COMMUNITY REFERENCE LABORATORY

FOR MARINE BIOTOXINS

REPORT ON THE CRLMB 2009 PROFICIENCY TESTING ON SAXITOXIN GROUP (PSP) TOXINS DETERMINATION (EU-NRLs AND SPANISH OFFICIAL CONTROL LABORATORIES)

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1. INTRODUCTION

Paralytic shellfish poisoning is caused by a group of approximately two dozen naturally occurring potent neurotoxins. These toxins specifically block the excitation current in nerve and muscle cells, finally resulting in paralysis and other illness in consumers of contaminated shellfish (Luckas et al., 2004).

Regulation (EC) N* 853/2004 of the European Parliament and of the Council lays down specific hygiene rules for food of animal origin. With regard to the "Health standards for live bivalve molluscs", it indicates that live bivalve molluscs placed on the market for human consumption must not contain marine biotoxins in total quantities (measured in the whole body or any part edible separately) that exceed 800 micrograms per kilogram for paralytic shellfish poison (PSP)

Commission Regulation (EC) N° 2074/2005 amended by **Commission Regulation (EC) Nº 1664/2006** indicates the recognised testing methods for marine biotoxins for the purpose of Regulations (EC) Nº 853/2004 and Nº 854/2004. With regard to the PSP detection method it is specified that: "The paralytic shellfish poison (PSP) content of edible parts of molluscs (the whole body or any part edible separately) must be detected in accordance with the biological testing method or any other intemationally recognised method. The so-called Lawrence method may also be used as an alternative method for the detection of those toxins as published in AOAC Official Method 2005.06 (Paralytic Shellfish Poisoning Toxins in Shellfish). If the results are challenged, the reference method shall be the biological method".

In the first meeting of the European network of National Reference Laboratories for Marine Biotoxins (EU NRLs) (Vigo, March 1996) the members of the NRLs agreed that the biological method described in the AOAC Official Methods of Analysis, method number 959.08 (mouse bioassay) should be the reference biological testing method. This biological testing method could be applied in association with any chemical method for PSP toxins. If the results were challenged, the reference method should be the biological method. The group recommended that the tolerance level (indicated by then in EU Directive 91/492/EEC) was expressed as 800 µg of saxitoxin equivalents/kg of meat. During the VII Meeting of EU-CRL/NRLs for Marine Biotoxins (Vigo, 2004) some questions about the standards for calibration of the PSP mouse bioassay arose and it was agreed that PSP toxin concentrations should be reported as µg equivalents STX dihydrochloride/100 g meat. However, at the X Meeting of EUCRL/NRLs for Marine Biotoxins (Cyprus, 2007) it was decided to report the PSP toxins concentration as µg STX dihydrochloride equivalents/kg shellfish meat.

The mouse bioassay (MBA) for the determination of PSP toxicity was firstly applied in 1937 to the assay of acidic extracts of mussels. In subsequent years, the general procedure was further standardised and validated in a series of intercomparative studies. This reference method (AOAC, 1990) is internationally recognized for quantifying PSP toxicity and it is used worldwide in PSP monitoring programmes, albeit with some variation in the acceptable regulatory limit for toxicity (Rodríguez-Velasco, 2008). At EU level, with regard to the detection of marine biotoxins, **Commission Regulation (EC) Nº 2074/2005** also mentions that "In addition to biological testing methods, alternative detection methods, such as chemical methods and in vitro assays, should be allowed if it is demonstrated that the performance of the chosen methods is at least as effective as the biological method and that their implementation provides an equivalent level of public health protection".

Other chemical methods used for the determination of PSP toxins are a the fluorimetric assay, high-performance liquid chromatography (HPLC) methods with fluorescence detection (FLD), using pre-column or post-column derivatization, liquid chromatography/mass spectrometry (LC/MS) methods, capillary electrophoresis and enzyme-linked immunosorbent assays (ELISA) (Ben-Gigirey and Villar-González, 2008). Functional assays can also show some potential for PSP toxins determination. Among these are: *in vitro* tissue culture bioassays, membrane potential-sensitive methods and receptor-based assays.

A HPLC-FLD method, involving pre-chromatographic oxidation of the PSP toxins was collaborative validated (Lawrence et al., 2005) and adopted as AOAC OMA 2005.06. After an interlaboratory study (18 EU laboratories) organized by the Community Reference Laboratory for Marine Biotoxins (EU CRLMB) to evaluate its "fitness for purpose" for the Official Control of PSP toxins, **Commission Regulation (EC) Nº 1664/2006**, amending Regulation (EC) No 2074/2005, recognized the method as an alternative method to the current reference method, mouse bioassay, for the detection of those PSP toxins as published in AOAC OMA 2005 06. If the results are challenged, the reference method shall be the biological method.

With the aim of evaluating the performance of the EU NRLs and the Spanish control laboratories and checking the equivalence of the methods used by the EU NRLs for the official control of PSP toxins in bivalves, intercalibration exercises on PSP toxins determination were organized by the CRLMB in 2006, 2007 and 2008.

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3. AIM OF THE EXERCISE

The aim of this exercise is to evaluate the ability of the EU-NRLs and the Spanish Official control laboratories to satisfactorily apply the recognised testing methods for PSP toxins control for the purpose of Regulations (EC) Nos. 853/2004 and 854/2004 stated in Annex III of Commission Regulation (EC) Nº 2074/2005, amended by Commission Regulation (EC) N° 1664/2006. At the same time, the equivalence of the different methods applied for PSP toxins determination will be studied.

