

## Supplementary Information

### Portable and point-of-care molecular detection of pathogenic *Vibrio* *parahaemolyticus* in shrimp

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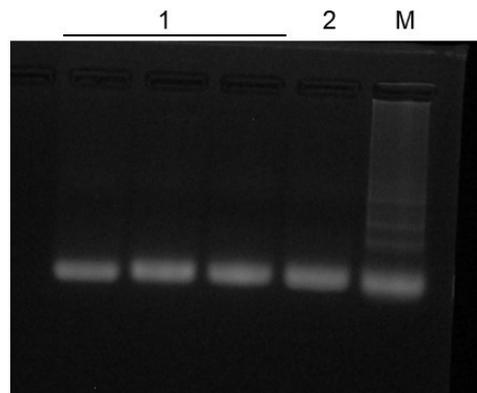
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**Table S1.** Primer set used for LAMP assay detecting *pirA* gene

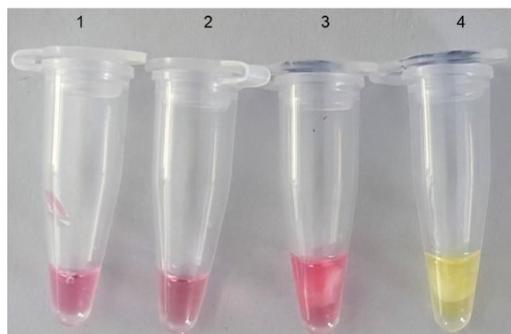
Bacteria	Gene	Primer name	Sequence	Length (bp)	5'pos	3'pos
<i>V. parahaemolyticus</i>	<i>pirA</i>	F3	CGTCACAGAAGTAGACAGC	19	113	131
		B3	CGTTGTAAATGGTAAGTTTCATCA	24	275	298
		FIP	CCACGTCCCGTATTCTCAATGT- AAACATACACCTATCATCCCG	43		
		BIP	GGAGCTTACCATTCAATACCAATG G-TTGTACCACATGTGATTTAGC	46		
		LB	GGTGCGCCATTTATGGCTGG	20	219	238

Optimization of LAMP condition for *pirA* primer set

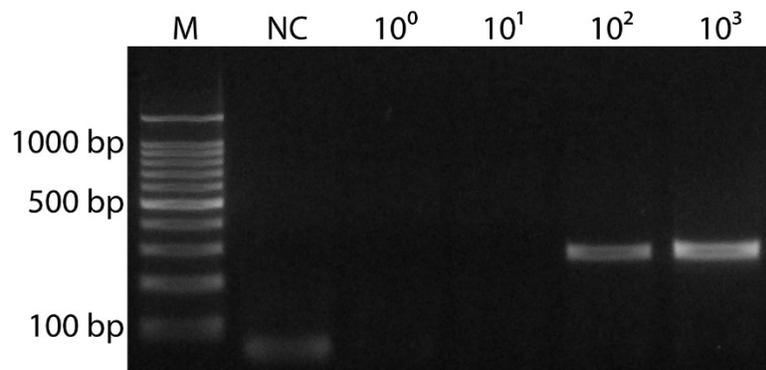


M: ladder, 1: Reaction performed at 60 °C, 2: Negative control.

**Fig. S1.** Agarose gel electrophoresis result of LAMP assay obtained using *pirA* primer set at different temperatures.

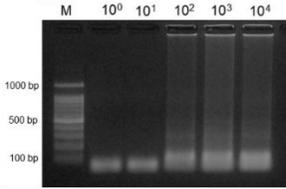
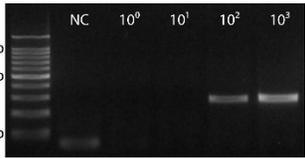


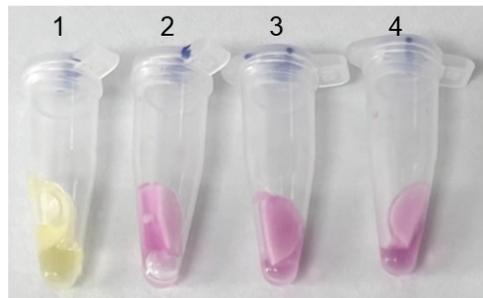
**Fig. S2.** Colorimetric results. 1, 2: No FTA card added. 3. FTA card not containing template was added. 4. FTA card containing template was added.



