2020 Proficiency Tests

European Union Reference Laboratory for Marine Biotoxins

SPECIFIC PROTOCOL

Coordination:

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1. Introduction

This protocol is complementary to the General Protocol on the 2020 Proficiency Tests of the European Union Reference Laboratory for Marine Biotoxins and contains the information and instructions about materials dispatch, methods of analysis and report of the results.

2. Test materials: information and instructions

For these PTs, you should receive by electronic mail: Specific Protocol, Arrival Form and Reporting File.
Dispatch Letter with individual Identity codes is included in the box containing test materials; Laboratories from Third Countries will receive the Identity code by email. Please remind that this code must be recorded in all the documents related to the study.
The test materials are sent by courier. Isothermal boxes are used for samples shipment in adequate conditions

EURLMB-2020-PT-ASP:

Tests materials consist on 2 samples approximately 10 g each of frozen scallops homogenates, labeled as EURLMB/20/A/01 and EURLMB/20/A/02.

EURLMB-2020-PT-LIPO:

Test materials consist on 2 samples approximately 5 g of sample each of frozen whole flesh mussel homogenates, labeled as EURLMB/20/L/01 and EURLMB/20/L/02 .

EURLMB 2020-PT-PSP:

Test materials consist on 2 samples of frozen whole flesh mussel homogenates labeled as EURLMB/20/P/01 and EURLMB/20/P/02.

The package must be checked upon reception, please make sure that the contents are correct and the samples are still frozen or refrigerated. The samples must be stored frozen at -18ºC or less, until the analysis, in any case it is advisable to perform the analysis as soon as possible. The contents of each container must be thoroughly mixed before weighing the sample for analysis.

Once the laboratory has received the material test, the **Arrival Form should be filled and sent back to the EURLMB by email** eurlmb@mscbs.es.

3. Methods of analysis

According to the Instructions provided in the General Protocol for these PTs, the methods used must be the Reference methods established in the EU legislation and accredited according to ISO 17025 .

The Reporting file should contain all the requested information about protocol, certified reference materials used for calibration purposes, etc.

4. Report of the results

A Reporting File consisting on an excel file is provided to report the results obtained. Participants are requested to submit this reporting file to the EURLMB by email eurlmb@mscbs.es. Deadline for submitting results is **22nd September 2020**. Results submitted later will not be included in the statistical evaluation.

4.1 EURLMB-2020-PT-ASP:

The results must be indicated as the sum of domoic acid (DA) and epidomoic acid (EA). The final result for each sample must be given in **mg/kg sample** (Regulation 853/2004/EC[1]. Indicate **only one result per sample.**

4.2 EURLMB-2020-PT-LIPO:

The methods used must detect at least the analogues stated in Regulation 074/2005/EC[2], amended by Regulation15/2011/EU[3].
In the Reporting file the participants were requested to report their results as follows:

- Free Okadaic acid group (OA, DTX-1 and DTX-2): µg free OA equivalents/kg.

[11] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin

[2] Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004

[3] Commission Regulation (EU) No 15/2011 of 10 January 2011 amending Regulation (EC) No 2074/2005 as regards recognised testing methods for detecting marine biotoxins in live bivalve molluscs

- Total Okadaic acid group, after hydrolysis (total OA, total DTX-1 and total DTX-2): µg total OA equivalents/kg.
- For Okadaic acid, dinophysistoxins and pectenotoxins together: µg OA equivalents/Kg
- For Azaspiracid group: µg AZA equivalents/Kg
- For Yessotoxin group: mg YTX equivalents/Kg.

Information about individual toxins concentrations (µg/kg) for the legislated groups is also requested.

Total toxicity equivalence is calculated using toxicity equivalent factors (TEFs) as recommended by EFSA which are indicated in the following table:

Toxin group	Analogue	TEF
OA group	OA	1
	DTX-1	1
	DTX-2	0.6
PTX group	PTX-1	1
	PTX-2	1
AZA group	AZA-1	1
	AZA-2	1.8
	AZA-3	1.4
YTX group	YTX	1
	Homo-YTX	1
	45-OH-YTX	1
	45-OH-homo-YTX	0.5

In the excel file provided TEFs are automatically applied to the final result for each group of lipophilic toxins.

4.3 EURLMB 2020-PT-PSP:

Final results should be reported in STX dihydrochloride equivalents/kg sample. Only one result per sample must be indicated.

Participants using HPLC/FLD (AOAC 2005.06 and 2011.02 OMA) are requested to indicate all the PSP toxins identified also indicating the individual concentrations of the quantified toxins in in µmol/kg. The individual toxin concentrations and total toxicity in µg STX dihydrochloride equivalents/kg sample will be calculate automatically.

The results should be expressed in the same way as for the official control either corrected or not corrected for recovery, using the corresponding sheets on the reporting file.

Laboratories reporting recovery corrected data are requested to indicate the procedure

commonly used for data correction, Please specify the approach used in the reporting file.

Toxicity factors for PSP toxins

It was agreed by the Group that EFSA TEFs should be used for calculating total toxicity. These values are included in Table determination of PSP toxins by AOAC OMA 2005.06 (HPLC method); you must use the EFSA Toxicity Equivalent Factors (TEFs) indicated in the following **Table**[4]**.** In the excel file provided TEFs are automatically applied to the final result.

Toxin	**TEF**	**Toxin**	**TEF**
STX	1.0	C2 (GTX8)	0.1
NEO	1.0	C4	0.1
GTX1	1.0	dc-STX	1.0
GTX2	0.4	dcNEO (GTX7)	0.4
GTX3	0.6	dcGTX2	0.2
GTX4	0.7	dcGTX3	0.4
GTX5 (B1)	0.1	11-hydroxy STX	0.3
GTX6 (B2)	0.1		

Also as agreed by the Group, for the calculation of coeluting epimer pairs when using AOAC OMA 2005.06, (dcGTX2 and dcGTX3; GTX1 and GTX4; GTX2 and GTX3, C1 and C2) the highest toxicity factor of the two epimers must be selected to calculate the toxicity contribution of each toxin,

For quantification of toxins

Full deatils for toxins quantification, etc. are given at the "EURLMB SOP for the analysis of Paralytic shellfish toxins (PST) by precolumn HPLC-FLD according to OMA AOAC 2005.06", version 1 June 2020

[4] Toxicity equivalency factors (TEFs) of STX-group toxins proposed by the CONTAM Panel (to be applied on a molar basis), as published in "Scientific Opinion of the Panel of Contaminants in the Food Chain on a request from the European Commission on Marine Biotoxins in Shellfish - Saxitoxin Group. *The EFSA Journal* (2009) 1019, 1-76".