Electronic Supplementary Material (ESI) for Analytical Methods.

Real-time LAMP-based dual-sample microfluidic chip for rapid and simultaneous detection of multiple waterborne pathogenic bacteria from coastal waters

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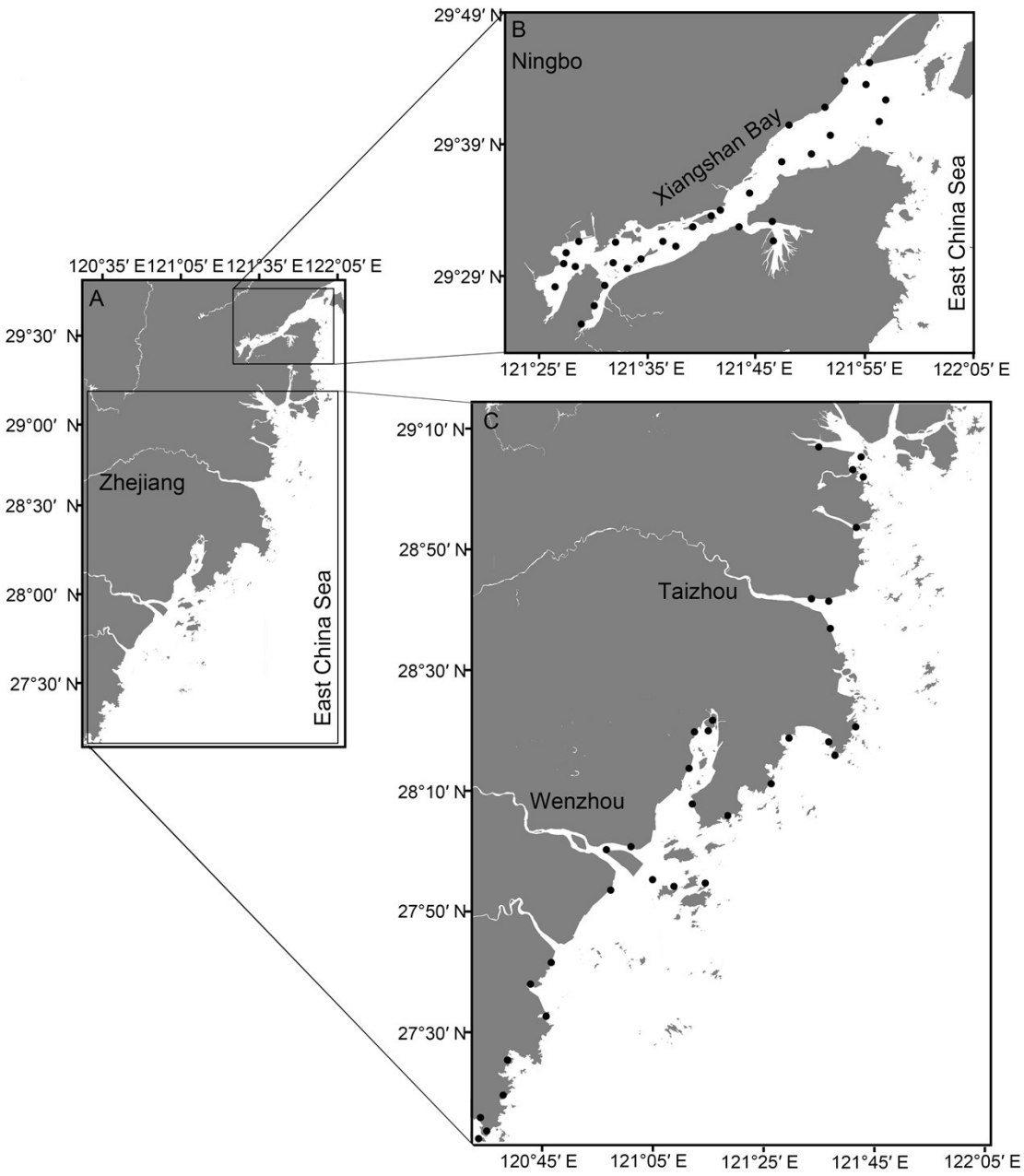