**Fig. S3.** Result of gel electrophoresis showing LOD of *V. parahaemolyticus*-causing AHPND on FTA card with conventional PCR amplification. NC: negative control.

**Table S2.** Comparison showing the LODs of *V. parahaemolyticus*-causing AHPND when LAMP and PCR were performed using templates serially diluted from  $10^0$  to  $10^3$

Criteria	LOD of LAMP amplification	LOD of PCR amplification																				
Definition	The lowest concentration of the genetic material which can be consistently detected in the serial dilution with the studied amplification																					
Target genes	<i>V. parahaemolyticus</i> -causing AHPND dropped on the FTA card																					
Polymerase	<i>Bst</i> polymerase	<i>Taq</i> polymerase																				
Running condition	<div style="border: 1px solid black; padding: 5px; width: fit-content; margin: auto;"> <p style="text-align: center; background-color: #FFD700; margin: 0;">LAMP amplification</p> <p style="text-align: center; margin: 5px 0 0 20px;">65°C</p> <hr style="border: 0.5px solid red; margin: 5px 0 0 20px;"/> <p style="text-align: center; margin: 0 20px 0 20px;">45 min</p> </div>	<table border="1" style="border-collapse: collapse; text-align: center; width: 100%;"> <thead> <tr style="background-color: #FFD700;"> <th>Pre-denaturation</th> <th>Denaturation</th> <th>Annealing</th> <th>Extension</th> <th>Final extension</th> </tr> </thead> <tbody> <tr> <td>95°C</td> <td>95°C</td> <td>52°C</td> <td>72°C</td> <td>72°C</td> </tr> <tr> <td>5 min</td> <td>30 s</td> <td>20 s</td> <td>20 s</td> <td>1 min</td> </tr> <tr> <td colspan="5" style="border-top: 1px solid black; border-bottom: 1px solid black;">35 cycles</td> </tr> </tbody> </table>	Pre-denaturation	Denaturation	Annealing	Extension	Final extension	95°C	95°C	52°C	72°C	72°C	5 min	30 s	20 s	20 s	1 min	35 cycles				
Pre-denaturation	Denaturation	Annealing	Extension	Final extension																		
95°C	95°C	52°C	72°C	72°C																		
5 min	30 s	20 s	20 s	1 min																		
35 cycles																						
Validation levels	$10^2$ CFU/ml of <i>V. parahaemolyticus</i> -causing AHPND																					
Gel electrophoresis																						



- 1: *Vibrio parahaemolyticus*
- 2: *Vibrio alginolyticus*
- 3: *Vibrio harveyi*
- 4: *Vibrio vulnificus*

**Fig. S4.** LAMP assay performed for specificity test determining *V. parahaemolyticus*-causing AHPND. All experiments were repeated three times.

**Table S3.** Raw G values corresponding to the data presented in Figure 3b

	G value	
	50 $\mu$ L	100 $\mu$ L
Replication 1	174.272	218.649
Replication 2	170.212	210.953
Replication 3	162.79	218.091
Negative control	164.396	178.977

**Table S4.** Raw G values corresponding to the data presented in Figure 4b

<i>V. harveyi</i>	<i>V. parahaemolyticus</i>
177.048	214.201
198.668	206.528
202.991	208.368

**Table S5.** Raw G-values corresponding to the data presented in Figure 7d

Non-spiked shrimp	Spiked shrimp
191.147	210.923
179.708	216.106
179.041	214.887