Supporting information

Multi-detection of Paralytic, Diarrheic and Amnesic Shellfish Toxins by an Inhibition Immunoassay Using a Microsphere-Flow Cytometry System

María Fraga[1], Natalia Vilariño[1], M Carmen Louzao[1], Paula Rodríguez[1], Katrina Campbell[2], Christopher T.Elliott[2] and Luis M. Botana[1]

Departamento de Farmacología[1], Facultad de Veterinaria, Universidad de Santiago de Compostela, 27002 Lugo, Spain

Institute for Global Food Security (IGFS)[2], School of Biological Sciences, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast, Northern Ireland, BT9 5AG

Table of contents:

Table S-1 …….. pS2

Table S-2 …….. pS3

Table S-3 ……... pS4

The present "Supporting information" contains data obtained along the experimental process. The information provided in the next three tables offers the possibility to review some numerical data that contribute to support the textual explanation. The first table indicates the homogeneity and stability of the three different batches of microspheres. The second table provides additional data regarding the lack of matrix interferences for mussels and scallops. The last table shows the stability of the toxin in different storage conditions.

Table S-1. Comparison of three different batches of STX-, OA- and DA–bound microspheres. Half maximal inhibitory concentration (IC_{50}) and the limits of the dynamic range (IC_{10} and IC_{90}) of calibration curves obtained with the different batches (arbitrarily named A, B and C) are shown. Data are expressed as concentrations in nM, with the equivalent in ng/mL in italics. Maximum and nonspecific binding signals are also shown as raw values in response units (RU) (mean ± SEM, n = 3, * statistically significant versus the other two batches).

	Batch	**IC_{10} (nM, *ng/mL*)**	**IC_{50} (nM, *ng/mL*)**	**IC_{90} (nM, *ng/mL*)**	**Maximum binding (RU)**	**Minimum binding (RU)**
STX	**A**	1.4 ± 0.6 *0.5 ± 0.2*	15 ± 3 *5.6 ± 1.1*	186 ± 37 *69 ± 14*	290 ± 26*	10 ± 1
	B	3.6 ± 0.5 *1.1 ± 0.2*	19 ± 2 *7.1 ± 0.7*	133 ± 9 *42 ± 3*	409 ± 32	14 ± 1
	C	1.8 ± 0.1 *0.5 ± 0.0*	11 ± 0.9 *4.3 ± 0.3*	91 ± 17 *28 ± 5*	492 ± 31	10 ± 2
OA	**A**	0.6 ± 0.1 *0.5 ± 0.1*	2.4 ± 0.3 *2.0 ± 0.3*	7.4 ± 1.7 *6.2 ± 1.4*	2337 ± 224*	4.8 ± 0.7*
	B	0.6 ± 0.0 *0.5 ± 0.0*	1.7 ± 0.2 *1.4 ± 0.1*	4.7 ± 0.7 *3.9 ± 0.6*	1175 ± 65	2.7 ± 0.2
	C	0.5 ± 0.1 *0.4 ± 0.1*	1.8 ± 0.2 *1.5 ± 0.2*	6.0 ± 0.6 *5.0 ± 0.5*	1167 ± 47	2.3 ± 0.3
DA	**A**	2.4 ± 0.8 *0.8 ± 0.2*	19 ± 3 *5.8 ± 0.8*	147 ± 18 *46 ± 6*	6503 ± 191	103 ± 12
	B	6.4 ± 0.7 *2.0 ± 0.2*	30 ± 3 *9.3 ± 1.0*	173 ± 21 *54 ± 7*	6205 ± 399	100 ± 10
	C	3.2 ± 1.8 *1.0 ± 0.6*	20 ± 9 *6.3 ± 2.7*	164 ± 59 *51 ± 18*	7089 ± 341	118 ± 29

Table S-2. Effect of different mussel and scallop matrixes on maximum binding signal. Maximum binding signals (expressed as raw data in response units, RU) obtained with the STX-OA-DA multiplexed immunoassay in PBS-BT and extracts of 10 mussel samples (M1-10) and 8 scallop samples (S1-8) (mean ± SEM of 2 replicates, * statistically significant versus blank).

Mussel extracts			
Maximum binding	**STX**	**OA**	**DA**
PBS-BT	234±30	2557±236	4761±751
M1	257±1	2598±93	4283±307
M2	300±26	2487±102	4548±570
M3	323±1	2753±17	5497±78
M4	329±20	2736±99	5284±504
M5	320±3	2872±79	5256±338
M6	274±11	2735±46	5416±253
M7	344±15	2770±14	5385±489
M8	240±32	2815±179	5583±551
M9	229±24	2190±151	4427±736
M10	303±3	2766±11	4802±378
Scallop extracts			
Maximum binding	**STX**	**OA**	**DA**
PBS-BT	140±12	1146±20	4133±4
S1	149±4	1242±36	3820±143
S2	158±4	1113±10	4661±412
S3	153±2	1160±15	3821±126
S4	154±15	1164±20	2854±146*
S5	153±2	1267±26	3995±245
S6	155±2	1270±11	2212±116*
S7	146±10	1201±5	3926±271
S8	157±0.2	1176±58	3862±492

Table S-3. Stability of STX, OA and DA in PBS-BT and mussel extract. PBS-BT and mussel extract were spiked with STX, OA and DA at 8 µg/L for STX, 1.6 µg/L for OA and 200 µg/L for DA. Toxin solutions were divided in aliquots and analyzed fresh, or after storage of 1 or 2 weeks at +4 ˚C or -20 ˚C. The results are expressed as percentage of toxin recovered after storage versus amount measured in fresh sample (mean ± SEM of 10 replicates, * statistically significant).

		1 week		**2 weeks**	
		+4 ˚C	**-20 ˚C**	**+4 ˚C**	**-20 ˚C**
STX	**PBS-BT**	106±2	110±23	131±2	137±2
	Mussel	95±3	98±3	78±9*	81±9*
OA	**PBS-BT**	100±1	99±1	57±1*	57±1*
	Mussel	138±2	137±3	96±11*	101±11
DA	**PBS-BT**	85±2	119±4	88±3	101±1
	Mussel	94±1	102±3	123±3	124±3