) 100 μM HNB; (B) 150 μM HNB; (C) 200 μM HNB; (D) 250 μM HNB; (E) 300 μM HNB; (F) 350 μM HNB; (G) 400 μM HNB; Positives, three replicate tests of VP template (1×10^7 CFU/ mL). NTC, three replicate tests of nuclease-free water. Error bars represented the standard deviation value of each test. At the top-right, the images were captured under the blue light (λ = 455 nm) and the nature light.

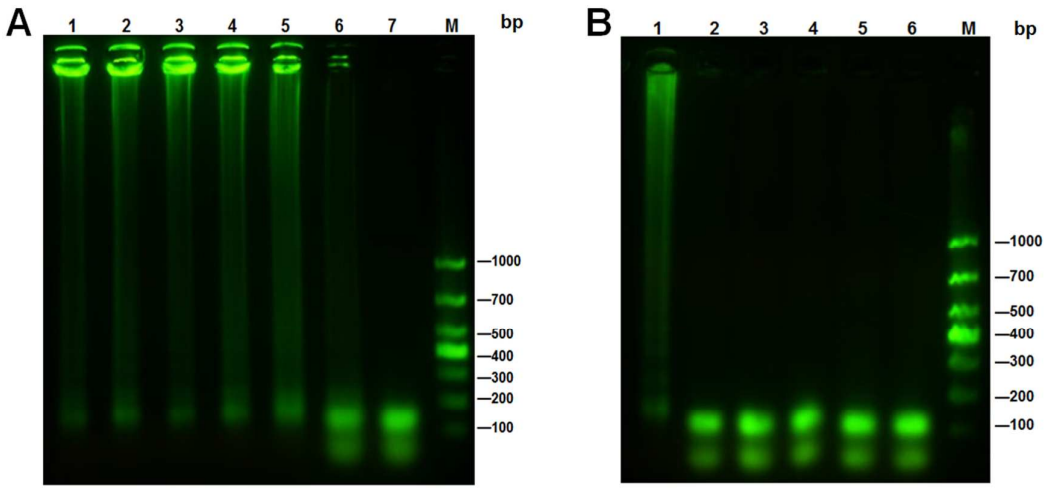


Figure S-2. 2% agarose gel electrophoresis of the MD-LAMP products. (A) Electrophoresis result of the products of gradient VP template concentrations. 1, 10^8 CFU/ mL; 2, 10^7 CFU/ mL; 3, 10^6 CFU/ mL; 4, 10^5 CFU/ mL; 5, 10^4 CFU/ mL; 6, 10^3 CFU/ mL; 7, nuclear-free water; M, DNA marker. (B) Electrophoresis result of the products of different bacteria. 1, *Vibrio parahemolyticus*; 2, *Salmonella typhimurium*; 3, *Shigella flexneri*; 4, *Listeria monocytogenes*; 5, *Staphylococcus aureus*; 6, nuclear-free water; M, DNA marker.