This Proficiency Testing on Saxitoxin Goup toxins (PSP toxins) determination was organized by the CRLMB between June and November 2009.

The materials for the study were two samples labelled **CRLMB/09/P/01** and **CRLMB/09/P/02**, consisting of frozen homogenates of mussels (whole body).

4. SCHEDULE AND INSTRUCTIONS

On May 1st 2009, the National Reference Laboratories and Spanish official control laboratories contact points were informed by electronic mail by the coordinator about the

CRLMB-2009-Proficiency Testing Programme and invited to participate in the exercises by submitting a provided Registration Form. They were also informed that, as a result of the agreements of the XI Meeting of EUCRL/NRLs for Marine Bioloxins (Slovenia, October 2008), additional non-NRL laboratories from United Kingdom and Ireland were invited too to take part in the 2009 programme.

Those concerned participants were asked to send back the completed Registration Form before May 15th 2009.

The Programme for the CRLMB 2009 Proficiency Testing also included information about the objective of the exercises, the test materials, instructions, the evaluation of results and reports and the foreseen timetable for each exercise.

On June 17th 2009, the study coordinator confirmed registration by electronic mail and informed participants that, according to the Programme, materials for the CRLMB-2009 Proficiency Testing on PSP toxins will be dispatched on June 22nd 2009. That day samples were sent by urgent courier, conditioned with dry ice, together with a letter with instructions and the Laboratory Code for the exercise. At the same time, participants received by electronic email dispatch details, the Protocol for the exercise, the Arrival Form and the Reporting Sheet.

In the Protocol, participants were asked to inspect the package after arrival to make sure that contents were correct and samples were still frozen and intact and to fill and send the "Arrival Form" to the CRLMB as soon as they received the samples. Most laboratories (twelve out of nineteen) received the samples in one day, six laboratories received them in two days and one in three days. All the participants received samples in good state and frozen.

Participants were requested to store the samples frozen (≤-18°C), immediately upon receipt, until defrosting just before the analysis. Analysis should be performed as soon as posible. For that, the contents of each container had to be thoroughly mixed before weighing for analysis.

PSP toxin analysis had to be carried out by the method usually employed at the laboratory for PSP control. Participants were told that it would be very useful if they could analyse samples by MBA and HPLC. A Reporting Sheet (excel file with two sheets: one for mouse bioassay results and other for HPLC results) was provided requiring the details of the method followed by the laboratory, the standards employed, results and comments or relevant information.

Participants had to report their results for total toxicity in µg equivalents STX dihydrochloride/kg meat. For HPLC methods, sample results were also asked for the individual toxins quantified (in µmol/kg).

Deadline for results submission was August 22nd 2009.

5. MATERIALS PREPARATION

Sample labelled CRLMB/09/P/01 consisted of a naturally contaminated blue mussel homogenate (whole body). Mussels (Mytilus edulis), collected in April 2008 in Hvaler, were kindly facilitated by the Norweigan NRL. The toxic episode was caused by *Alexandrium* sp. Mussels, received frozen, clean and without shell, were defrosted, homogenised and distributed in plastic containers with approximately 38 g, frozen and stored at -24ºC until analysis/day of shipment. Preliminary analysis by HPLC (AOAC OMA 2005.06) revealed the presence of GTX2,3, GTX1,4, STX and C1,2 (traces) and a level of total PSP toxicity below the legal limit.

Sample labelled **CRLMB/09/P/02** consisted of a mussel homogenate (whole body) of naturally contaminated mussels diluted with blank mussels. Toxic mussels (*Mytilus galloprovincialis*), collected in October 2007 during a *Gymnodinium* sp. bloom, were kindly provided by Portuguese NRL. Blank mussels (*Mytilus galloprovincialis*), from Northwest Spain (Galicia), were acquired at a retail market. All samples were thoroughly cleaned outside/inside with freah water to remove sand or other foreign material. tissues removed from the shell homogenated and frozen until their preparation. For material preparation mussels were thawed and mixed using an Ultraturrax. The homogenate was distibuted in aliquots of approximately 38 g frozen and stored at -24ºC until analysis/day of ahipment. Preliminary analyais by HPLC (AOAC OMA 2006 06) showed the presence of GTX6, GTX5, C1,2, dcNEO, dcSTX and dcGTX2,3 with a total PSP toxicity below the legal limit.

Homogeneity and stability checks for both materials were performed at the CRLMB by MBA, according to “The International Harmonized Protocal for the Proficiency Testing of Analytical Chemistry Laboratories” (Thompson et al 2006) and the ISO 13528:2005 Homogenety checks were carried out in five aliquots, taking two test portions from each aliquot for analysis The total toxicity results showed a value of 605 µg equivalents STX.2HCl/kg meat (S.D 34, n=10) for sample CRLMB/09/P/01 and 385 µg equivalents STX.2HCl/kg meat (S.D 8, n=10) for sample CRLMB/09/P/02. For the stability test, three portions (taking two portions each) were kept under transport circumstances and analysed under repeatability conditions. The coefficients of variation between the six

portions tested were 4% and 5% for samples CRLMB/09/P/01 and CRLMB/O9/P/O2, respectively.

6. METHODS USED BY PARTICIPANTS

The Reporting Sheet facilitated for results submission required the reporting of the method followed by the laboratory, the standards employed, the results and comments or relevant information for both methods, mouse bioassay or other alternative method.

