Supplementary Information

Multiplex Electrochemical Genosensor for Identifying Toxigenic *Vibrio cholerae* Serogroups O1 and O139

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Table S1. Oligonucleotide sequence of primers, capture probes, and synthetic targets

Oligonucleotide	Sequence (5′ → 3′)	Length (mer)	Amplicon size (bp)
Primers			
LaO1-FX	6-FAM-AAC AAG TCG TTC GCC TTA TTA AC	23	155
LaO1-RL	AAT GAT AGA ACC GCC GCC AAG T	22	
LaCT-FX	6-FAM-CAG TCC TCA TCC AGA TGA AC	20	221
LaCT-RL	GCT CTT CCC TCC AAG CTC TAT GC	23	
LaO139-FX	6-FAM-ACG CAC GCT TAT CAA TAC AG	20	289
LaO139-RL	CGA TCC CGA ATC AAG ATC AAC TGT	24	
IAC-FX	6-FAM-TGT AGG TTG TCA TCC ATC AG	20	112
IAC-RL	TTC TAC TTC AAC ACA AAC AGC CTT C	25	
Thiol-modified capture probes			
CP_O1	HS-$(CH_2)_6$-ATG ATA GAA CCG CCG CCA AG	20	NA
CP_CT	HS-$(CH_2)_6$-TCC CTC CAA GCT CTA TGC TC	20	NA
CP_O139	HS-$(CH_2)_6$-CCC GAA TCA AGA TCA ACT GTT	20	NA
CP_IAC	HS-$(CH_2)_6$-CTT CAA CAC AAA CAG CCT TCA	21	NA
Synthetic targets			
ST_O1	6-FAM-CTT GGC GGC GGT TCT ATC AT	20	NA
ST_CT	6-FAM-GAG CAT AGA GCT TGG AGG GA	20	NA
ST_O139	6-FAM-AAC AGT TGA TCT TGA TTC GGG	21	NA
ST_IAC	6-FAM-TGA AGG CTG TTT GTG TTG AAG	21	NA

FX, forward excess primer; RL, reverse limiting primer; CP, capture probe; ST, synthetic target; 6-FAM, 6-carboxyfluorescein; NA, not applicable.

Table S2. Results interpretation of electrochemical signals from multiplex genosensor

Bacterial strains	**Electrochemical signals from multiplex genosensor**				
	CP_IAC*	**CP_O1**	**CP_CT**	**CP_O139**	**Interpretation**
Toxigenic *V. cholerae* O1	-/+	+	+	-	Positive for toxigenic *V. cholerae* O1
Toxigenic *V. cholerae* O139	-/+	-	+	+	Positive for toxigenic *V. cholerae* O139
Bacterial strains other than toxigenic *V. cholerae* O1 & O139 (Negative results)	+	-	-	-	Negative for toxigenic *V. cholerae* O1 & O139
Invalid negative results	-	-	-	-	Invalid negative results

CP, capture probe, -, negative signal; +, positive signal.

* IAC was incorporated into the mLATE-PCR to rule out false negative results. In the absence of target DNA, the IAC template must be amplified to indicate proper functioning of the PCR system. Therefore, a valid negative result must be accompanied by a positive signal for CP_IAC.

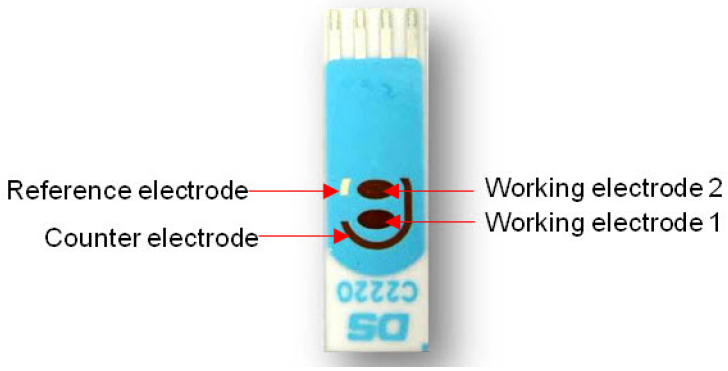

Figure S1. Four-electrode configuration of the screen-printed gold electrode bisensor (SPGEB). The SPGEB consisted of two oval-shaped gold working electrodes surrounded by a common silver reference electrode and a gold counter electrode.

