GENERAL PROTOCOL

2023 Proficiency Testing Schemes

European Union Reference Laboratory for Monitoring of Marine Biotoxins

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1. Introduction

In compliance with the duties of the European Union Reference Laboratories for feed and food described in Article 94 of Regulation 625/2017/EC[1], the European Union Reference Laboratory for Monitoring of Marine Biotoxins (EURLMB) organizes a series of Proficiency Tests (PTs) for the determination of the three groups of marine biotoxins (PSP, ASP and LP) regulated in the EU Legislation.

This protocol contains the general procedures for the EURLMB Proficiency Testing Schemes. These PTs are addressed to the National Reference Laboratories of the EU Member States (NRLs). Laboratories for the official control of marine biotoxins in third countries may be also accepted to participate in these PTs after a formal request evaluated on a case-by-case basis with the EU Commission approval.

The aim of these PTs is to obtain information regarding the quality and comparability of the data reported by the participant laboratories and also to evaluate the laboratories ability to satisfactorily apply the recognized methods for marine biotoxins analysis, for the purposes of Regulation (EC) 853/2004[2] , Regulation (EU) 786/2013[3,] Regulation (EU) 2021/1374[4], Regulation (EU) 2019/627[5], Regulation (EU) 2021/1709[6]. Moreover, in cases when alternative methods could be applied, their equivalence will be also assessed.

2. Participants

According to Art. 94 of EU Regulation 625/2017/EC all EU NRLs for Marine Biotoxins are obliged to participatein the EURLMB PTs. NRLs and Laboratories for official control of marine biotoxins in Third Countries might be also accepted to participate.
NRLs are invited to participate in the trials and request to submit their Registration Form

[1] Regulation (EC) No 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls performed toensure the verification of compliance with feed and food law, animal rules on animal health and welfare, plant health and plant protection products

[2] 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

[3] Commission Regulation (EU) No 786/2013 of 16 August 2013 amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Council as regards the permitted limits of yessotoxins in live bivalve molluscs Text with EEA relevance

[4] Regulations Commission Delegated Regulation (EU) No 2021/1374 of 12 April 2021 amending Annex III to Regulation (EC) No 853/2004

[5] Commission Implementing Regulation (EU) No 2019/627 f 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls

[6] Commission Implementing Regulation (EU) No 2021/1709 of 23 September 2021 amending Implementing Regulation (EU) 2019/627 as regards uniform practical arrangements for the performance of official controls on products of animal origin

by electronic email to the EURLMB: eurlmb@aesan.gob.es within the announced deadline.
Since the participation of EU NRLs in the EURLMB PTs is mandatory, those NRLs unable to participate must provide the adequate justification and also indicate the laboratory that is going to carry out this task on their behalf.

3. Confidentiality and communication

The proprietor of the PTs data is DG-SANTE[7] and as such must have access to all the information on the PTs results. In order to ensure the confidentiality of the data, a laboratory code will be provided to participants. This laboratory code will not be linked to the name of the laboratories in the final report.
Communication between participating laboratories during the test on matters concerning a PT exercise is not permitted from the start of the PT exercise until the distribution of the report.
The official language used in PTs is English.

4. Announcement

Participants will be contacted by email, at least one month before the distribution of the test materials, General Protocol and Registration Form will be included in this email. Confirmation to registered participants will be sent byemail immediately after deadline.

5. Marine biotoxins groups and test materials

Proficiency testing will be conducted for the analysis of the three groups of toxins regulated in the EU (Lipophilic, Amnesic and Paralytic shellfish toxins).
EURLMB-2023-PT-ASP: Proficiency Test for ASP toxins determination.
EURLMB-2023-PT-LIPO: Proficiency Test for Lipophilic toxins determination.
EURLMB 2023-PT-PSP: Proficiency Test for PSP toxins determination.
The materials provided will include shellfish tissue homogenates prepared at the EURLMB from non-contaminated and/or naturally contaminated shellfish samples and adequately tested for homogeneity and stability. Test materials will be dispatched

7 DG-SANTÉ = European Commission, Health and Food Safety Directorate-General

frozen with dry ice or with frozen gel-pack; (depending on the country transportation requirements).

6. Communication of material dispatch

Confirmation of samples dispatch and all related information will be sent by email. All documents required for PTs such as Specific protocol (including instructions for material storage, methods to be used, etc.), Arrival Form, Reporting File and any other information of interest will be also included in this email. Participants receive a letter with individual identity codes which will be included in the box containing the test materials. The identity code must be recorded in all the documentation related to the study.

7. Homogeneity

Homogeneity studies for test materials are conducted at the EURLMB by recognized testing methods. Materials are tested for homogeneity before distributionto participants. Homogeneity tests involve the analysis of two replicates, taken fromten or eight units randomly chosen. Data obtained for the homogeneity tests are statistically evaluated according to the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, published by IUPAC[8]. Cochran's Test and Homogeneity test are used.

