Supporting Information for

A rotary valve-assisted fluidic system coupling with CRISPR/Cas12a for fully integrated nucleic acids detection

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Real-time PCR assay for V. parahaemolyticus

The real-time PCR assay was carried out in 25 μL reaction mixtures. The mixtures contained 1 × Ex Taq Buffer for Taq DNA polymerase (Takara Biomedical Technology Co., Ltd., Beijing, China), 0.2 mM dNTP each (Sangon, Shanghai, China), 0.4 μM F-tlh, 0.4 μM R-tlh, 0.625 U Ex Taq DNA polymerase (Takara Biomedical Technology Co., Ltd., Beijing, China), 4 μM SYTO 9 fluorescent stains (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 4 μL templates. And 20 μL mineral oil was added over the reaction mixtures to avoid evaporation. The thermal-cycled program was 95 ºC for 5 min, 40 cycles of 95 ºC for 15 s and 60 ºC for 30 s. The amplification reaction was performed in a QuantStudio 3 Real-time PCR system (Thermo Fisher Scientific Inc., Waltham, MA, USA). The cycle threshold (C_t) value reflected the initial number of the cycles at which a detectable fluorescent signal was generated. The amplicons could be further analyzed by melting curve. The melting process was performed at an ascent rate of 0.15 ºC/s from 70 ºC to 95 ºC and the fluorescent signal was recorded every 2 s. The primers were synthesized by Sangon (Shanghai, China) and the detailed information about the primers can be seen in **Table S2**.

The magnetic bead-based laboratory method

Detailed operation process was as follows: 300 μL of cultured bacterial strains were first centrifuged at 10000 rpm for 2 min in 1.5 mL centrifuge tube and the supernatant was discarded. The precipitated bacterial strains were resuspended by 250 μL of lysis reagents. The lysis reagents (pH 7.6-8.0) were consisted of 4 M guanidinium isothiocyanate (GITC), 20 mM ethylenediaminetetraacetic acid (EDTA), 2 % sarkosyl and 50 mM Tris-HCl. Then, 300 μL of isopropanol and 2 μL of magnetic beads (Sera-Mag SpeedBeads Carboxyl Magnetic Beads, 50 mg/mL) were added into the tube. The tube was shaken at room temperature for 15 min. A magnetic stand was used to settle the magnetic beads and the supernatant was discarded. Afterwards, the magnetic beads were washed twice with 300 μL of 80 % ethanol. Later, the supernatant was completely removed, and the magnetic beads needed to be dried for about 10 min to ensure that the remaining ethanol could be removed thoroughly. Finally, 50 μL of nuclease-free water was added into the tube to resuspend the magnetic beads for 5 min. The magnetic beads were settled by the magnetic stand and the supernatant containing nucleic acids was collected for subsequent experiments.

The agarose gel electrophoresis analysis

After amplification, the amplicons were electrophoresed at 110 V on a 3 % (w/v) agarose gel (pre-supplemented with GoldView Ⅱ, Solarbio Science & Technology Co., Ltd, Beijing, China) in 1 × TAE buffer. A 50 bp DNA ladder (Sangon, Shanghai, China) was employed as marker. The gel was photographed with a ChemiDoc XRS+ System (BioRad, CA, USA).

The detailed lyophilization steps for LAMP reagents

The one lyophilized protection solution (Solution 1), which consisted of 30 % (wt) D-(+)-trehalose dihydrate ($C_{12}H_{22}O_{11}\cdot 2H_2O$) and 10 % (wt) pullulan (($C_{37}H_{62}O_{30}$)n), was first prepared. During preparation, after the pullulan was fully dissolved, the D-(+)-trehalose dihydrate was then added. All of them were purchased from Macklin Biochemical Co., Ltd, Shanghai, China. Subsequently, another lyophilized protection solution consisting of 60 % (wt) Mannitol ($C_6H_{14}O_6$) was prepared (Solution 2). Mannitol was purchased from Shanghai yuanye Bio-Technology Co., Ltd, China. Afterwards, the LAMP mixtures (total 12 μL) was prepared (Solution 3), which contained 5 μL of 10 × ThermoPol buffer, 32 U *Bst* DNA polymerase, 8 mM $MgSO_4$, 6 mM dNTP, 3.2 M betaine and primer mixtures consisting of 6.4 μM FIP-tlh, 6.4 μM BIP-tlh, 0.8 μM F3-tlh, 0.8 μM B3-tlh, 1.6 μM LF-tlh and 1.6 μM LB-tlh.