The methods used by participants for this exercise were: mouse bioassay (MBA), HPLC-FLD methods with precolumn or post-column derivatization, ELISA Ridascreen® Fast PSP SC and Jellet Rapid Test for PSP. Some participants summarised the method employed while others reported the bibliographic references of the method (with/without additional indications).

Several laboratories reported they apply modifications to a published method Some laboratories that use commerclal kits referred to the name of the kit.

Nineteen out of twenty-seven participant laboratories use the MBA for the official control of the PSP biotoxins and they basically follow the 959.08 AOAC Official Method of Analysis. **Table 1** shows a summary of the MBA methods followed by participants, together with information on the standards used, the mice employed, the standardisation of the method, etc. In the table it is seen that some laboratories reported modifications to the 959.08 AOAC Official Method.

Thirteen out of the twenty-seven participants employed HPLC-FLD methods. Seven laboratories employed HPLC as the only method, while six used HPLC and MBA. HPLC-FLD methods are summarized in Table 2 together with information on the standards used and some comments about samples analysis received from participants. Most laboratories followed AOAC OMA 2005.06 (Lawrence HPLC method); some of them with modifications. However, one laboratory followed a post-column derivatization HPLC-FLD method. One laboratory reported the use of ELISA Ridascreen® Fast PSP SC Kit for Official PSP Control combined to Jellet Rapid Test for PSP.

TABLE 1. SUMMARY OF MOUSE BIOASSAY FEATURES

Laboratory code	Method	Standard for standardization	Mice strain, sex and weight range	[1]CF., [2]D.LS., [3]D.A	pH of extract
0901	AOAC OMA 959.08	NRC-CRM-STX-e lot 20060419	NMRI mice 19-21 g	C.F D.L.S 19/03/2009 D.A 01/07/2009	3-4
0905	AOAC OMA 959.08	FDA Reference Standard STX	Swiss CD1	C.F.: 024 D.L.S 25/09/2008 D.A 25/06/2009	CRLMB/09/P/01 3.04 CRLMB/09/P/02 3.09
0906	AOAC OMA 959.08	NRC CRM STX-e	CD 1strain	C.F.: 019 D.L.S 08/05/2009 D.A 08/07/2009	CRLMB/09/P/01 3.21 CRLMB/09/P/02 3.22
0907	"REFERENCE MBA"	-	Balbic	C.F.: 020 D.L.S 05/06/2009 D.A 10/07/2009	CRLMB/09/P/01 3.01 CRLMB/09/P/02 3.96
0908	AOAC OMA 959.08	NRC CRM STX-e	20-22-20 g	C.F.: 026 D.L.S 08/05/2009 D.A 08/07/2009	3.00
0910	AOAC OMA 959.08	NRC CRM STX-e	S/IOPS CD1 male (16-18) g (ICR)	C.F.: 019 D.L.S 01/01/2009 D.A 06/08/2009	3.00
0913	AOAC OMA 959.08	STX.diHCl (FDA)	CR-CD1	C.F.: 019 D.L.S 06/07/2004 D.A 11/08/2009	CRLMB/09/P/01 3.06 CRLMB/09/P/02 2.98
0914	Modified AOAC, 959.08	Not applicable	Female NMRI	C.F.: 026 D.L.S 03/05/2004 D.A 11/08/2009	3.00
0915	-	-	NMRI Female	-	3.00
0916	AOAC OMA 959.08	NRC CRM Saxitoxin	CD-1	C.F.: 017 D.L.S 06/05/2009 D.A 11/08/2009	3.50
0918	AOAC OMA 959.08	-	Swiss	C.F.: - D.L.S - D.A 09/07/2009	3.10
0919	AOAC OMA 959.08	FDA	SWISS-NMRI	C.F.: 021 D.L.S 03/03/2009 D.A 21/07/2009	CRLMB/09/P/01 3.20 CRLMB/09/P/02 2.70
0921	AOAC OMA 959.08	FDA Reference Standard Saxitoxin dihydrochloride	ICR (CD-1)	C.F.: 020 D.L.S 30/06/2009 D.A 8-9/7/2009	CRLMB/09/P/01 2.92 CRLMB/09/P/02 2.89

[1] C.F.: Conversion Factor
[2] D.L.S.: Date of last standardization
[3] D.A: Date of analisys

0922	AOAC OMA 959.08 with minor modiications[4]	NRC-CRM-STX-e	Albino Swiss, outbred	C.F.: 018 D.L.S 14/04/2009 D.A 1-2/7/2009	CRLMB/09/P/01 3.00 CRLMB/09/P/02 2.80
0923	MOUSSE BIOASSAY	NRC-CRM-STX-e	Hsd:ICR (CD-1)	C.F.: 019 D.L.S 30/04/2009 D.A 24/06/2009	CRLMB/09/P/01 3.63 CRLMB/09/P/02 3.22
0924	PNT-A-008/AOAC OMA 959.08	STX diHCl FDA	CD-1	C.F.: 022 D.L.S 30/04/2009 D.A 24/06/2009	CRLMB/09/P/01 2.96 CRLMB/09/P/02 3.02
0925	LNRBM-PSP01 method base don AOAC 959.08	NRC CRM STX-e lot 20060419	IOPS OF1 (male, 18/20g)	C.F.: 019 D.L.S 17/06/2009 D.A 29/06/2009	3.00
0926	AOAC OMA 959.08	NRC	CD1	C.F.: 018 D.L.S 13/05/2009 D.A 10/08/2009	3.00
0927	"Reference mouse bioassay"	Washington Seafood Lab, USA	ICR	C.F.: 021 D.L.S 10/07/2009 D.A 13/07/2009	CRLMB/09/P/01 2.40 CRLMB/09/P/02 2.50

