

Supporting Information

A novel qPCR method for simultaneous detection and quantification of viable pathogenic and non-pathogenic *Vibrio parahaemolyticus* (*tlh*⁺, *tdh*⁺, *ureR*⁺)

Ben Niu,^{1†} Bin Hong,^{1†} Zhaohuan Zhang^{1†}, Lili Mu^a, Haiquan Liu^{1,2,3,4}

Yingjie Pan^{1,2,3}, YongZhao^{1,2,3*}

¹ College of Food Science and Technology, Shanghai Ocean University, 999# Hu Cheng Huan Road, Shanghai, 201306, China

² Laboratory of Quality & Safety Risk Assessment for Aquatic Products on Storage and Preservation (Shanghai), Ministry of Agriculture, 999# Hu Cheng Huan Road, Shanghai, 201306, China

³ Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, 999# Hu Cheng Huan Road, Shanghai, 201306, China

⁴ Engineering Research Center of Food Thermal-processing Technology, Shanghai Ocean University, Shanghai 201306, China

Table S1 The gene information (*tlh*, *tdh*, and *trh*) of clinical *Vibrio parahaemolyticus*.

Strains Name	<i>tlh</i> ⁺	<i>tdh</i> ⁺	<i>trh</i> ⁺
V. pc1	+	+	-
V. pc2	+	+	-
V. pc15	+	+	-
V. pc16	+	+	-
V. pc17	+	+	-
V. pc18	+	-	+
V. pc19	+	+	-
V. pc20	+	+	-
V. pc21	+	+	-
V. pc22	+	+	-
V. pc25	+	+	-
V. pc26	+	+	-
V. pc27	+	+	-
V. pc28	+	+	-
V. pc29	+	+	-
V. pc32	+	+	-
V. pc33	+	+	-
V. pc34	+	+	-
V. pc35	+	+	-
V. pc36	+	-	+
V. pc37	+	+	-
V. pc38	+	+	-
V. pc40	+	+	-
V. pc41	+	+	-
V. pc42	+	+	-
V. pc43	+	+	+
V. pc44	+	-	-
V. pc45	+	+	-
V. pc46	+	+	-
V. pc47	+	+	-
V. pc48	+	+	-
V. pc49	+	+	+
V. pc50	+	+	-
V. pc51	+	+	-
V. pc54	+	+	+
V. pc55	+	+	-
V. pc85	+	-	+
V. pc89	+	+	-
V. pc90	+	+	-
V. pc94	+	-	+
V. pc97	+	+	-
V. pc100	+	+	-

Table S2 Comparison of qPCR, RNA-qPCR and Plate counting assays with the developed novel qPCR

Name	mechanism	Time	Labor	Accuracy	DOL	Throughput	Cost	Remarks
Plate counting	Colony-Forming Units	4-5 day	High	Low	10 CFU/100mL	Low	Low	False-negative results in case of VBNC cell.
qPCR	DNA amplification	~4 h	Moderate	Moderate	10 ² cell/mL	High	High	No discrimination between dead and viable cells.
RNA-qPCR	DNA amplification	>4 h	Moderate	Moderate	10 ² cell/mL	High	High	Difficulty on RNA extraction
novel-qPCR	DNA amplification	~4 h	Moderate	High	10 ² cell/mL	High	High	No