Then, the 20 μL of solution 1, 5 μL of solution 2 and 12 μL of solution 3 were mixed. The mixtures stood at 4 ºC for 2 hours to make the protection solution and LAMP mixtures mixed thoroughly. After mixing, the solution mixtures were stored at -20 ºC for 6 h and then were stored at -80 ºC for 36-48 h. Finally, the mixtures were freeze-dried for 4-5 h by the freeze dryer (SCIENTZ-10N, Ningbo Scientz Biotechnology Co., Ltd, China). Thus, the lyophilized LAMP reagents were obtained.

The preparation procedure of spiked shrimp samples

Fresh shrimp samples purchased from Hangzhou local market were first de-headed and de-contaminated by immersing in 75 % ethanol for 2 min. Then, the shrimp samples were washed by nuclease-free water and placed in a biological safety hood under UV light for 30 min. Afterwards, the sterile shrimp samples were respectively incubated with a series of gradient concentrations of *V. parahaemolyticus* (O3:K6 and O4:K8) for 30 min under ambient temperature, which made the shrimp samples fully attached by *V. parahaemolyticus*. Thus, the preparation of spiked shrimp samples was completed, and disposable Q-tips were used to collect the samples by fully wiping the shrimps.

Table S1. The information about the bacterial strains used in this study.

Strain	Strain ID or serotypes	PCR for tlh	Source[a]
Vibrio parahaemolyticus	O3:K6	P	Lin'an CDC (China)
Vibrio parahaemolyticus	O4:K8	P	Lin'an CDC (China)
Vibrio parahaemolyticus	ATCC33846	P	ATCC
Vibrio parahaemolyticus	ATCC33847	P	ATCC
Vibrio parahaemolyticus	ATCC17802	P	ATCC
Vibrio harveyi	ATCC14126	N	ATCC
Vibrio vulnificus	ATCC27562	N	ATCC
Vibrio cholerae	ATCC14035	N	ATCC
Vibrio cincinnatiensis	ATCC35912	N	ATCC
Vibrio mimicus	ATCC33653	N	ATCC
Vibrio natriegens	ATCC14048	N	ATCC
Vibrio fluvialis	ATCC33809	N	ATCC
Vibrio vulnificus	ATCC29306	N	ATCC
E. coli O157:H7	ATCC43889	N	ATCC
Staphylococcus aureus	ATCC29213	N	ATCC
Aspergillus brasiliensis	ATCC16404	N	ATCC
Pseudomonas aeruginosa	ATCC9027	N	ATCC
Listeria monocytogenes	ATCC54006	N	ATCC
Candida albicans	ATCC10231	N	ATCC
Escherichia coli	ATCC25922	N	ATCC
Bacillus subtilis	ATCC6633	N	ATCC
Escherichia coli	ATCC8739	N	ATCC

P/N: Positive/Negative results

[a] Lin'an CDC: Lin'an Center for Disease Control and Prevention; ATCC: American Type Culture Collection.

105 **Table S2.** Sequences of primers and gRNA-tlh

Category	Sequence name	Sequence information	Reference
Primers used in LAMP assay	F3-tlh	CGCTGACAATCGCTTCTCAT	[1]
	B3-tlh	GTTCTTCGCTTTGGCAATGT	
	FIP-tlh	CTGTCACCGAGTGCAACCACTTAACC ACACGATCTGGAGCA	
	BIP-tlh	GCATCACAATGGCGCTTCCCACCGTT GGAGAAGTGACCTA	
	LF-tlh	TGTTGATTTGATCTGGCTGC	
	LB-tlh	TAACCCGAACAGCTGGTTC	
Primers used in PCR assay	F-tlh	GACATTACGTTCTTCGCCGC	
	R-tlh	GTTCTTCGCCAGTTTTGCGT	
gRNA used in CRISPR/Cas12a	gRNA-tlh	UAAUUUCUACUAAGUGUAGAUUUG AUUUGAUCUGGCUGCAUU	[2]

