PROFICIENCY TESTING FOR THE FOOD SECTOR: THE EXPERIENCE OF THE EUROPEAN UNION REFERENCE LABORATORY FOR CHEMICAL ELEMENTS IN FOOD OF ANIMAL ORIGIN

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Abstract – The consistent production of reliable analytical results can be a demanding task for laboratories that must select a strategy to guarantee the quality of the released data. Among the possible strategies the participation in Proficiency Testings (PTs) on regular basis is one of the best ways to monitor the laboratory's performance both against its own requirements and the norm of other laboratories. In case of a consolidated network of participants, PTs can be used to harmonize their level of performance as well.

The European Union Reference Laboratory for chemical elements in food of animal origin (EURL-CEFAO), organizes PTs since 2006. Over the time, the EURL experience in preparing adequate PT materials and in releasing reliable assigned values have constantly improved making the scheme particularly valuable. The recurrent repetition of exercises on the same analyte/matrix combination, the participation of European outstanding laboratories in the EURL-CEFAO PTs and the continuous follow-up activity (e.g. use of control charts of z-score) resulted in a steady and satisfactory general performance of the network as well as in the harmonization of the results submitted by participants.

Keywords: Proficiency Testing, European Union Reference Laboratory, interlaboratory comparisons, chemical elements

1. INTRODUCTION

Nowadays participation in Proficiency Testings (PTs) is regarded as an essential tool to assure the quality of the analytical results produced by laboratories. All over the world the majority of accreditation bodies considers the regular participation in inter-laboratory comparisons as a key element for ensuring the reliability of results obtained by laboratories accredited according to ISO/IEC 17025/2005 [1]. As a result, the demand of specific schemes has increased over the last years, though the supply has not increased accordingly in all fields. Furthermore, the laboratory has to carefully select the PT schemes in which participate taking into account its specific needs (matrix/analytes combination; values of concentration of the analytes; physical form of the samples). This aspect can further reduce the availability of adequate commercial PTs.

The determination of chemical elements in food of animal origin is an example where the availability of commercial PTs is quite inadequate to meet the demand. The European Union Reference Laboratory for Chemical Elements in Food of Animal Origin (EURL-CEFAO), in compliance with its duties listed in Regulation (EC) No. 882/2004 [2], has focused on the activity of proficiency testing provider (PTP) to cover this particular area. The EURL scheme is intended for those EU National Reference Laboratories (NRLs) appointed as outstanding laboratories in their respective EU Member States.

Compliance with international standards, accreditation of the PT scheme, experience of PTP in the organization of inter-laboratory comparisons, high level of technical competence of the organizer and reliability of the assigned values are the prominent requirements for a well-qualified scheme. In order to provide participants with PTs which are highly valued by accreditation bodies, the EURL-CEFAO has been accredited as PTP since 2009 (according to ISO Guide 43-1 [3] and ISO/IEC 17043:2010 [4]), though exercises have been

organized by the laboratory since 2006. Over time, the EURL has developed and optimized a number of internal procedures with the view to produce adequate PT test items with the matrices of interest (milk, meat, fish, offal, honey, infant formula) in different forms (liquid, frozen and freeze-dried). It has gained a great experience in the evaluation of the materials adequacy for PT purposes (i.e. stability, homogeneity) as well as in the statistical analysis of the results. The scheme is also conceived to monitor the long-term performance of participants by periodical repetition of the same matrix/analytes combination. This general strategy has continuously improved the laboratories participating in the exercises in terms of performance and harmonization of results. In fact, though the success/failure of the participants is evaluated by a more restrictive criterion than the one commonly used in the food sector, the percentage of satisfactory results has increased over the PTs.

Likewise, the results submitted by participants have reached a good level of agreement, the parameters related to data dispersion becoming more and more narrow. Last but not least, the use of control charts for z-scores allowed most laboratories to monitor their ability to produce reliable results and reach a steady performance.

2. ORGANIZATION OF EURL-CEFAO PROFICIENCY TESTINGS

PTs organized by the EURLs are addressed to expert laboratories in the EU Member States (i.e., NRLs), that are obliged to participate in the PTs carried out by the pertinent EURL.

During the decade from 2006 to 2015, the EURL-CEFAO PTs had been mainly planned as a long term program pursuing the main objective of promoting the harmonization of performance within the network of NRLs. In the early stage, three PTs were yearly organized including two samples at different levels of concentration in some of the exercises (e.g. 10th PT on meat; 11th PT on milk). Starting from 2011, the number of PTs per year has been reduced due to the high and steady performance reached by the network. Nowadays, the EURL-CEFAO's activity is planned so as to conduct two exercises: one to be completed in the first part of the year and the other in the second part.