TABLE 2. SUMMARY OF HPLC-FLD METHODS USED BY PARTICIPANTS

0902	AOAC Official Method 2005.06	NRC-CRM-STX-e NRC-CRM-NEO-b NRC-CRM-C1&2 NRC-CRM-GTX1&4-b NRC-CRM-GTX2&3-b NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcNEO-b NRC-CRM-dcGTX2&3-b	
0904	Refined AOAC Official Method 2005.06 Ref.: Turner et al., (2009) J.AOAC Vol 92(1)p. 190-207	NRC-CRM-STX-e NRC-CRM-NEO-b NRC-CRM-C1&2 NRC-CRM-GTX1&4-b NRC-CRM-GTX2&3-b NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcNEO-b NRC-CRM-dcGTX2&3-b	Sample CRLMB/09/P/01 V. smal dcGTX2,3 primary peak in F2, not quantified or removed from GTX1,4 contribution due to size and S/N of peak. Sample CRLMB/09/P/02: 1) C3,4 toxin (small peak), not quantified due to lack of standard 2) difficult to get the hydrolysed F2 pH-adjusted to a "sensible" pH 3) small NEO and dcNEO peaks in F3 (very low S/N (<1.5), not quantified

[4] Modifications as recomended im "Manual on Harmful Marine Microalgae" IOC Manuals and Guides nº33, pp.213-228 (Mammalian bioassays)

0905	AOAC Official Method 2005.06	NRC-CRM-STX-e NRC-CRM-NEO-b NRC-CRM-C1&2 NRC-CRM-GTX1&4-b NRC-CRM-GTX2&3-b NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcNEO-b NRC-CRM-dcGTX2&3-b	Sample CRLMB/09/P/02: 1) C3&4 presence
0906	AOAC Official Method 2005.06	NRC-CRM-STX-e NRC-CRM-NEO-c NRC-CRM-C1&2 NRC-CRM-GTX1&4-b NRC-CRM-GTX2&3-c NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcNEO-b NRC-CRM-dcGTX2&3-b	Results are not corrected Sample CRLMB/09/P/01 NEO <LOQ Sample CRLMB/09/P/02 (C3,4 identified in F1 after periodate oxidation, not quantified due to the lack of a C3,4 standard)
0909	AOAC Official Method 2005.06	NRC-CRM-STX-e NRC-CRM-NEO-c NRC-CRM-C1&2 NRC-CRM-GTX1&4-b NRC-CRM-GTX2&3-c NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcNEO-b NRC-CRM-dcGTX2&3-b	
0910	AOAC Official Method 2005.06	NRC-CRM-STX-e NRC-CRM-GTX2&3-c NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcGTX2&3-b	Results corrected for the recovery
0911	AOAC Official Method 2005.06	NRC-CRM-STX-e NRC-CRM-NEO-c NRC-CRM-C1&2 NRC-CRM-GTX2&3-c NRC-CRM-GTX5-b NRC-CRM-dcSTX	The method is not fully implemented in the laboratory
0912	AOAC Official Method 2005.06	NRC-CRM-STX-e NRC-CRM-NEO-c NRC-CRM-C1&2 NRC-CRM-GTX1&4-b NRC-CRM-GTX2&3-c NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcGTX2&3-b	Other standars are used for dcNEO (lot 20030731), GTX1&4-b (lot 20020711) & dcGTX2&3 (lot20021016). Observed ratio GTX2/GTX3 in GTX2&3 in calibrant of NRC Canada changed, when comparing GTX2&3-b and GTX2&3-c. Due to differences in fluorescence intesities of GTX2 and GTX3, this may have consequences for analytical results. For simple CRLMB/09/P/01 results calculated base don calibrant GTX2&3-c. If results would have been calculated based

			on GTX2&3-b total estimated toxicity of simple would have been 4% higher Similar effects could occur for other grouped calibrants. If ratios changed
0915	Post-column derivatization method base don Oshima Ref.: Asp et al 2004 Toxicom 43 319-327	NRC-CRM-STX-e NRC-CRM-NEO-b NRC-CRM-C1&2 NRC-CRM-GTX1&4-c NRC-CRM-GTX2&3-b NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcNEO-b NRC-CRM-dcGTX2&3-b	
0916	AOAC Official Method 2005.06	NRC-CRM-STX-e NRC-CRM-NEO-c NRC-CRM-C1&2 NRC-CRM-GTX1&4-c NRC-CRM-GTX2&3-b NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcNEO-b NRC-CRM-dcGTX2&3-b	
0917	AOAC Official Method 2005.06	NRC-CRM-STX-e NRC-CRM-NEO-c NRC-CRM-C1&2 NRC-CRM-GTX1&4-c NRC-CRM-GTX2&3-c NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcNEO-b NRC-CRM-dcGTX2&3-b	
0919	AOAC Official Method 2005.06	NRC-CRM-STX-e NRC-CRM-NEO-c NRC-CRM-C1&2 NRC-CRM-GTX1&4-c NRC-CRM-GTX2&3-c NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcNEO-b NRC-CRM-dcGTX2&3-b	Sample CRLMB/09/P/02: C3,4 identified, not quantified due to the lack of standard
0920	AOAC Official Method 2005.06	NRC-CRM-STX-e NRC-CRM-NEO-c NRC-CRM-C1&2 NRC-CRM-GTX1&4-c NRC-CRM-GTX2&3-c NRC-CRM-GTX5-b NRC-CRM-dcSTX NRC-CRM-dcNEO-b NRC-CRM-dcGTX2&3-b	Both samples were stored in a fridge that was out of order for 24 hours and during that period the Tª reached at 17°C and maintained at this Tº for many hours. The samples were frosted again and examined 2 weeks later