Fig. S1 Map of the Xiangshan Bay, Taizhou, and Wenzhou coastal region (A) showing the sampling regions of fisheries, residential areas, or industrial parks (B and C). 64 sampling sites were marked using black dots

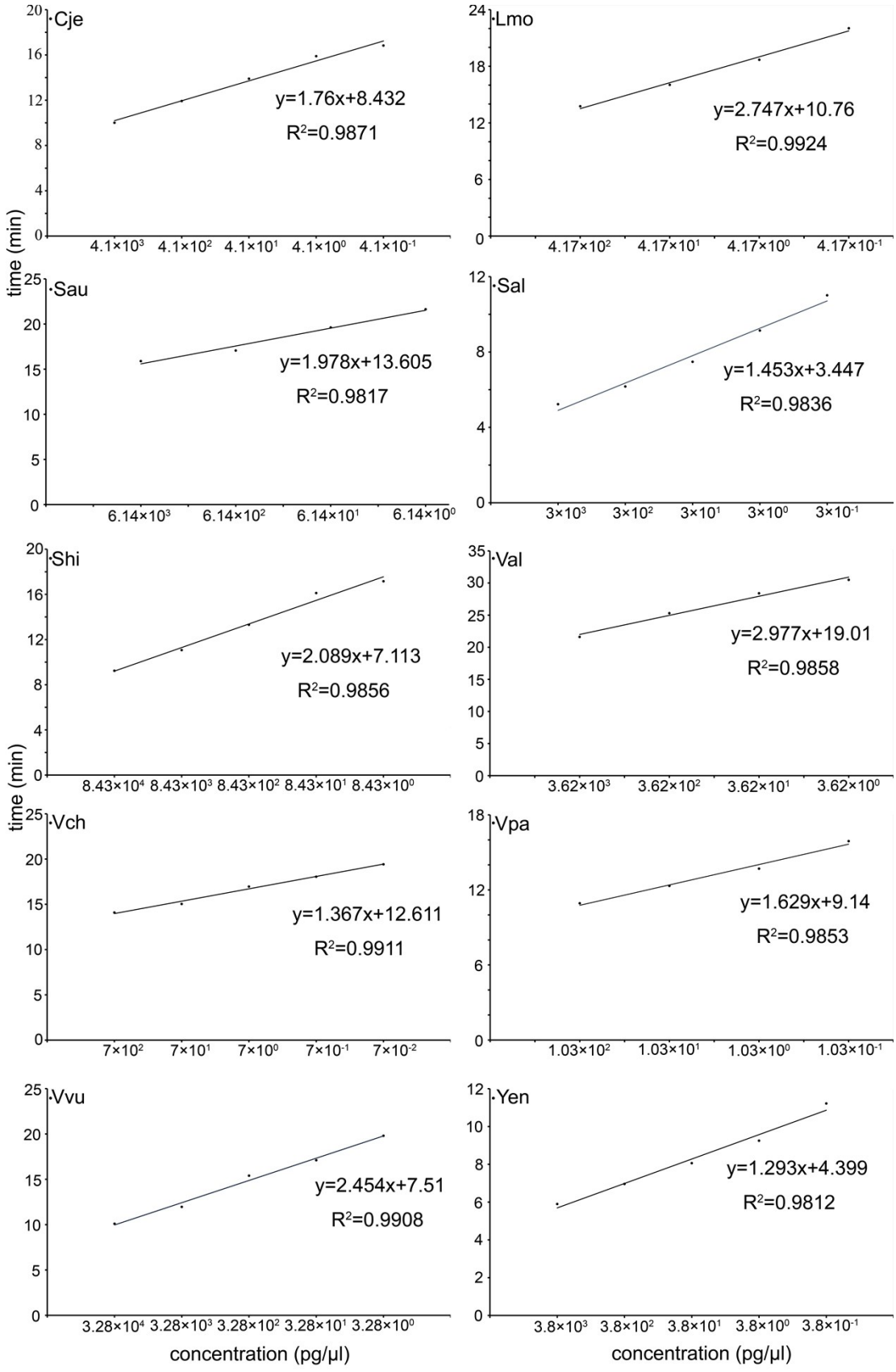

Fig. S2 The limits of detection (LOD) of the in-tube LAMP. The correlation between time to amplification and amount of target DNA was determined using the in-tube

LAMP. Serially diluted gDNA of each microbe is presented on the X-axis, and time is presented on the Y-axis.

Table S1 Microbial species used in this study.

Bacteria	Isolate/Strain	Source
Aeromonas hydrophila	ATCC 13444	Purchased from American Type Culture Collection (ATCC)
Aeromonas hydrophila	ATCC 7966	Purchased from China General Microbiological Culture Collection Center (CGMCC)
Aeromonas hydrophila	ayu-Ah0201	Isolated and kept in our laboratory
Campylobacter jejuni	ATCC 29428	Purchased from ATCC
Campylobacter jejuni	ATCC 33291	Provided by Prof. Yan-Jun Zhang (Zhejiang Provincial Center for Disease Control and Prevention, China)
Campylobacter jejuni	-	Provided by Prof. Yan-Jun Zhang
Edwardsiella tarda	MCCC 235	Purchased from Marine Culture Collection of China (MCCC)
Edwardsiella tarda	Et-CD	Provided by Prof. Mao-Cang Yan (Zhejiang Mariculture Research Institute, China
Escherichia coli	ATCC 9637	Purchased from ATCC
Listeria monocytogenes	ATCC 19115	Purchased from Guangdong Microbial Culture Collection Center (GDCCC)
Listeria monocytogenes	ATCC 700302	Purchased from ATCC
Listeria monocytogenes	10403S	Provided by Prof. Wei-Huan Fang (College of Animal Sciences, Zhejiang University,