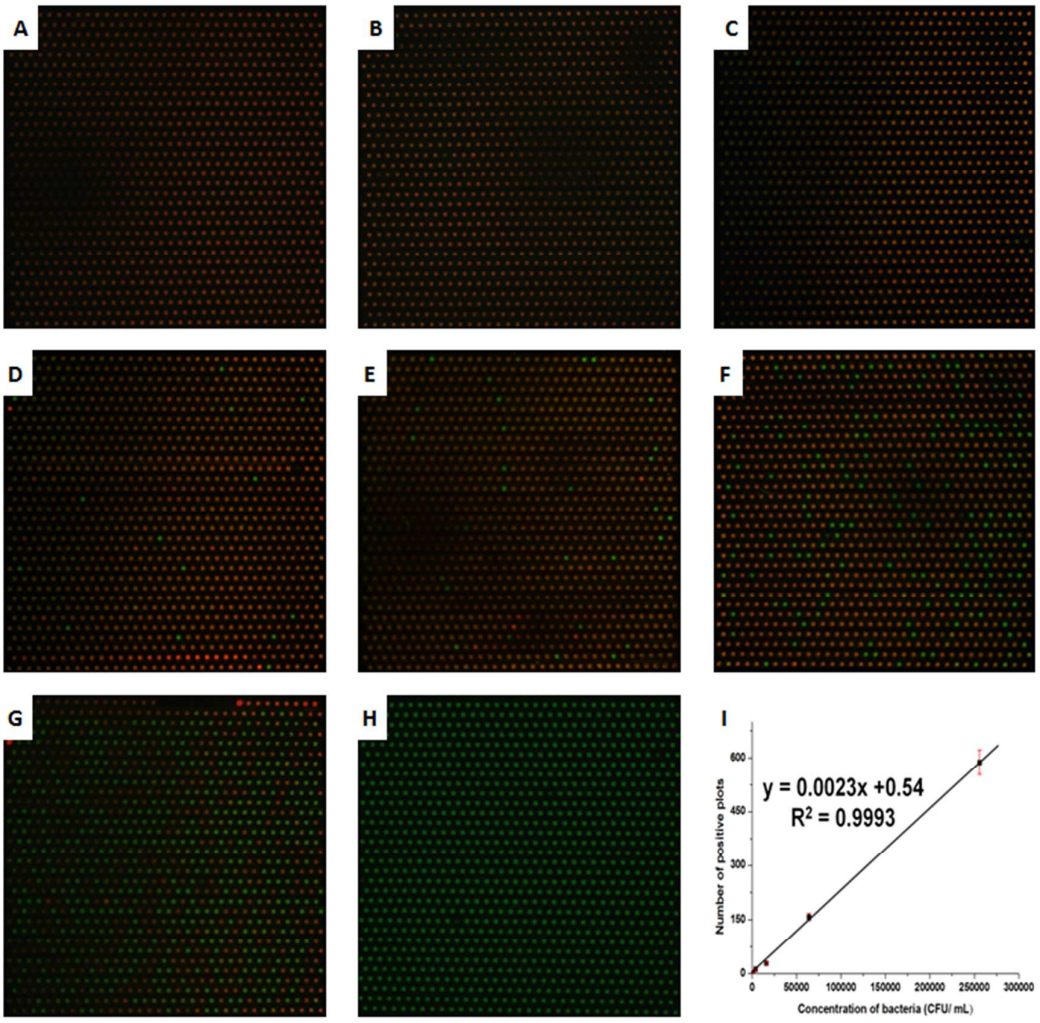
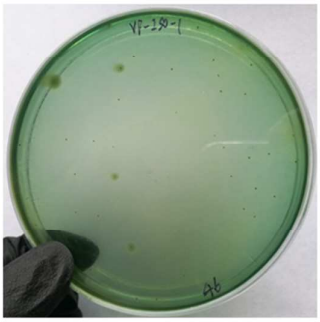
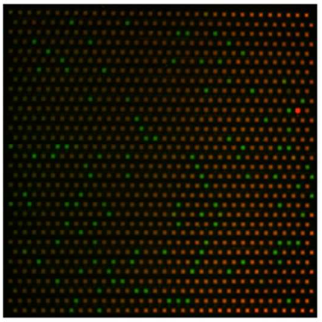
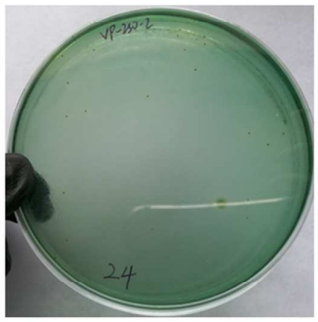
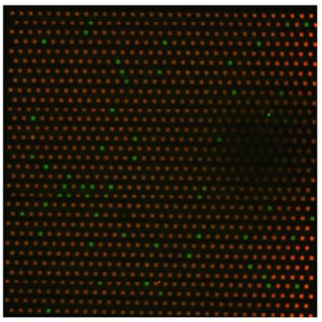
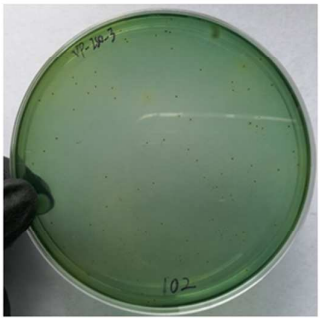
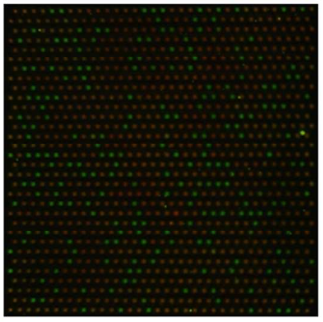
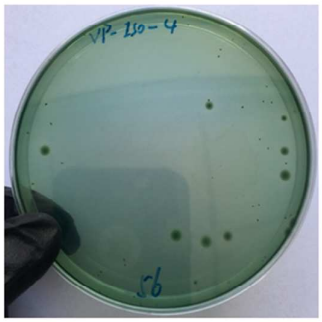
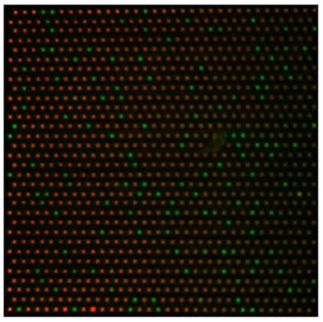