Over the years, the participants have been provided with a scheme to give them the possibility:

- to check and improve the performance of their analytical methods;
- to verify the effectiveness of corrective actions undertaken (when necessary) through the repetition of the same matrix;
- to have a long term follow-up of their performance;
- to promote the improvement of Quality Control System

Arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), molybdenum (Mo) and lead (Pb) are the chemical elements that have been proposed in the exercises.

The scheme of PTs organized in the last ten year period is reported in Table 1.

Table 1. Summary of the PTs organized by the EURL-CEFAO in the decade 2006-2015. As and Hg are to be read as total As and total Hg

Year	Matrix	Physical form	Analytes
2006	Meat	Freeze-dried	As, Cd, Pb
	Milk	Freeze-dried	As, Cd, Pb
2007	Meat	Freeze-dried	As, Cd, Pb
	Milk	Liquid	As, Cd, Pb
	Fish	Freeze-dried	As, Cd, Pb, Hg
2008	Liver	Freeze-dried	As, Cd, Pb
	Milk	Liquid	As, Cd, Pb
	Meat	Freeze-dried	As, Cd, Pb
2009	Fish	Freeze-dried	As, Cd, Pb, Hg
	Milk	Liquid	As, Cd, Pb
2010	Meat	Frozen	As, Cd, Pb, Hg
	Milk	Liquid	As, Cd, Pb
	Fish	Frozen	As, Cd, Pb, Hg
2011	Meat	Freeze-dried	As, Cd, Pb
	Liver	Frozen	As, Cd, Pb
2012	Milk	Liquid	As, Cd, Pb
	Infant formula	Powder	Cd, Pb
2013	Meat	Frozen	Cd, Cu, Hg, Pb
	Honey	Semi-liquid	Cd, Pb
2014	Kidney	Frozen	Cd, Cu, Hg, Pb
	Mussels	Freeze-dried	As, Cd, Pb, Hg
2015	Infant formula	Powder	As, Cd, Pb, Mo
	Fish	Freeze-dried	As, Cd, Pb, Hg

PTs are usually based on chemical elements and foods for which a maximum level (ML) is set in pertinent regulations [5,6,7,8,9] but, as of 2012, new analytes/matrix combinations (e.g. infant

formula; honey) [10, 11] have been included in the exercises. These combinations are selected on the basis of information coming from the European Commission, i.e. discussion about introduction of new limits in the relevant regulations, emerging topics and specific demands from participants.

2.1. Preparation of material and assessment of its adequacy for PT purposes

The EURL-CEFAO has invested significant energy and resources in setting adequate internal procedures to produce PT test items in a form as similar as possible to the samples analysed by laboratories during their everyday work. Over the years, liquid samples (e.g., milk) [12], partially liquid samples (e.g., honey) and frozen samples (e.g., meat, fish, offal) have been proposed as PT test items. The analytes concentration is often adjusted around values of interest. This adjustment is performed either spiking the starting material, purchased at retail stores, with standard solutions of chemical elements or diluting it with a suitable similar matrix. The former is used if the basal content of analytes is lower than the concentration value planned for PT, the latter is used if the basal content is greater than the concentration value foreseen in the final material (e.g., mercury in fish). Freeze-dried samples are periodically included in the exercises so as to supply the NRLs with samples easy to handle and store that they can use for their internal scope (e.g., quality control) as well.

Once PT test items have been prepared, they have to be tested for stability and homogeneity. In fact, the samples have to be stable for the duration of PT and the degree of homogeneity should be enough to guarantee that differences between test items will not significantly affect the evaluation of participants' performance.

The EURL-CEFAO evaluates homogeneity against restrictive criteria, so the analytical methods used to perform this evaluation have to be extremely precise. As a consequence, an integral part of its work is to develop and to validate analytical procedures adequate to highlight any difference among the test items [13]. In fact, it is necessary to avoid that a possible difference among the samples is covered up by the analytical variability of the method used. Therefore, if the method is precise enough the between-samples difference can be attributed to the heterogeneity of the material.

This specific aspect should make the participant feel more confident about the quality of the material produced by the EURL-CEFAO and its adequacy for the PT purpose.

As an example, the analytical results of the homogeneity test for Pb in the 22nd PT (infant formula) are represented in graphic form (Figure 1).