106

Table S3 Comparison of our established method, other reported methods by LAMP and commercial methods for pathogen detection

Targets	Fully integrated	D-time	D-cost	Sensitivity (per reaction)	Operation process	Ref.
Vibrio parahaemolyticus	Yes	80 min	< $4 (USD)	31 copies	No centrifugation; No pumping	This work
Other reported methods						
Vibrio parahaemolyticus	No	60 min	< $4 (USD)	2.63 CFU	Complicated manual operation	[1]
Vibrio parahaemolyticus	No	> 80 min	< $4 (USD)	~ 2000 copies	Complicated manual operation	[3]
Vibrio parahaemolyticus	No	< 60 min	> $5 (USD)	100 copies	Centrifugation	[4]
Vibrio parahaemolyticus	No	> 70 min	< $4 (USD)	41.8 copies	Complicated manual operation	[5]
Salmonella	Yes	> 70 min	> $15 (USD)	~ 50 CFU	Pumping	[6]
Salmonella	Yes	90 min	> $10 (USD)	0.34 CFU	Pumping	[7]
Vibrio parahaemolyticus	Yes	60 min	> $10 (USD)	100 CFU	Centrifugation	[8]
Escherichia coli O157:H7	No	> 60 min	120 (USD)	2 copies	Complicated manual operation	[9]
Commercial methods						
Adenovirus	Yes; FilmArray pouch	~ 60 min	> $80 (USD)	60 CFU	/	[10]
Ebola virus	Yes; GeneXpert	100 min	> $30 (USD)	7.3 copies	/	[11]
Influenza virus	Yes; Cobas Liat	~ 20 min	> $20 (USD)	~ 250 copies	/	[12]

D-time: detection time from sampling to results; D-cost: detection cost per test.