7. EVALUATION OF RESULTS

The deadline for results submission was August 22nd 2009 Only two participant laboratories registered for the exercise submitted their results after the deadline. As an exception, their results were also considered for evaluation.

Initial review of data: valid data

Results were initially reviewed to remove non valid data.

Only quantitative data were valid for evaluation. Thus, results from Laboratory 0903 by Jellet Rapid Test (qualitative) and from Laboratory 0914 by MBA (due to their license for animal experiments they perform a modified qualitative version of the official method) were not taken into account for evaluation. In the same way, results for sample CRLMB/09/P/02 from Laboratories 0901 (< 30), 0910 (3 mice alive), 0918 (<350) and 0920 (negative) were excluded from evaluation.

With regard to quantitative, results from Laboratory 0915 for sample CRLMB/09/P/02, using an HPLC post-column method, were considered as nonvalid since they wrongly identified the individual PSP toxins (they did not identify present toxins and wrongly identified non-present toxins). Similarly, results from Laboratory 0911 for both samples tested were considered as non-valid and excluded from evaluation since they wrongly identified toxins and even wrongly calculated total PSP toxicity from the individual toxins quantification provided.

Determination of the assigned value for each sample

The determination of the assigned value was carried out by *The Consensus from Participants", after removal of non valid data. To minimize the influence of extreme results, outliers were identified and excluded for calculations. The assigned value was then calculated as the mean from participants" results.

Outliers identification was carried out by the Dixon test (a. = 0.05).

Determination of the standard deviation for proficiency assessment

The approach followed to determine the standard deviation for proficiency assessment was to derive it from the results obtained in previous precision experiments (ISO 13528:2005). Precision data obtained in the previous proficiency testing for PSP toxins determination organised by the CRLMB and in the collaborative study of Lawrence et col. (2005) were taken into account.

Performance statistics

For each sample z-scores were calculated (ISO/IEC Guide 43-1:1997) for all valid data. For the evaluation of laboratory performance it was considered that if $|z| \leq 2$ the result was satisfactory; if $2 < |z| < 3$ the result was questionable; if unsatisfactory $|z| \geq 3$ the result was unsatisfactory.

8. RESULTS

Raw results facilitated by participants for total PSP toxicity are summarized in **Table 3**. **Tables 4 and 5** show proficiency results after evaluation of valid results for samples **CRLMB/09/P/01** and **CRLMB/09/P/02**, respectively, indicating the assigned value, S.D., RSD_R% and target standard deviation for assessment, as well as z-scores for each laboratory.

TABLE 3. RESULTS PROVIDED BY PARTICIPANTS FOR THE CRLMB 2009 PROFICIENCY TESTING ON SAXITOXIN GROUP (PSP TOXINS) DETERMINATION (TOTAL TOXICITY)

Laboratory code	Method	µg STX 2HCl equivalents/kg	
		CRLMB/09/P/01	CRLMB/09/P/02
0901	MBA AOAC 959.08	890	<30*
0902	HPLC AOAC 2005.06	1214	504
0903	ELISA Ridascreen	1324	578
0904	JELLET RAPID TEST	POSITIVE*	POSITIVE*
	HPLC AOAC 2005.06 (Refined)	660	235
0905	MBA AOAC 959.08	696	465
	HPLC AOAC 2005.06	718	261
0906	MBA AOAC 959.08	720	540
	HPLC AOAC 2005.06	732	353
0907	MBA (no details provided)	620	392
0908	MBA AOAC 959.08	500	420
0909	HPLC AOAC 2005.06	474	2223
0910	MBA AOAC 959.08	496	3 mice alive*
	HPLC AOAC 2005.06	671	505
0911	HPLC AOAC 2005.06	370*	2258*
0912	HPLC AOAC 2005.06	879	264
0913	MBA AOAC 959.08	742	476
0914	MBA AOAC 959.08 (modified)	>800*	<800*
0915	MBA (no details provided)	490	374
	HPLC POSTCOLUMN	770	412*
0916	MBA AOAC 959.08	435	356
	HPLC AOAC 2005.06	596	262

0917	HPLC AOAC 2005.06	1250	322
0918	MBA AOAC 959.08	530	<350*
0919	MBA AOAC 959.08	570	680
	HPLC AOAC 2005.06	652	424
0920	HPLC AOAC 2005.06	490	Negative*
0921	MBA AOAC 959.08	630	450
0922	MBA AOAC 959.08	438	411
0923	MBA AOAC 959.08	490	360
0924	MBA AOAC 959.08	486	735
0925	MBA AOAC 959.08	498	399
0926	MBA AOAC 959.08	563	375
0927	MBA (no details provided)	684	450