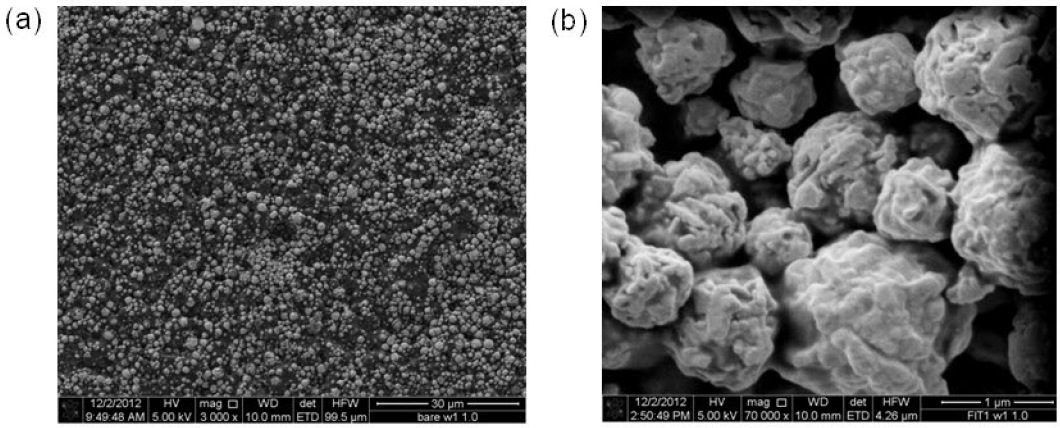

Figure S2. Field emission scanning electron microscope images of the gold working electrode showing the grain-like structure of the rugous surface. Magnification: (a) 3,000 × and (b) 70,000 ×.