Figure S1 The schematic diagram of LAMP reaction coupling with CRISPR/Cas12a

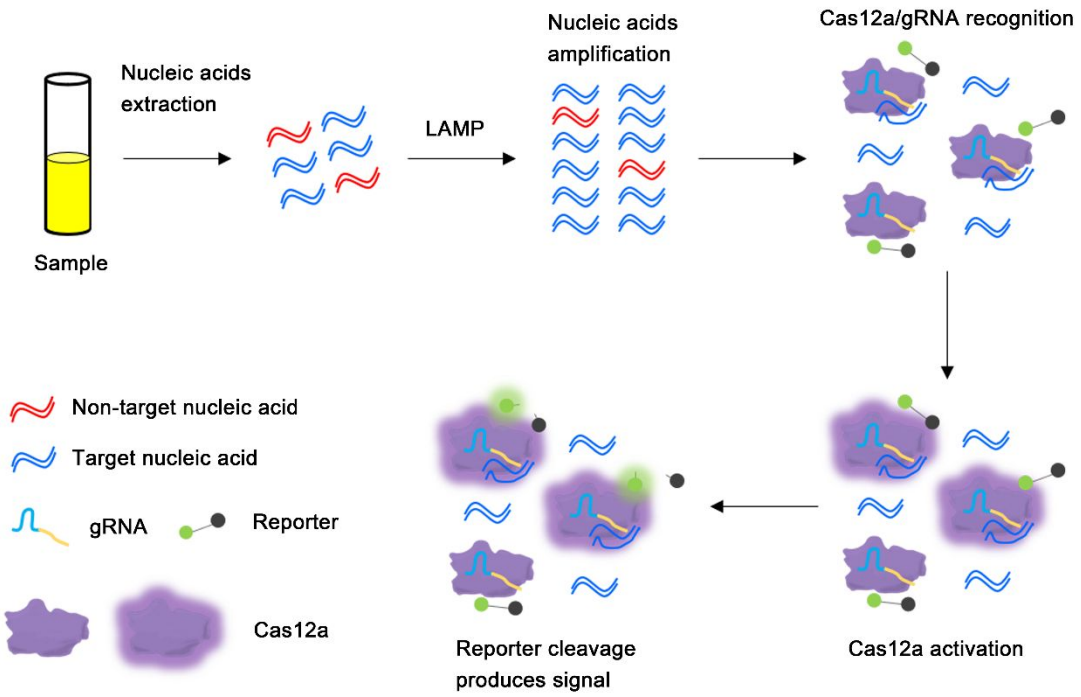


Figure S2 The photos of fluidic chip. **a)** The appearance of fluidic chip. **b)** The top and side views of fluidic chip.

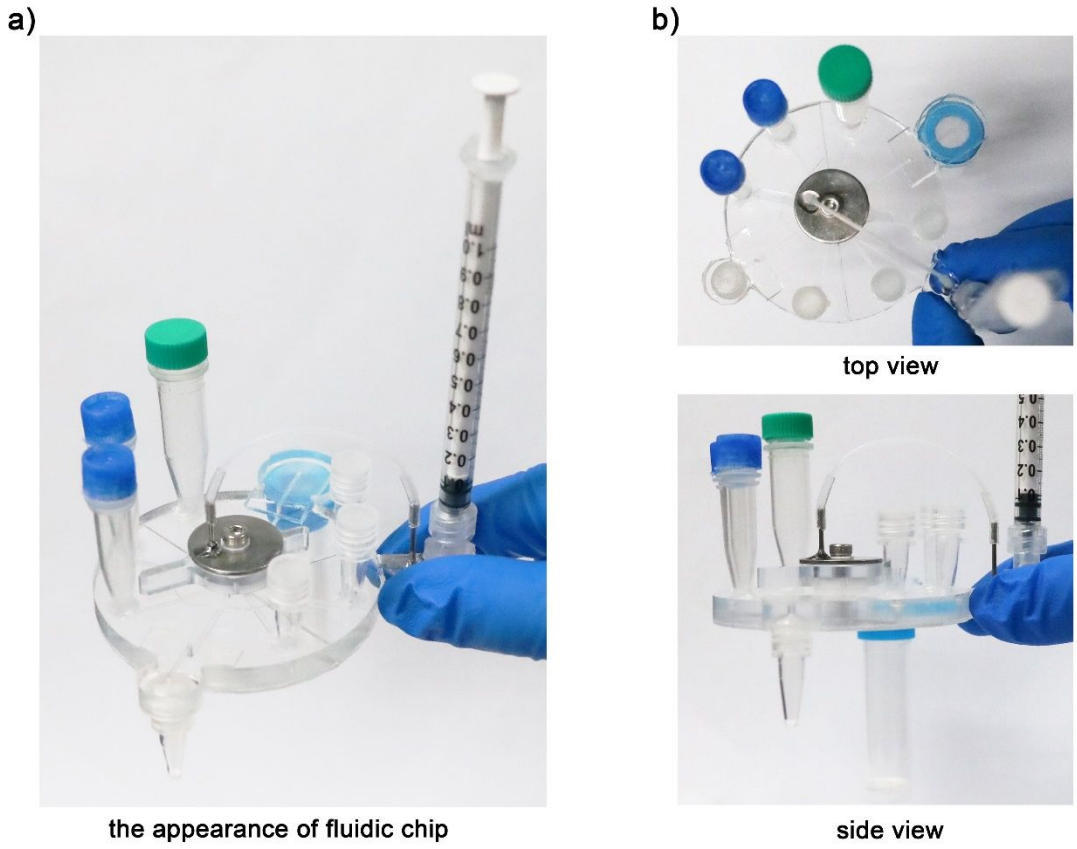


Figure S3 The nucleic acids extraction process with the fluidic chip (45 min) and the whole operation process from sampling to results completed in 80 min.