		China)
Listeria monocytogenes	ZJ364	Provided by Prof. Wei-Huan Fang
Nocardia seriolae	ATCC 43993	Purchased from ATCC
Nocardia seriolae	W060622	Provided by Prof. Guo-Liang Wang (College of Marine Sciences, Ningbo University, China)
Photobacterium damselae	Mm041	Isolated and kept in our laboratory
Pseudomonas aeruginosa	ATCC 9027	Purchased from GDCCC
Pseudomonas putida	MCCC 1A01082	Purchased from MCCC
Proteus vulgaris	ATCC 13315	Purchased from ATCC
Staphylococcus aureus	ATCC 6538	Purchased from GDCCC
Staphylococcus aureus	ATCC 27325	Purchased from ATCC
Salmonella enterica	ATCC 9150	Purchased from ATCC
Salmonella enterica	ATCC 50041	Provided by Prof. Yan-Jun Zhang
Shigella flexneri	ATCC 12022	Provided by Prof. Yan-Jun Zhang
Streptococcus iniae	ATCC 29178	Purchased from ATCC
Streptococcus iniae	SO-2	Provided by Prof. Dong Qian (College of Marine Sciences, Ningbo University, China)
Vibrio alginolyticus	ATCC 17749	Purchased from ATCC
Vibrio alginolyticus	ATCC 33787	Purchased from ATCC
Vibrio alginolyticus	MCCC 1B00091	Purchased from MCCC
Vibrio anguillarum	ATCC 19264	Purchased from ATCC
Vibrio anguillarum	ayu-H080701	Isolated and kept in our laboratory
Vibrio campbellii	GL1008_W02	Isolated and kept in our laboratory
Vibrio campbellii	GL1008_W16	Isolated and kept in our laboratory
Vibrio cholerae	GL1010_S02	Isolated and kept in our laboratory
Vibrio cholerae	-	Provided by Prof. Yan-Jun Zhang
Vibrio cholerae	CMCC 4819	Provided by Prof. Yan-Jun Zhang
Vibrio coralliilyticus	GL1009_W34	Isolated and kept in our laboratory
Vibrio natriegens	GL1009_W34	Isolated and kept in our

