A loop-mediated isothermal amplification-based method for the visual detection of *Vibrio parahaemolyticus* within only 1 hour, from shrimp sampling to results

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Materials and regents

A total of 26 bacterial strains including 5 *V. parahaemolyticus* isolates, 10 non-*parahaemolyticus Vibrio* isolates and 11 non-*Vibrio* bacteria were used in this study. As shown in **Table S1**, All strains were purchased from American Type Culture Collection (ATCC), USA, except *V. parahaemolyticus* KP9 and *V. parahaemolyticus* ZJ9N (kindly provided by School of Animal Science, Zhejiang University). All the natural seafood samples were purchased from local market of Hangzhou, China.

Regents of sodium hydroxide (NaOH) and agarose were both purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). TIANamp bacteria DNA kit was purchased from Tiangen Biotech Co., Ltd. (Beijing, China) for standard DNA extraction. TaKaRa Taq™ Hot Start amplification kit was purchased from Takara Bio Inc. (Dalian, China) for PCR amplification. For LAMP amplification, *Bst* DNA polymerase and thermoPol buffer were purchased from New England Biolabs Inc., (Ipswich, MA). Betaine was purchased from Sigma-Aldrich Co. LLC. (St Louis, MO, USA). dNTP (2.5 mM each) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). SYTO 9 was purchased from Thermo Fisher Scientific Inc. (Waltham, MA USA). $MgSO_4$ was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Thermostable inorganic pyrophosphatase was purchased from New England Biolabs Inc., (Ipswich, MA).

Primer designed for PCR amplification.

The primers recognized *V. parahaemolyticus*-specific thermolabile haemolysin (*tlh*)

gene (GenBank NO. M36437) were designed by Primer premier 5.0. The *tlh*-F (5'-GACATTACGTTCTTCGCCGC-3') and *tlh*-R (5'-GTTCTTCGCCAGTTTTGCGT–3') were used as PCR primers and the length of DNA amplicon was 354 bp. PCR reaction was carried out with TaKaRa Taq™ Hot Start amplification kit (Takara Bio Inc., Dalian, China) for 30 cycles in a MyiQ2 Real Time PCR Detection System (BioRad, Hercules, CA, USA).

Boiling method.

100 μL of *V. parahaemolyticus* suspension was heated at 95 °C for 10 min. After cell debris pelleted by centrifugation at 20,000 g and 4 °C for 5 min, 1 μL of the supernatant was used as DNA template for PCR and LAMP assay.

Commercial kit.

TIANamp bacteria DNA kit (Tiangen Biotech Co., Ltd, Beijing, China) was used for DNA extraction and purification according to its operation manual. DNA templates were dissolved in 200 μL tris-EDTA (TE) buffer and stored at -20 °C. For LAMP and PCR assay, 1 μL DNA was used as template.

67

68 **Table S1 Bacterial isolates used in this study.**

Strain	Strain ID	*tlh* amplification		Source[a]
		LAMP	PCR	
Vibro parahaemolyticus	**ATCC17802**	**P**	**P**	**ATCC**
Vibro parahaemolyticus	**ATCC33846**	**P**	**P**	**ATCC**
Vibro parahaemolyticus	**ATCC33847**	**P**	**P**	**ATCC**
Vibro parahaemolyticus	**KP9**	**P**	**P**	**ZJ**
Vibro parahaemolyticus	**ZJ9N**	**P**	**P**	**ZJ**
Vibrio harveyi	**ATCC14126**	**N**	**N**	**ATCC**
Vibrio vulnificus	**ATCC27562**	**N**	**N**	**ATCC**
Vibrio vulnificus	**ATCC29306**	**N**	**N**	**ATCC**
Vibrio vulnificus	**ATCC33816**	**N**	**N**	**ATCC**
Vibrio cholerae	**ATCC14035**	**N**	**N**	**ATCC**
Vibrio cincinnatiensis	**ATCC35912**	**N**	**N**	**ATCC**
Vibrio fluvialis	**ATCC 33809**	**N**	**N**	**ATCC**
Vibrio mimicus	**ATCC33653**	**N**	**N**	**ATCC**
Vibrio mimicus	**ATCC33655**	**N**	**N**	**ATCC**
Vibrio natriegens	**ATCC14048**	**N**	**N**	**ATCC**
E. coli O157:H7	**ATCC43889**	**N**	**N**	**ATCC**
Escherichia coli	**ATCC25922**	**N**	**N**	**ATCC**
Staphylococcus aureus subsp. aureus	**ATCC6538**	**N**	**N**	**ATCC**
Bacillus subtilis	**ATCC6633**	**N**	**N**	**ATCC**
Candida albicans	**ATCC10231**	**N**	**N**	**ATCC**
Salmonella typhimurium	**ATCC14028**	**N**	**N**	**ATCC**
Aspergillus brasiliensis	**ATCC16404**	**N**	**N**	**ATCC**
Escherichia coli	**ATCC8739**	**N**	**N**	**ATCC**
Pseudomonas aeruginosa	**ATCC9027**	**N**	**N**	**ATCC**
Listeria monocytogenes	**ATCC19115**	**N**	**N**	**ATCC**

69 P/N, positive/negative results.

70 [a]ATCC, American Type Culture Collection. ZJ, provided by School of Animal
71 Science, Zhejiang University.