*Not valid for evaluation

TABLE 4. PROFICIENCY ASSESMENT RESULTS FOR SAMPLE CRLMB/09/P/01 IN THE CRLMB 2009 PROFICIENCY TESTING ON SAXITOXIN GROUP (PSP TOXINS) DETERMINATION (TOTAL TOXICITY)

SAMPLE CRLMB/09/P/01, assigned value: 674 µg eq. STX.2HCl/kg S.D. (n=31): 231 µg/kg,RSD_R%: 34, Target σ: 202 (target RSD_R:%: 30.0)			
Laboratory code	**Method**	**µg STX 2HCl equivalents/kg**	**z-score**
0901	MBA AOAC 959.08	890	**1.065**
0902	HPLC AOAC 2005.06	1214	**2.667**
0903	ELISA Ridascreen	1324	**3.210**
0904	HPLC AOAC 2005.06 (Refined)	660	**-0.071**
0905	MBA AOAC 959.08	696	**0.107**
	HPLC AOAC 2005.06	718	**0.213**
0906	MBA AOAC 959.08	720	**0.225**
	HPLC AOAC 2005.06	732	**0.284**
0907	MBA (no details provided)	620	**-0.269**
0908	MBA AOAC 959.08	500	**-0.862**
0909	HPLC AOAC 2005.06	474	**-0.991**
0910	MBA AOAC 959.08	496	**-0.882**
	HPLC AOAC 2005.06	671	**-0.017**
0912	HPLC AOAC 2005.06	879	**1.013**
0913	MBA AOAC 959.08	742	**0.334**
0915	MBA (no details provided)	490	**-0.912**
	HPLC POSTCOLUMN	770	**0.472**
0916	MBA AOAC 959.08	435	**-1.183**
	HPLC AOAC 2005.06	596	**-0.389**
0917	HPLC AOAC 2005.06	1250	**2.845**
0918	MBA AOAC 959.08	530	**-0.714**
0919	MBA AOAC 959.08	570	**-0.516**

	HPLC AOAC 2005.06	652	**-0.111**
0920	HPLC AOAC 2005.06	490	**-0.912**
0921	MBA AOAC 959.08	630	**-0.220**
0922	MBA AOAC 959.08	438	**-1.169**
0923	MBA AOAC 959.08	490	**-0.912**
0924	MBA AOAC 959.08	486	**-0931**
0925	MBA AOAC 959.08	498	**-0.872**
0926	MBA AOAC 959.08	563	**-0.551**
0927	MBA (no details provided)	684	**0.047**

SAMPLE CRLMB/09/P/01

Quantitative results for this sample ranged from 435 µg STX 2HCl equivalents/kg to 1324 µg STX.2HCl equivalents/kg (**Table 3**). Taking into account the legal limit for these toxins group (800 µg STX.2HCl equivalents/kg), twenty-seven out of thirty-four results would assign a "negative result' (below the legal limit) and seven would assign a "positive result" (above the legal limit).

Thirty-one out of thirty-four results provided for this sample were valid for evaluation. The two qualitative results non valid for evaluation were a "positive" result (by Jellet Rapid Test) from Laboratory 0903 and a result >800 µg STX.2HCl equivalents/kg by MBA from Laboratory 0914. On the other hand, result from Laboratory 0911 was excluded since they wrongly identified PSP toxins in the sample and even wrongly calculated total PSP toxicity.

Results from those laboratories that reported valid data were included in the statistical evaluation of results for total PSP toxicity. By applying Dixon test ($\alpha = 0.05$), no outliers were identified for sample CRLMB/09/P/01. The assigned value, RSD_R% and target o for this sample are shown in **Table 4**. For performance statistics the z-scores were calculated (**Table 4 and Figure 1**). Result from Laboratory 0903, which employed the ELISA Ridascreen®, was found as unsatisfactory and results from Laboratories 0917 and 0902, applying the HPLC AOAC 2005.06 Official Method, were considered questionable.

FIGURE 1. Z-SCORES OBTAINED BY PARTICIPANTS IN THE CRLMB 2009 PROFICIENCY TESTING ON SAXITOXIN GROUP (PSP TOXINS) DETERMINATION FOR SAMPLE CRLMB/09/P/01 (TOTAL TOXICITY)