		laboratory
Vibrio fischeri	ATCC 700601	Purchased from ATCC
Vibrio fluvialis	ATCC 33809	Purchased from CGMCC
Vibrio harveyi	ATCC 14126	Purchased from ATCC
Vibrio harveyi	ATCC 33866	Purchased from CGMCC
Vibrio harveyi	ATCC 33842	Purchased from CGMCC
Vibrio ichthyoenteri	GL1012_S01	Isolated and kept in our laboratory
Vibrio orientalis	GL1010_W10	Isolated and kept in our laboratory
Vibrio orientalis	GL1010_S07	Isolated and kept in our laboratory
Vibrio parahaemolyticus	ATCC 33845	Provided by Prof. Yan-Jun Zhang
Vibrio parahaemolyticus	ATCC 33847	Provided by Prof. Yan-Jun Zhang
Vibrio parahaemolyticus	O1	Provided by Prof. Yan-Jun Zhang
Vibrio parahaemolyticus	O2	Provided by Prof. Yan-Jun Zhang
Vibrio parahaemolyticus	O3	Provided by Prof. Yan-Jun Zhang
Vibrio rotiferianus	CAIM 577	Purchased from the Belgian Co-Ordinated Collection of Microorganisms (BCCM/LMG)
Vibrio rotiferianus	GL1009_W24	Isolated and kept in our laboratory
Vibrio splendidus	Vs-So	Provided by Prof. Mao-Cang Yan
Vibrio tubiashii	GL1011_C02	Isolated and kept in our laboratory
Vibrio vulnificus	ATCC 27562	Purchased from CGMCC
Yersinia enterocolitica	ATCC 23715	Provided by Prof. Yan-Jun Zhang

Table S2 Status of seawater samples collected in this study

Sampling Site / Samples Counts / Sampling Time	Xiangshan bay in Ningbo	Wenzhou	Taizhou [b]
September 2018	31	-	-
April 2019	30	-	-
July 2019	12 [a]	-	-
September 2019	-	18	15
April 2020	-	18	-

[a] These samples were collected due to local official temporary requirement.

[b] No samples were collected from these sampling sites in April 2020, due to epidemic prevention against COVID-19.

Table S3 Layout of the primer sets in the dual-sample chip corresponding to 10 tested bacteria

No. of the reaction wells [a]	Pathogens
1, 12	inner positive control (PC)
2, 13	*V. parahaemozyticus*
3, 14	*V. cholerae*
4, 15	*V. vulnificus*
5, 16	*V. alginolyticus*
6, 17	*S. enterica*
7, 18	*S. flexneri*
8, 19	*C. jejuni*
9, 20	*S. aureus*
10, 21	*Y. enterocolitica*
11, 22	*L. monocytogenes*

[a] The reaction wells from No. 1 to No. 11 are located in Half Ⅰ of the dual-sample chip; in contrast, the reaction wells from No. 12 to No. 22 are located in Half Ⅱ

Table S4 Comparison of on-chip LAMP assay with conventional microbiological methods and conventional PCR assays.