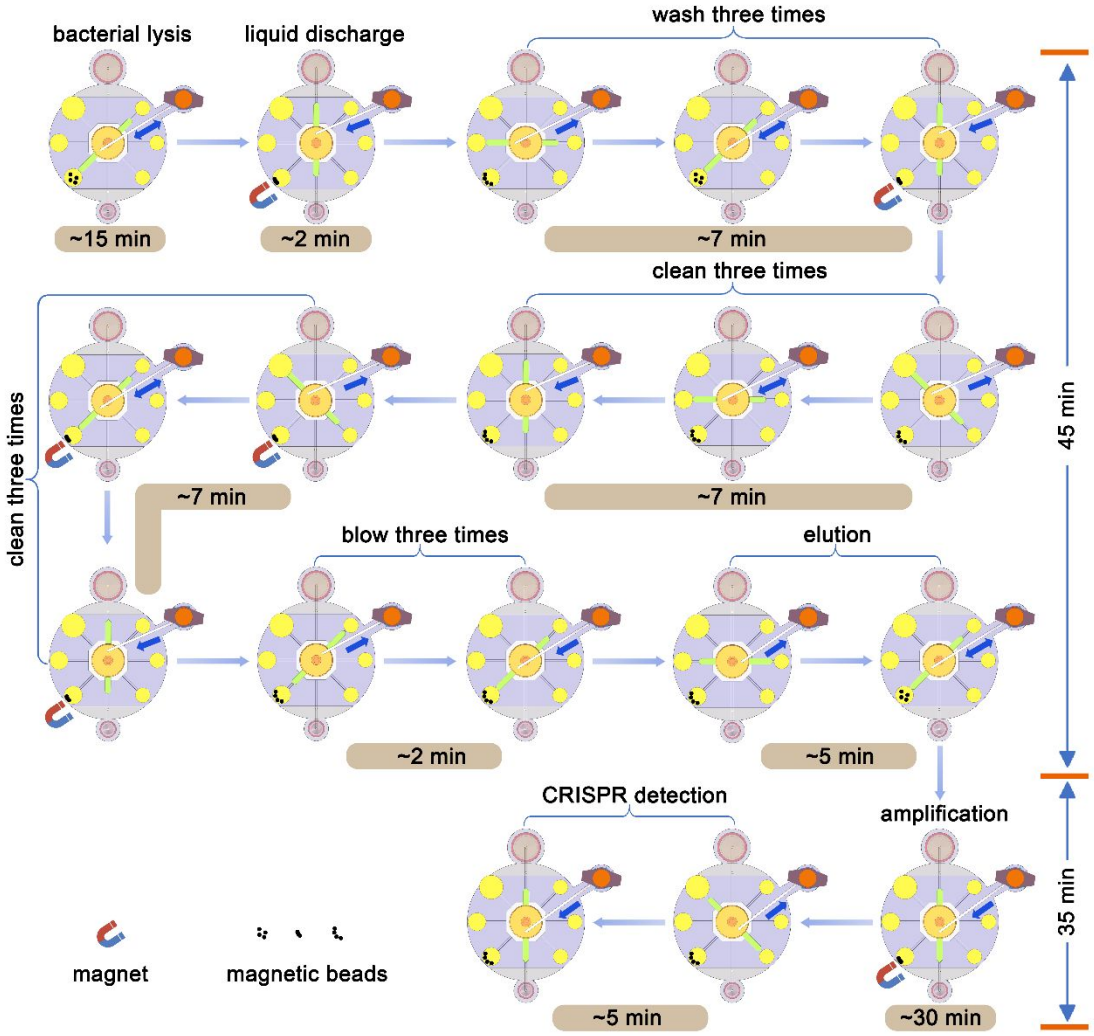


Figure S4 a) The gel image of LAMP amplicons with *V. parahaemolyticus* (O3:K6 and O4:K8) containing around 3.1 x 10^6 copies of target DNA as targets. M: marker; N: nuclease-free water. **b)** The gel image before CRISPR/Cas12a detection (b-1) and after CRISPR/Cas12a detection (b-2). LAMP reaction was conducted with *V. parahaemolyticus* (O3:K6) containing around 3.1 x 10^6 copies of target DNA as target.