Fig. S-3. The CMD-LAMP reactions with different concentrations of VP in food contaminated sample: (A) 0 CFU/mL; (B) 2.5×10^2 CFU/mL; (C) 1.0×10^3 CFU/mL; (D) 4.0×10^3 CFU/mL; (E) 1.6×10^4 CFU/mL; (F) 6.4×10^4 CFU/mL; (G) 2.56×10^5 CFU/mL; (H) 1.024×10^6 CFU/mL; (I) The linear relationship between number of positive plots and bacterial concentrations in food contaminated sample. Error bars represented the standard deviation value of each test with three replicates.

	Plate count method	CMD-LAMP method
Isolation-1 of *Vibrio parahemolyticus*		
Colony forming units （1000 fold dilution）	47.67± 4.73	Positive signal plots: 104.00± 9.64
Isolation-2 of *Vibrio parahemolyticus*		
Colony forming units （1000 fold dilution）	23.00± 2.65	Positive signal plots: 53.00± 3.61
	Plate count method	CMD-LAMP method
Isolation-3 of *Vibrio parahemolyticus*		
Colony forming units （1000 fold dilution）	102.33± 5.51	Positive signal plots: 220.33± 8.50
Isolation-4 of *Vibrio parahemolyticus*		
Colony forming units （1000 fold dilution）	57.33± 7.09	Positive signal plots: 127.00± 3.61

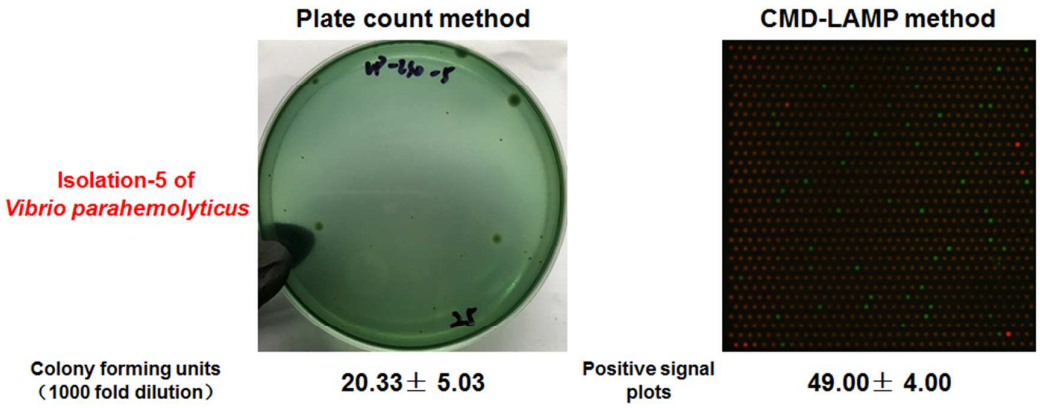


Figure S-4. Results for isolation strains of VP in food contaminated samples measured by plate count method (left) and CMD-LAMP method (right).

References:

1. Park, B. H.; Oh, S. J.; Jung, J. H.; Choi, G.; Seo, J. H.; Kim, D. H.; Lee, E. Y.; Seo, T. S., An integrated rotary microfluidic system with DNA extraction, loop-mediated isothermal amplification, and lateral flow strip based detection for point-of-care pathogen diagnostics. *Biosensors & bioelectronics* **2017**, *91*, 334-340.