Table S2 Estimation of the sensitivity of traditional sampling method for *V. parahaemolyticus* detection from spiked shrimp samples.

concentration of *V. parahaemolyticus* (CFU/g)	repeat	Ct value			mean of Ct values	SD	RSD (%)	mean of all Ct values	SD[r]	RSD(%)[r]	Time (min)[c]
		1	2	3							
	1	20.11	20.30	20.25	20.22	0.10	0.49				
1.25×10^6	2	20.11	20.15	20.03	20.10	0.06	0. 30	20.29	0.23	1.16	20.29
	3	20.67	20.42	20.56	20.55	0.13	0. 61				
	1	22.21	22.45	22.33	22.33	0.12	0. 54				
1.30×10^5	2	21.87	21.63	21.89	21.80	0.14	0. 66	22.04	0.27	1.23	22.04
	3	22.06	21.87	22.02	21.98	0.10	0. 46				
	1	24.97	25.03	24.82	24.94	0.11	0. 43				
4.50×10^4	2	27.43	27.39	27.21	27.34	0.12	0. 43	25.77	1.37	5.31	25.77
	3	24.91	25.02	25.11	25.01	0.10	0. 40				
	1	30.53	31.31	31.11	30.98	0.41	1.31				
3.00×10^3	2	32.07	32.18	33.02	32.42	0.52	1.60	31.58	0.75	2.38	31.58
	3	31.00	31.49	31.48	31.32	0.28	0. 89				
	1	43.61	_f	_f	43.61	_f	_f				
2.50×10^2	2	_f	42.55	_f	42.55	_f	_f	43.08	0.75	1.74	43.08
	3	_f	_f	_f	_f	_f	_f				
	1	_f	_f	_f	_f	_f	_f				
0.00×10^0	2	_f	_f	_f	_f	_f	_f	_f	_f	_f	_f
	3	_f	_f	_f	_f	_f	_f				

[r] Caculations based on all Ct values.

[f] No data.

[c] Every cycle takes 60s.

Table S3 Primers of LAMP and PCR for the detection of total *V. parahaemolyticus.*

		Sequence (5'-3')	Gene position
LAMP primers	**Tlh-FIP**	**CTGTCACCGAGTGCAACCACTTAACCACACGATCTGGAGCA**	**508-526/560-581**
	Tlh-BIP	**GCATCACAATGGCGCTTCCCACCGTTGGAGAAGTGACCTA**	**610-629/650-669**
	Tlh-F3	**CGCTGACAATCGCTTCTCAT**	**486-505**
	Tlh-B3	**GTTCTTCGCTTTGGCAATGT**	**686-705**
	Tlh-LF	**TGTTGATTTGATCTGGCTGC**	**540-559**
	Tlh-LB	**TAACCCGAACAGCTGGTTC**	**630-648**
PCR primers	**Tlh-F**	**GACATTACGTTCTTCGCCGC**	**469-488**
	Tlh-R	**GTTCTTCGCCAGTTTTGCGT**	**803-822**

Table S4 Amplification components for fluorescent LAMP assay.

Component	Final concentration
ThermoPol Buffer	1X
$MgSO_4$	6 mM
FIP and BIP	1.6 μM (each)
F3 and B3	0.2 μM (each)
LF and LB	0.4 μM (each)
Betaine	0.8 M
dNTP	1.4 mM
Bst DNA polymerase	8 U
SYTO 9	4 μM
DNA template	1 μL
Sterile water	up to 50 μL

Table S5 Detection of *V. parahaemolyticus* in natural seafood samples.

Sample type	No. of samples	Visual detection method	PCR	Culture-based method
Shrimp	32	3	3[a]	3[a]
Cuttlefish	11	2	2[a]	2[a]
Oyster	10	4	3[a]	4[a]
Jellyfish	15	2	2[a]	2[a]
Crab	28	4	4[a]	4[a]
Sleevefish	16	3	3[a]	3[a]
Lobster	17	2	2[a]	2[a]
Weever	12	2	1[a]	1[a]
Razor clam	33	3	3[a]	3[a]
Clam	42	3	3[a]	2[a]
Scallop	23	2	1[a]	2[a]
Porphyra	36	2	2[a]	2[a]
Laminaria japonica	28	3	3[a]	3[a]
Total	303	35	32	33
Positive rate	-	11.6 %	10.6 %	10.9 %

[a], LAMP positive.

Table S6 LAMP amplification components for visual detection.

Component	Final concentration
ThermoPol Buffer	1X
$MgSO_4$	6 mM
FIP and BIP	1.6 μM (each)
F3 and B3	0.2 μM (each)
LF and LB	0.4 μM (each)
Betaine	0.8 M
dNTP	1.4 mM
Bst DNA polymerase	8 U
SYTO 9	4 μM
Thermostable inorganic pyrophosphatase	0.1 U
DNA template	1 μL
Sterile water	up to 50 μL