Sample collection region	Sample number	Conventional microbiological methods	On-chip LAMP	PCR assays
Xiangshan Bay	HX-1901-003	*V. parahaemolyticus*	*V. parahaemolyticus*	*V. parahaemolyticus*
	HX-1901-007	*V. parahaemolyticus*	*V. parahaemolyticus*	Negative
	HX-1901-008	*V. parahaemolyticus*	*V. parahaemolyticus*	Negative
	HX-1901-0014	*V. parahaemolyticus*	*V. parahaemolyticus*	*V. parahaemolyticus*
	HX-1901-022	*V. parahaemolyticus*	Negative	Negative
	HX-1901-0030	*V. parahaemolyticus*	*V. parahaemolyticus*	*V. parahaemolyticus*
	HX-1904-004	*V. parahaemolyticus*	*V. parahaemolyticus*	*V. parahaemolyticus*
	HX-1904-007	*V. parahaemolyticus*	*V. parahaemolyticus*	*V. parahaemolyticus*
	HX-1904-009	*V. parahaemolyticus*	*V. parahaemolyticus*	*V. parahaemolyticus*
	HX-1904-0016	*V. parahaemolyticus*	*V. parahaemolyticus*	*V. parahaemolyticus*
	HX-1904-0027	Negative	*V. vulnificus*	Negative
	HX-1904-0028	Negative	Negative	*V. alginolyticus*
	HX-1904-0030	*V. vulnificus*	*V. vulnificus*	*V. vulnificus*
	HX-1907-006	*V. vulnificus*	*V. vulnificus*	*V. vulnificus*
	HX-1901-001	Negative	Negative	Negative
	HX-1901-002	Negative	Negative	Negative
	HX-1901-004	Negative	Negative	Negative
	HX-1901-005	Negative	Negative	Negative
	HX-1901-006	Negative	Negative	Negative
	HX-1901-009	Negative	Negative	Negative
	HX-1901-010	Negative	Negative	Negative

HX-1901-011	Negative	Negative	Negative
HX-1901-012	Negative	Negative	Negative
HX-1901-013	Negative	Negative	Negative
HX-1901-015	Negative	Negative	Negative
HX-1901-016	Negative	Negative	Negative
HX-1901-017	Negative	Negative	Negative
HX-1901-018	Negative	Negative	Negative
HX-1901-019	Negative	Negative	Negative
HX-1901-020	Negative	Negative	Negative
HX-1901-021	Negative	Negative	Negative
HX-1901-023	Negative	Negative	Negative
HX-1901-024	Negative	Negative	Negative
HX-1901-025	Negative	Negative	Negative
HX-1901-026	Negative	Negative	Negative
HX-1901-027	Negative	Negative	Negative
HX-1901-028	Negative	Negative	Negative
HX-1901-029	Negative	Negative	Negative
HX-1901-031	Negative	Negative	Negative
HX-1904-001	Negative	Negative	Negative
HX-1904-002	Negative	Negative	Negative
HX-1904-003	Negative	Negative	Negative
HX-1904-005	Negative	Negative	Negative
HX-1904-006	Negative	Negative	Negative
HX-1904-008	Negative	Negative	Negative
HX-1904-010	Negative	Negative	Negative

HX-1904-011	Negative	Negative	Negative
HX-1904-012	Negative	Negative	Negative
HX-1904-013	Negative	Negative	Negative
HX-1904-014	Negative	Negative	Negative
HX-1904-015	Negative	Negative	Negative
HX-1904-017	Negative	Negative	Negative
HX-1904-018	Negative	Negative	Negative
HX-1904-019	Negative	Negative	Negative
HX-1904-020	Negative	Negative	Negative
HX-1904-021	Negative	Negative	Negative
HX-1904-022	Negative	Negative	Negative
HX-1904-023	Negative	Negative	Negative
HX-1904-024	Negative	Negative	Negative
HX-1904-025	Negative	Negative	Negative
HX-1904-026	Negative	Negative	Negative
HX-1904-029	Negative	Negative	Negative
HX-1907-001	Negative	Negative	Negative
HX-1907-002	Negative	Negative	Negative
HX-1907-003	Negative	Negative	Negative
HX-1907-004	Negative	Negative	Negative
HX-1907-005	Negative	Negative	Negative
HX-1907-007	Negative	Negative	Negative
HX-1907-008	Negative	Negative	Negative
HX-1907-009	Negative	Negative	Negative
HX-1907-010	Negative	Negative	Negative