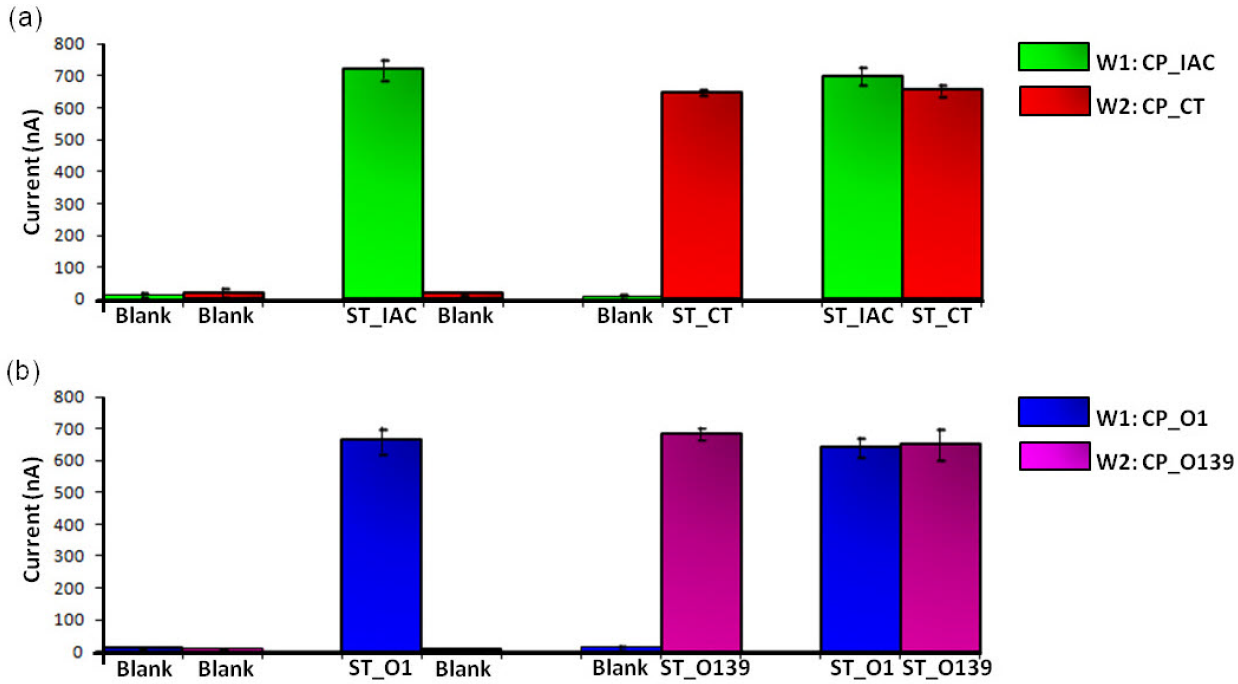

Figure S3. Diffusion analysis of electroactive products between adjacent electrodes on the SPGEB. The data are expressed as the mean ± SD (*n*=3). W1, working electrode 1; W2, working electrode 2; CP, capture probe; ST, synthetic target.

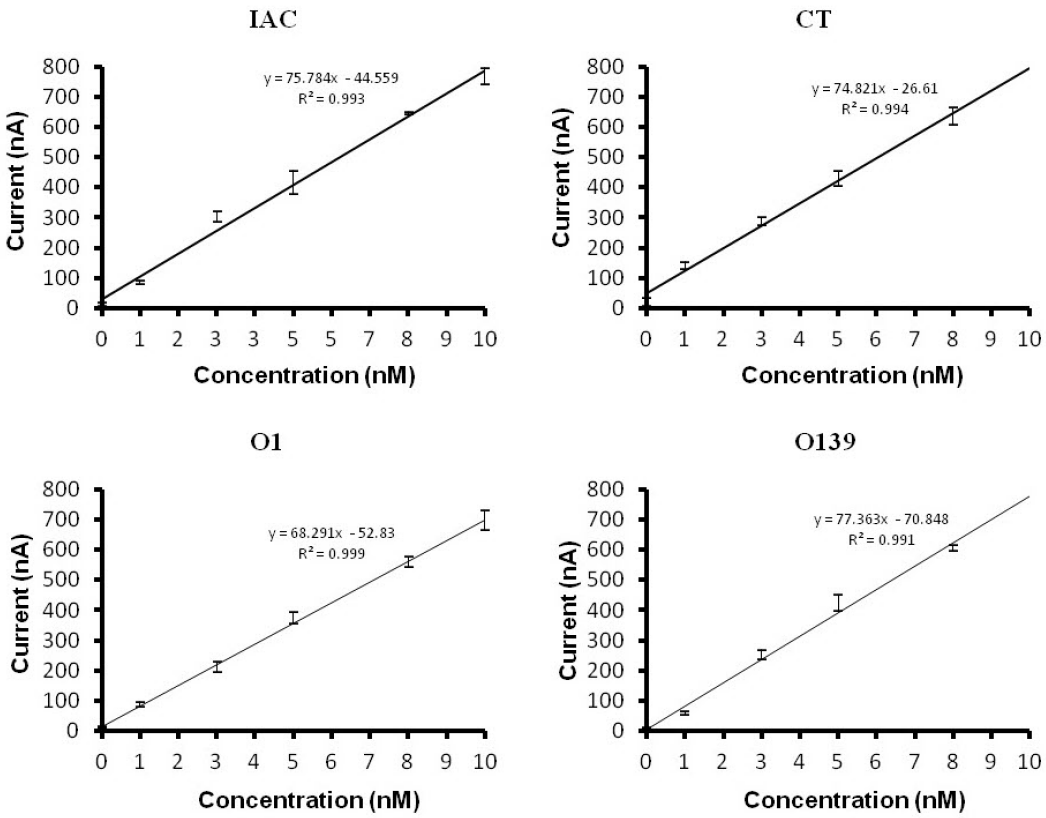

Figure S4. Calibration plots for various targets. Each point represents the mean of three replicates while the error bars indicate ± SD.