8. Stability

Stability studies are conducted at the EURLMB by recognized testing methods. Data obtained for the stability tests are statistically evaluated according to the International Harmonized Protocol for the Proficiency Testing, IUPAC. The stability tests involve the analysis of two batches of three replicates. The first batchis analized shortly before the test material shipment and the second one right after the deadline for the submission of results. As the duration of the shipment might vary between labs/countries, the EURLMB will conduct stability tests at conditions simulating the shipment with dry ice and with frozen gel-pack. These test materials will be kept under ambient conditions and will be analysed immediately after the confirmation of the latest reception.

8 The international harmonized protocol for the proficiency testing of analytical chemistry laboratories, 2006

9. Methods

Participation in PTs will be accepted when methods used are the ones established in the EU Legislation and methods internationally validated through collaborative trials.In any case it is mandatory that laboratories participate with the methods they use for official control. Other recognized alternative methods could be accepted as long asthey fulfil the requirements above indicated.

10. General procedure for reporting results

Participant laboratories are responsible for reporting their results to the EURLMBwithin the stipulated deadline. Results must be reported using the provided Reporting File. **All the requested information included in the reporting file must be completed.**

11. Evaluation of results

Statistical evaluation of results will be carried out according ISO 13528:2015[9]. Only quantitative results of the toxins present in the sample will be taken into account for statistical evaluation.

11.1 Determination of the assigned value for each sample

The determination of the assigned value is carried out by consensus among participants. The assigned value X of a test material used in a round of a proficiency test is the robust average of the results reported by all the participants in the round, calculated using Algorithm A. This algorithm yields robust values of the average and standard deviation of the data to which it is applied.

11.2 Determination of standard uncertainty of the assigned value U_X of the assigned value

When the assigned value is derived as a robust average calculated using Algorithm A, the standard uncertainty of the assigned value X is estimated as:

$$u_X = \frac{1.25 \times s^*}{\sqrt{p}}$$

Where s* is the robust standard deviation calculated using Algorithm A and p number of participants, respectively.

[9] ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparisons, International

If $U_X \leq 0.3\sigma$, then the uncertainty of the assigned value is negligible and does not need be included in the interpretation of the results of the proficiency test.

11.3 Determination of the standard deviation for proficiency assessment

The statistical approach applied by EURLMB in the Proficiency Testing was revised by the EURLMB WG of experts. The Working Group focused particularly on the determination of the standard deviation for proficiency assessment (target σ) in the PTs for the different groups of marine biotoxins. The Working Group agreed on the calculation of the target σ following the Horwitz-Thompson equation (Thompson et al.,2006.

Horwitz gives a general model for reproducibility of analytical methods that may be used to derive the following expression for the reproducibility standard deviation:

$$\sigma = 0.02 \times c^{0.8495}$$

Where c, is the concentration of the chemical species determined in percent, mass fraction.

Thompson (2000) demonstrated that Horwitz equation is neither applicable to the lower concentration range (<120 µg/kg) nor as to the higher concentration range (>138 g/kg) and suggested another model for those ranges. Therefore for analyte concentrations <120 µg/kg the target σ will be calculated as follows:

$$\sigma = 0.22 \times c$$

11.4 Score and evaluation criteria

z and z' score are used for the evaluation of individual laboratory performance in accordance with ISO 13528.

z-score is calculated using the following formula:

$$Z = \frac{(x - X)}{\sigma}$$

Where σ is the standard deviation for proficiency assessment.

z-score will be interpreted in the following way, as is set in the ISO 17043:2010[10]

[10] ISO/IEC 17043:2010. Conformity assessment – General requirements for proficiency testing

$|z| \leq 2{,}0$: Satisfactory

$2{,}0 < |z| < 3{,}0$: Questionable

$|z| \geq 3{,}0$: Unsatisfactory

According to ISO 13528 if the uncertainty of the assigned value does not comply with this criterion $U_x \leq 0.3\ \sigma$, the uncertainty is significant and therefore has to be taken into account in the interpretation of the results of the proficiency test. This can be done by calculating the z´-score as follows:

$$Z' = \frac{(x - X)}{\sqrt{(\sigma^2 + u_x^2)}}$$

Where u_x is the standard uncertainty of the assigned value X.

z´ score shall be interpreted in the same way as z-score using the same critical values as mentioned before.

12. Report

Final report will be sent to participants at the end of the study and always before the annual workshop, for participants to be able to have their results on time for discussion during the workshop. The final report also includes assigned values and z-score for all toxins present in the test materials. This final report will be also sent to DG-SANTE.

13. Feedback

At any time before, during or after the PT participants have the possibility to contact the EURLMB for further information or clarifications or to communicate any possible error.

14. Follow-up activities

According to instructions from DG-SANTE, the “Protocol for management of underperformance in comparative testing and/or lack of collaboration of National Reference Laboratories (NRLs) with EU Reference Laboratories (EURLs) activities”, appropriate actions must be taken if the results of comparative tests reveal underperformance or if NRLs fail to collaborate properly with the corresponding designated EURL.

15. Timetable

Schedule for PTs is indicated in this Table.

PROFICIENCY TESTING TRIAL	Participants registration deadline	Materials Dispatch	Results submission	Report available
EURLMB-2023-PT-LIPO	**April 10th- 18th**	**May 22nd**	**July 21st**	**Sept. 29th**
EURLMB-2023-PT-ASP				
EURLMB-2023-PT-PSP				