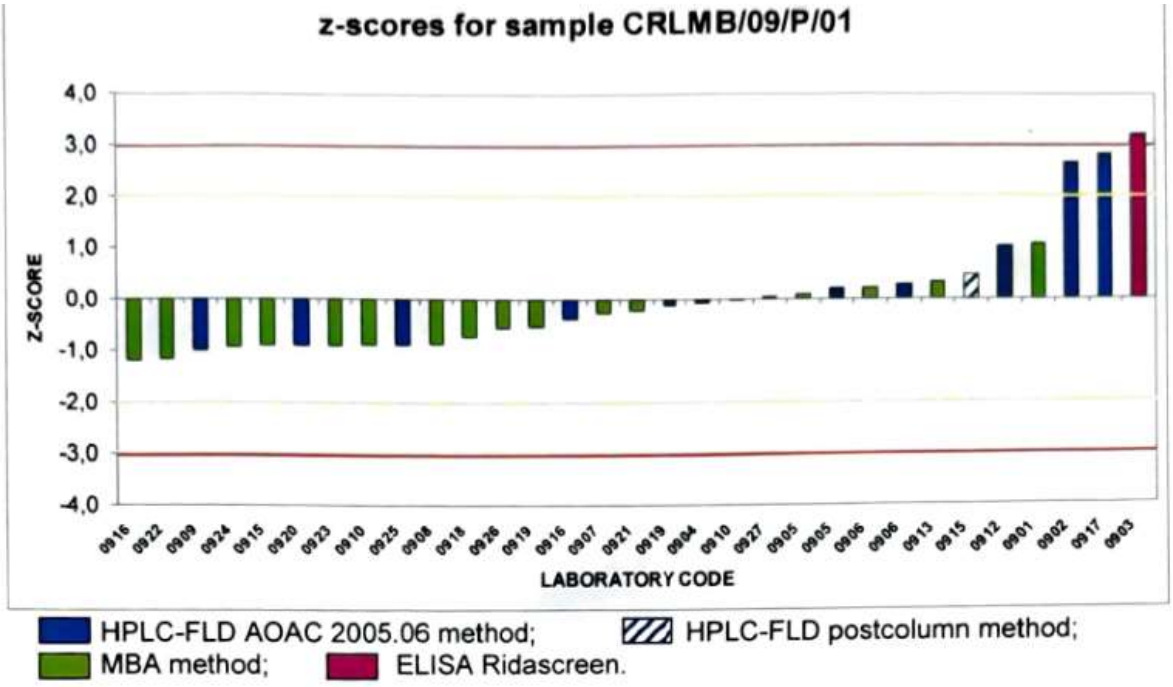


TABLE 5. PROFICIENCY ASSESMENT RESULTS FOR SAMPLE CRLMB/09/P/02 IN THE CRLMB 2009 PROFICIENCY TESTING ON SAXITOXIN GROUP (PSP TOXINS) DETERMINATION (TOTAL TOXICITY)

SAMPLE CRLMB/09/P/02, assigned value: 424 µg eq. STX.2HCl/kg S.D. (n=25): 123 µg/kg,RSD$_R$%: 29, Target σ: 127 (target RSD$_R$:%: 30.0)			
Laboratory code	**Method**	**µg STX 2HCl equivalents/kg**	**z-score**
0902	HPLC AOAC 2005.06	504	**0.632**
0903	ELISA Ridascreen	578	**1.214**
0904	HPLC AOAC 2005.06 (Refined)	235	**-1.484**
0905	MBA AOAC 959.08	465	**0.325**
	HPLC AOAC 2005.06	261	**-1.280**
0906	MBA AOAC 959.08	540	**0.916**
	HPLC AOAC 2005.06	353	**-0.556**
0907	MBA (no details provided)	392	**-0.249**
0908	MBA AOAC 959.08	420	**-0.029**
0909	HPLC AOAC 2005.06	2223*	**14.158**
0910	HPLC AOAC 2005.06	505	**0.640**
0912	HPLC AOAC 2005.06	264	**-1.256**
0913	MBA AOAC 959.08	476	**0.412**
0915	MBA (no details provided)	374	**-0.391**

0916	MBA AOAC 959.08	356	**-0.532**
	HPLC AOAC 2005.06	262	**-1.271**
0917	HPLC AOAC 2005.06	322	**-0.800**
0919	MBA AOAC 959.08	680	**2.017**
	HPLC AOAC 2005.06	424	**0.003**
0921	MBA AOAC 959.08	450	**0.207**
0922	MBA AOAC 959.08	411	**-0.099**
0923	MBA AOAC 959.08	360	**-0.501**
0924	MBA AOAC 959.08	735	**2.450**
0925	MBA AOAC 959.08	399	**-0.194**
0926	MBA AOAC 959.08	375	**-0.383**
0927	MBA (no details provided)	450	**0.207**