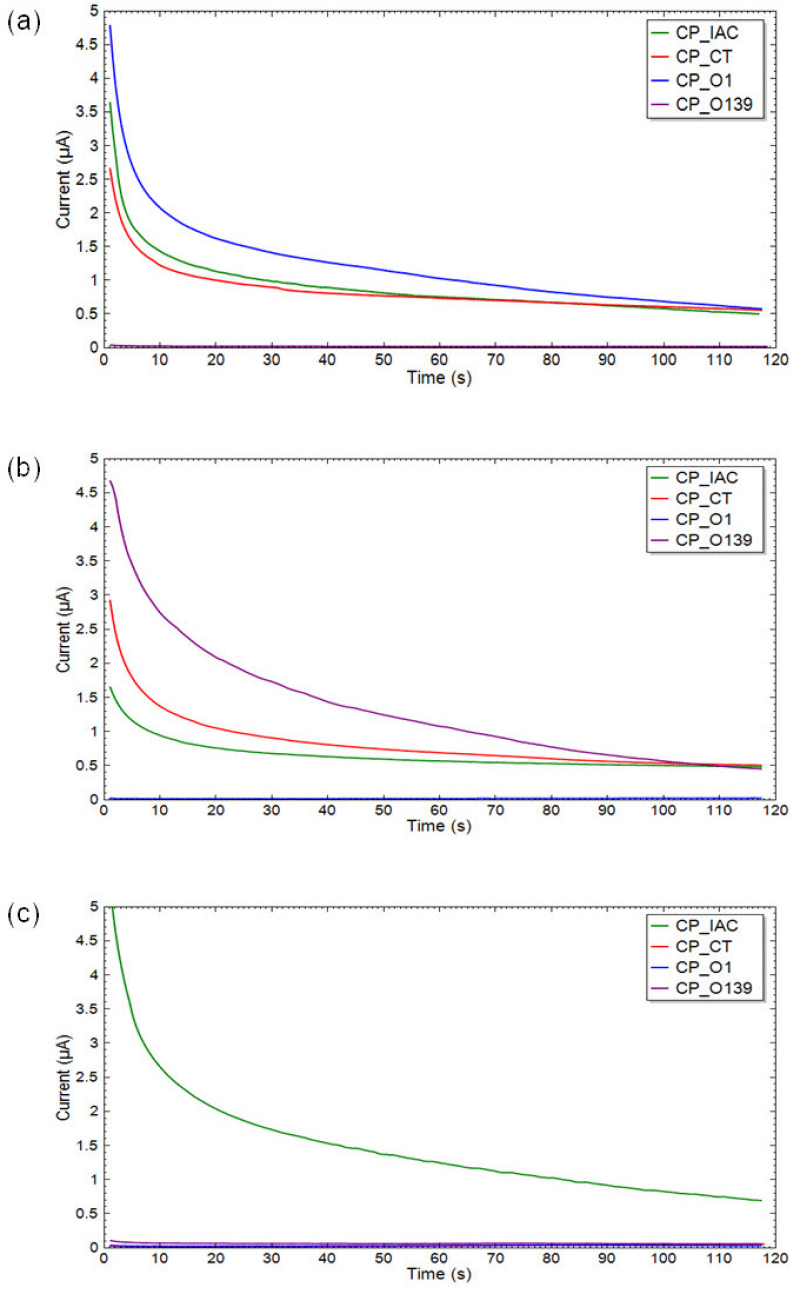

Figure S5. Representative amperometric responses obtained for (a) toxigenic *V. cholerae* O1, (b) toxigenic *V. cholerae* O139 and (c) negative for toxigenic *V. cholerae* O1 & O139.