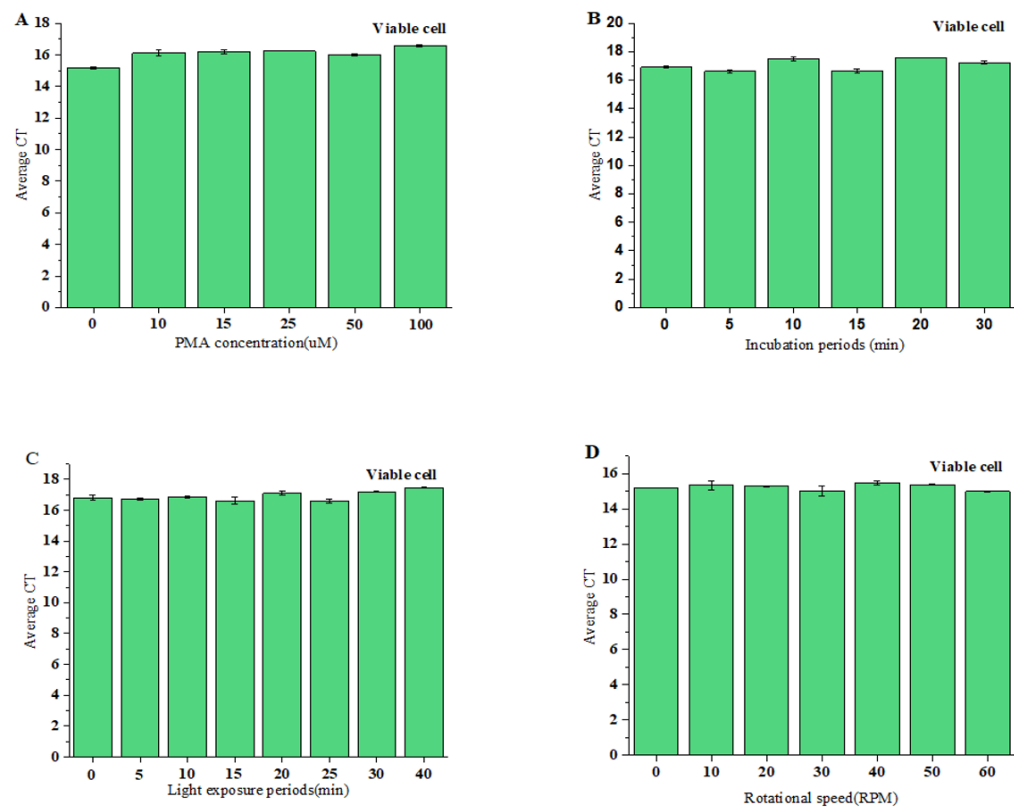


Fig. S1 PMA pretreatment on viable cell: concentration (A), incubation time (B), light exposure (C), and rotation rate (D).

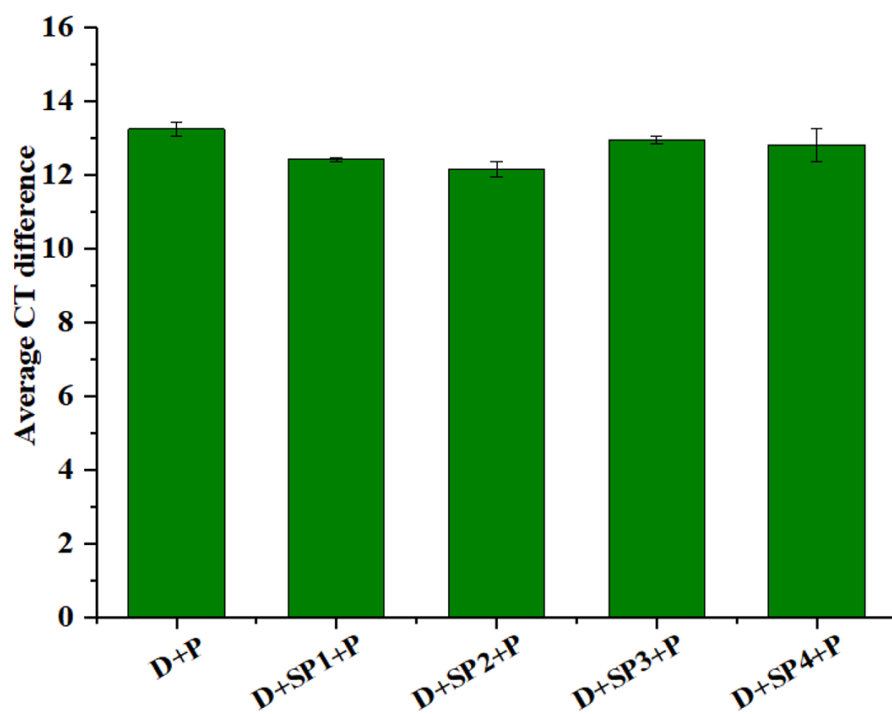


Fig. S2 The novel qPCR inhibition of practical sample. (D: dead cell, P: PMA, SP1: shrimp, SP2: calm, SP3: water collected from shrimp bucket, SP4: water collected from calm bucket)