*Outlier result

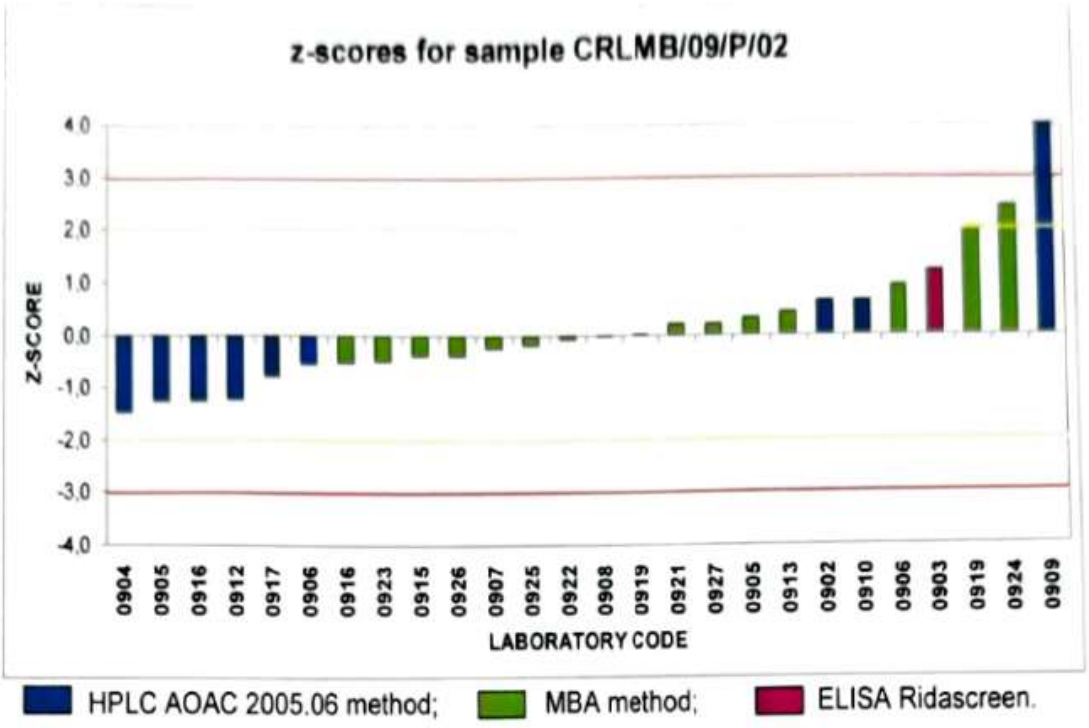
SAMPLE CRLMB/09/P/02

Quantitative results for this sample ranged from 235 ¡g STX2HCI equivalents/kg to 2223 pg STX 2HCI equivalents/kg (Table 3) Taking into account the legal limit for these toxins group (800 yg STX.2HCI equivalents/kg), all laboratories with the exception of Laboratory 0909 would assign a “negative result" to this sample.

Twenty-six out of thirty-four results provided for this sample were valid for evaluation. Qualitative results from Laboratories 0901, 0910, 0914 and 0918 by MBA (<30, “3 mice alive”, <350 and <800, respectively), Laboratory 0903 by Jellet Rapid Test (*positive”) and Laboratory 0920 by HPLC AOAC 2005.06 method (‘negative”) were considered as non valid. On the other hand, results from Laboratory 0915, using an-HPLC post-column method, were excluded before statistical evaluation due to the wrong identification of PSP toxins present in the sample (they did not identify present toxins and wrongly identified non-present toxins). In the same way, results provided by Laboratory 0911 by HPLC AOAC 2005.06 were excluded since they only indentified two toxins in the sample (GTX1,4, not present and even when they reported not standard available and GTX5) and they wrongly calculated PSP toxicity from the individual toxins results reported.

Results from those laboratories that reported valid data were included in the statistical evaluation of results for total PSP toxicity. By applying Dixon test (a = 0.05), result from Laboratory 0909 was identified as outlier for sample CRLMB/09/P/02. The assigned value, S.D., RSDr% and target o for this sample are shown in **Table 5.** For performance statistics the z-scores were calculated (**Table 5 and Figure 2**). Only Laboratory 0909, applying the HPLC AOAC 2005.06 Official Method, had an unsatisfactory result, while Laboratories 0919 and 0924, both using MBA, obtained questionable results.

FIGURE 2. Z-SCORES OBTAINED BY PARTICIPANTS IN THE CRLMB 2009 PROFICIENCY TESTING ON SAXITOXIN GROUP (PSP TOXINS) DETERMINATION FOR SAMPLE CRLMB/09/P/02 (TOTAL TOXICITY)



9. CONCLUSIONS

Two samples were tested by 27 participants in the CRLMB 2009 Proficiency Testing for Saxitoxin group (PSP toxins) determination, providing a total of 34 results by mouse bioassay, HPLC methods, an-ELISA not-validated method and a qualitative test (Jellet Rapid Test).

Results from Laboratory 0911 using the HPLC AOAC 2005.06 method were considered as non-valid since they wrongly identified PSP toxins and wrongly calculated PSP total toxicity. It is remarkable that this participant noted that the method is not fully implemented in their laboratory.

For sample **CRLMB/09/P/01**, a naturally contaminated mussel sample (*Mytilus edulis*), collected in Norway during an *Alexandrium* sp. bloom, all the 31 valid quantitative results were considered for statistical evaluation. The assigned value for total PSP toxicity for this sample was 674 µg STX.2HCl equivalents/kg, with a Relative Stardard Deviation (RSD_R%) of 34%. In the Proficiency Assesment of this sample (target σ: 202 µg/kg), 28 results were satisfactory, 2 results, from Laboratories 0917 and 0902, by the HPLC AOAC 2005.06 method, were found questionable (2< | z-score | <3) and only the result

from Laboratory 0903, using the ELISA Ridascreen kit, was unsatisfactory (|zscore|> 3) The two qualitative results provided for this sample were “positive” (Laboratory 0903 using the Jellet Rapid Test and Laboratory 0914, using mouse bioassay) that, according to the assigned value, would be a "false positive” result with regard to the regulatory limit applicable.

Sample **CRLMB/09/P/02** was PSP contaminated mussel sample (Mytilus galloprovinciallis), prepared from a sample collected in Portugal during a Gymnodinium sp. bloom. For this sample, results from Laboratory 0916, using a HPLC post-colum method, was considered as non-valid due to the wrong identification of toxins and other result was identified as outlier. The assigned value (25 results) for total PSP toxicity for this sample was 424 µg STX.2HCl equivalents/kg, with a Relative Stardard Deviation (RSD_R%) of 29%. In the Proficiency Assesment of this sample (target σ: 127 µg/kg), 23 results were satisfactory, 2 results (Laboratories 0919 and 0924 by mouse bioassay) were questionable (2<|z-score]<3) and only the result from Laboratory 0909, using the HPLC AOAC 2005.06 method (and previously identified as outlier), was unsatisfactory (|z-score|> 3). Five out of the six qualitative results provided for this sample were “negative” that would be in accordance with the assigned value, in terms of the regulatory limit applicable; although result from Laboratory 0903 using the Jellet Rapid Test (“positive”) would be a “false positive".

10. ACKNOWLEDGEMENTS

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