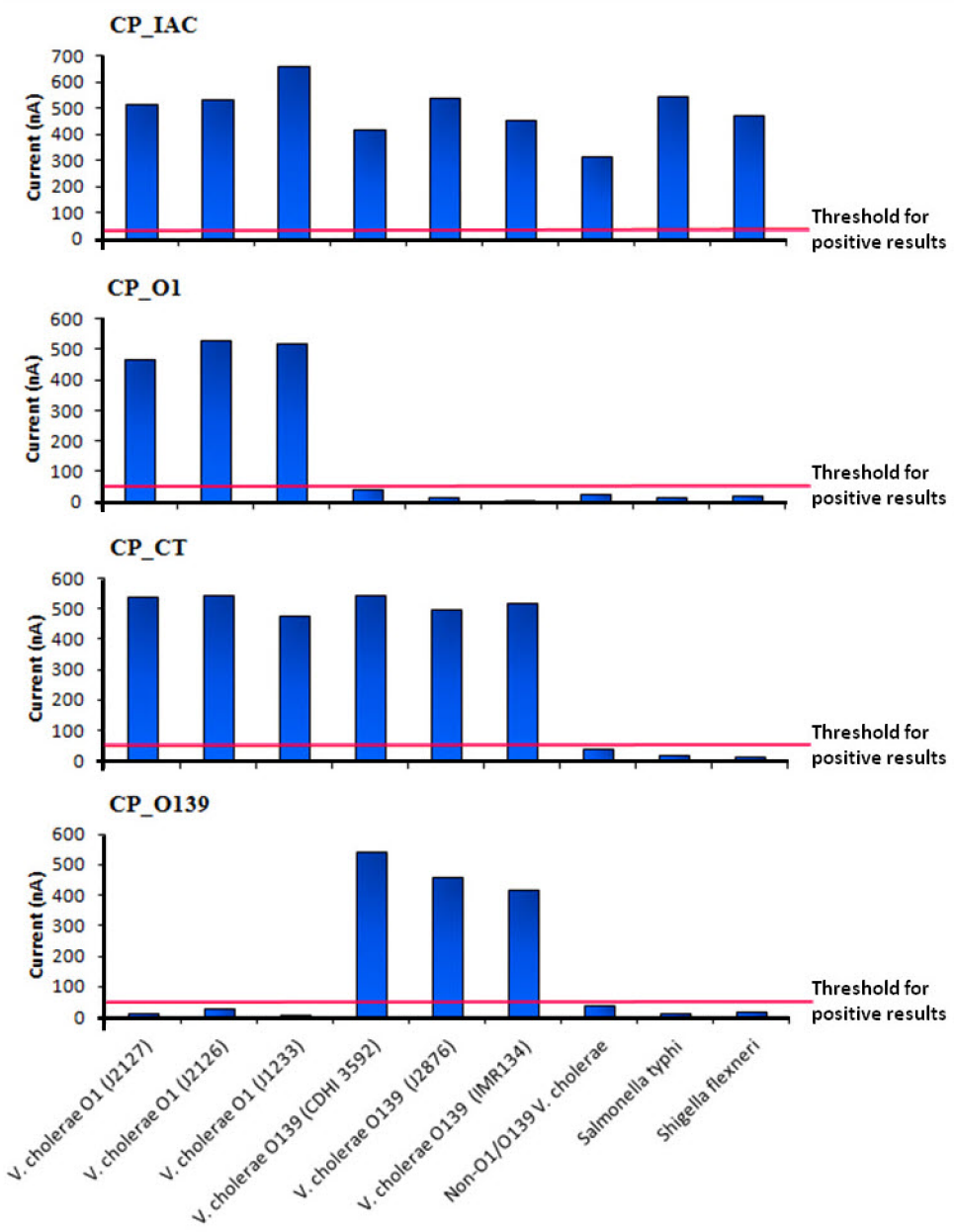

Figure S6. Evaluation result of the multiplex genosensor using spiked stool samples for representative strains of bacteria tested. The horizontal red line indicates the threshold value for positive results.