	HX-1907-011	Negative	Negative	Negative
	HX-1907-012	Negative	Negative	Negative
	WZ-2005-002	*V. vulnificus*	*V. vulnificus*	Negative
	WZ-2005-003	*V. vulnificus*	*V. vulnificus*	*V. vulnificus*
	WZ-2005-007	*V. vulnificus*	*V. vulnificus*	*V. vulnificus*
	WZ-2005-0010	*V. vulnificus*	Negative	Negative
	WZ-2005-016	*V. vulnificus*	*V. vulnificus*	*V. vulnificus*
	WZ-2006-001	*V. vulnificus*	*V. vulnificus*	*V. vulnificus*
	WZ-2006-002	*V. vulnificus*	*V. vulnificus*	*V. vulnificus*
	WZ-2006-003	*V. vulnificus*	*V. vulnificus*	*V. vulnificus*
	WZ-2008-004	*V. vulnificu*, *L. monocytogenes*	*V. vulnificu, L. monocytogenes*	*V. vulnificu, L. monocytogenes*
	WZ-2008-006	Negative	*V. parahaemolyticus*	*V. parahaemolyticus*
Wenzhou	WZ-2008-007	Negative	Negative	*V. parahaemolyticus*
	WZ-2005-001	Negative	Negative	Negative
	WZ-2005-003	Negative	Negative	Negative
	WZ-2005-005	Negative	Negative	Negative
	WZ-2005-006	Negative	Negative	Negative
	WZ-2005-008	Negative	Negative	Negative
	WZ-2005-009	Negative	Negative	Negative
	WZ-2005-011	Negative	Negative	Negative
	WZ-2005-012	Negative	Negative	Negative
	WZ-2005-013	Negative	Negative	Negative
	WZ-2005-014	Negative	Negative	Negative
	WZ-2005-015	Negative	Negative	Negative

	WZ-2005-017	Negative	Negative	Negative
	WZ-2005-018	Negative	Negative	Negative
	WZ-2006-004	Negative	Negative	Negative
	WZ-2006-005	Negative	Negative	Negative
	WZ-2006-006	Negative	Negative	Negative
	WZ-2006-007	Negative	Negative	Negative
	WZ-2006-008	Negative	Negative	Negative
	WZ-2008-001	Negative	Negative	Negative
	WZ-2008-002	Negative	Negative	Negative
	WZ-2008-003	Negative	Negative	Negative
	WZ-2008-005	Negative	Negative	Negative
	WZ-2008-008	Negative	Negative	Negative
	WZ-2008-009	Negative	Negative	Negative
	WZ-2008-0010	Negative	Negative	Negative
	TZ-1910-001	*V. alginolyticus*	*V. alginolyticus*	Negative
	TZ-1910-004	*V. parahaemolyticus, S. enterica*	*V. parahaemolyticus, S. enterica*	*V. parahaemolyticus, S. enterica*
	TZ-1910-009	*V. parahaemolyticus, S. enterica*	*V. parahaemolyticus, S. enterica*	*V. parahaemolyticus, S. enterica*
Taizhou	TZ-1910-0013	*V. parahaemolyticus, S. enterica*	*V. parahaemolyticus, S. enterica*	*V. parahaemolyticus, S. enterica*
	TZ-1910-0015	Negative	Negative	*V. parahaemolyticus*
	TZ-1910-002	Negative	Negative	Negative
	TZ-1910-003	Negative	Negative	Negative
	TZ-1910-005	Negative	Negative	Negative

	TZ-1910-006	Negative	Negative	Negative
	TZ-1910-007	Negative	Negative	Negative
	TZ-1910-008	Negative	Negative	Negative
	TZ-1910-010	Negative	Negative	Negative
	TZ-1910-011	Negative	Negative	Negative
	TZ-1910-012	Negative	Negative	Negative
	TZ-1910-014	Negative	Negative	Negative

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