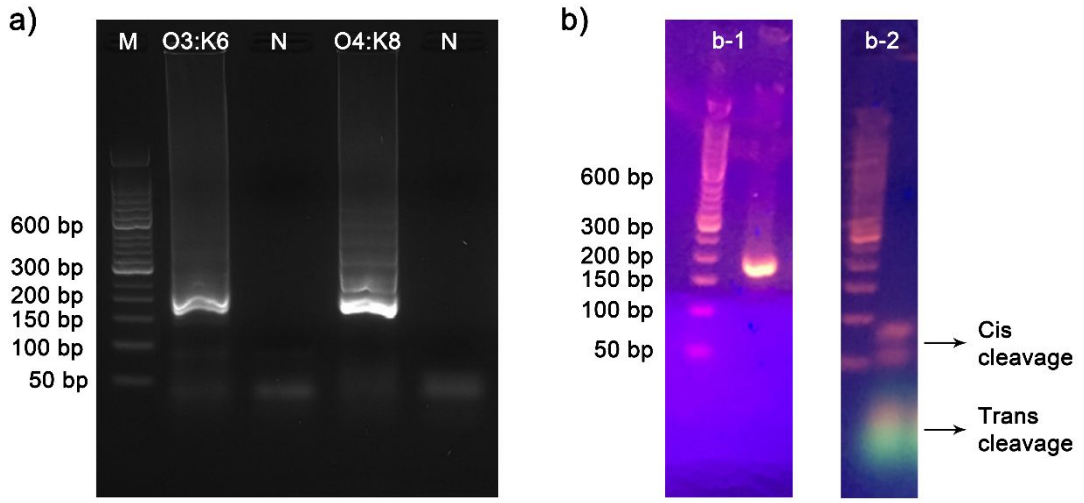
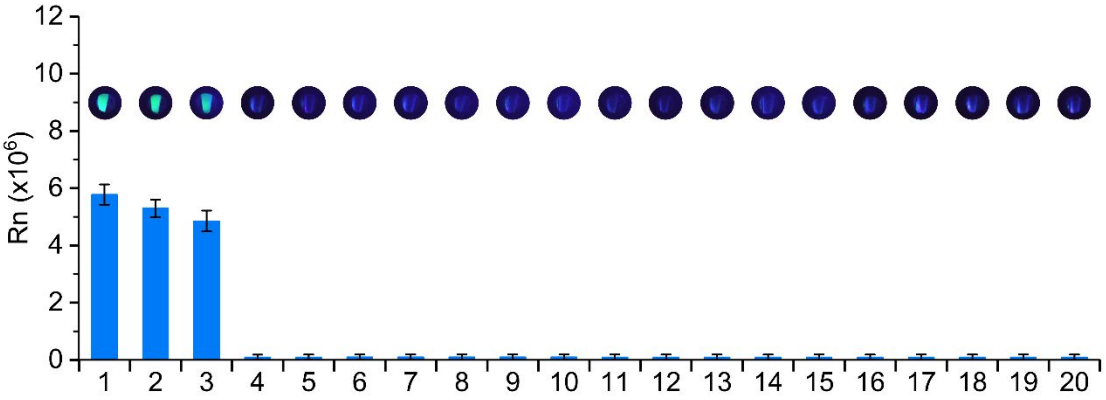


Figure S5 Results of specificity test by the fluidic chip coupling with CRISPR/Cas12a (The detection concentration of bacteria was adjusted to 1.0 x 10^6 copies of target DNA per reaction; The fluorescent signal intensity acquired by QuantStudio 3 Real-time PCR system after the CRISPR reagents were mixed with LAMP reaction mixtures for 5 min at 37 ºC)



1: *Vibrio parahaemolyticus* (ATCC33846)
2: *Vibrio parahaemolyticus* (ATCC33847)
3: *Vibrio parahaemolyticus* (ATCC17802)
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17: *Candida albicans* (ATCC10231)
18: *Escherichia coli* (ATCC25922)
19: *Bacillus subtilis* (ATCC6633)
20: *Escherichia coli* (ATCC8739)

Captions for Supporting Videos S1-S2

Supporting video S1. The sealing performance of fluidic chip.

Supporting video S2. The liquid stirring performance of fluidic chip.

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