2. Zhou, S.; Gao, Z. X.; Zhang, M. Development of a quadruplex loop-mediated isothermal amplification assay for field detection of four Vibrio species associated with fish disease. *SpringerPlus* **2016**, *5*, 1104.

3. Arunrut N.; Kampeera J.; Sirithammajak S. Sensitive visual detection of AHPND bacteria using Loop-Mediated Isothermal Amplification combined with DNA-functionalized gold nanoparticles as probes. *PloS One* **2016**, *11*, e0151769.

4. Wang Y.; Li D.; Wang Y. Rapid and sensitive detection of Vibrio parahaemolyticus and Vibrio vulnificus by multiple endonuclease restriction Real-Time Loop-Mediated Isothermal Amplification technique. *Molecules* **2016**, *21*, E111.

5. Zeng, J.; Wei, H.; Zhang, L.; Liu, X.; Zhang, H.; Cheng, J.; Ma, D.; Zhang, X.; Fu, P.; Liu, L., Rapid detection of Vibrio parahaemolyticus in raw oysters using immunomagnetic separation combined with loop-mediated isothermal amplification. *International journal of food microbiology* **2014**, *174*, 123-8.

6. Wang L.; Shi L.; Su J. Detection of Vibrio parahaemolyticus in food samples using in situ loop-mediated isothermal amplification method. *Gene*, **2013**, *515*, 421-5.

7. Prompamorn P.; Sithigorngul P.; Rukpratanporn S. The development of loop-mediated isothermal amplification combined with lateral flow dipstick for

detection of Vibrio parahaemolyticus. *Lett. Appl. Microbiol.* **2011**, *52*, 344-351.

8. Chen S.; Ge B. Development of a toxR-based loop-mediated isothermal amplification assay for detecting Vibrio parahaemolyticus. *BMC Microbiol.* **2010**, *10*, 41.

9. Yamazaki, W.; Kumeda, Y.; Misawa, N.; Nakaguchi, Y.; Nishibuchi, M., Development of a loop-mediated isothermal amplification assay for sensitive and rapid detection of the tdh and trh genes of Vibrio parahaemolyticus and related Vibrio species. *Applied and environmental microbiology* **2010**, *76*, 820-8.

10. Yamazaki, W.; Ishibashi, M.; Kawahara, R. Development of a loop-mediated isothermal amplification assay for sensitive and rapid detection of Vibrio parahaemolyticus. *BMC Microbiol.* **2008**, *8*, 163-170.

11. Escalante-Maldonado, O.; Kayali, A. Y.; Yamazaki, W.; Vuddhakul, V.; Nakaguchi, Y.; Nishibuchi, M., Improvement of the quantitation method for the tdh (+) Vibrio parahaemolyticus in molluscan shellfish based on most-probable- number, immunomagnetic separation, and loop-mediated isothermal amplification. *Frontiers in microbiology* **2015**, *6*, 270.

12. Di, H.; Ye, L.; Neogi, S. B. Development and evaluation of a loop-mediated isothermal amplification assay combined with enrichment culture for rapid detection of very low numbers of Vibrio parahaemolyticus in seafood samples. *Biol. Pharm. Bull.* **2015**, *38*, 82-87.

13. Tanaka N.; Iwade Y.; Yamazaki W. Most-probable-number loop-mediated isothermal amplification-based procedure enhanced with K antigen-specific

immunomagnetic separation for quantifying tdh(+) Vibrio parahaemolyticus in molluscan Shellfish. *J. Food Prot.* **2014**, *77*, 1078-85.

14. Yamazaki, M.; Aoki, H.; Iwade, Y.; Matsumoto, M.; Yamada, K.; Yamamoto, H.; Suzuki, M.; Hiramatsu, R.; Minagawa, H., An enrichment medium for increasing a very small number of vibrio parahaemolyticus cells to the detection limit of the loop-mediated isothermal amplification (LAMP) assay. *Japanese journal of infectious diseases* **2012**, *65*, 111-6.

15. Nemoto J.; Ikedo M.; Kojima T. Development and evaluation of a loop-mediated isothermal amplification assay for rapid and sensitive detection of Vibrio parahaemolyticus. *J. Food Prot.* **2011**, *74*, 1462-7.