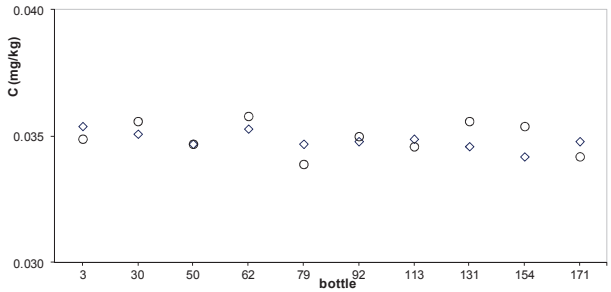

Fig. 1. Homogeneity test for Pb (22nd PT): the analysis is performed in duplicate and the values of concentration (C) obtained for the first (open circle) and the second (open diamond) test portion are reported.

2.2. Key elements for statistical evaluation of participants' results

The main step to evaluate the performance of a laboratory participating in a PT consists in setting two "reference values", namely the assigned value (x_{pt}) and the standard deviation for proficiency assessment (σ_{pt}). Among the different possible approaches to establish these parameters, the EURL-CEFAO has chosen the consensus from participants to derive the assigned value and the fitness for purpose to set σ_{pt}. The "consensus" is commonly used by PTPs that, however, should be aware of some disadvantages related to this approach. In particular, the possibility of assigning a biased value should be taken into account and minimized. As for the EURL-CEFAO, the probability that a bias occurs in the assigned value is extremely low. Firstly, because the results are considered also from an analytical point of view and rejected *a priori* in case of doubts about how they have been produced. Secondly, because the possible presence of statistical subpopulations, linked to the analytical techniques used, is thoroughly investigated and adequately evaluated.

As far as σ_{pt} is concerned, the value used is lower than that from the Horwitz–Thompson equation (σ_H) and is set according to specific algorithms developed by the EURL-CEFAO. These equations

have been set taking into account type of matrix as well as type and concentration value of the analyte. The resulting σ_{pt} corresponds to the level of performance that the participants in the PT are expected to achieve. Furthermore, they have been conceived to correspond to low enough values taking into account the expertise and the role of the NRLs. Both x_{pt} and σ_{pt} are usually set by applying robust statistics following a procedure compliant with ISO 13528:2015 [14].

2.3. Reliability of the assigned value

The reliability of the assigned value can be considered a strength point of the EURL-CEFAO scheme. This is due to the high competence of the laboratories belonging to the network as well as the statistical procedure followed to derive x_{pt}. In confirmation of this reliability, the assigned value, the value obtained from homogeneity test and the expected value are usually comparable. Furthermore, some studies have been conducted to derive x_{pt} from a calibration against the reference value of suitable certified reference materials (CRMs). The values assigned using this procedure resulted comparable to the values obtained from consensus, demonstrating the adequacy of the statistical procedure applied to derive the assigned value. As an example, the expected value (based on the gravimetric spiking), the assigned value, the value from homogeneity test and the value assigned against CRMs are compared in Table 2 for two PTs on milk (14th and 16th PT). CRMs used for deriving the assigned value ($x_{pt\text{-}CRM}$) were BCR063R and ERM-BD 150.

Table 2. Comparison among expected value (x_{exp}), value from homogeneity test (x_{hom}), assigned value (x_{pt}) and value assigned against CRM ($x_{pt\text{-}CRM}$). Each value is reported with the expanded uncertainty (k=2) and all values are expressed in µg/kg

Analyte (PT)	x_{exp}	x_{hom}	x_{pt}	$x_{pt\text{-}CRM}$
Cd (14th PT)	6.0±0.04	6.4±0.6	6.0±0.4	n.e.
Pb (14th PT)	24.0±0.2	27.0±2.2	25.2±1.0	26.5±2.8
Cd (16th PT)	4.98±0.04	4.90±0.98	5.15±0.28	5.3±1.5
Pb (16th PT)	27.3±0.3	28.6±2.3	28.0±1.2	31.0±2.0

2.4. Performance of participants

In compliance with ISO 13528:2015, the participant's performance is evaluated in terms of z-score that is calculated according to formula (1).

$$z_i = \frac{(x_i - x_{pt})}{\sigma_{pt}} \quad (1)$$

where x_i is the PT result submitted by the participant. Based on the value of z_i, the performance can be acceptable ($|z| \leq 2.0$), questionable ($2.0 < |z| < 3.0$) or unacceptable ($|z| \geq 3.0$). Over the EURL-CEFAO PTs, percentage of laboratories satisfactorily performing has been increased till to reach a steady good performance for almost all analytes/matrix combinations.

The EURL-CEFAO uses Shewhart control charts as a graphical means to combine performance scores over several PTs. This visual presentation is far more explicative than the interpretation of a single numeric score. Therefore, this is useful to participants to check their performance over the time, also detecting likely trends, as well as to the PTP to monitor the general performance of the network, also pointing out possible crucial points of the exercises.

The charts are updated after each PT and distributed to participants so they can promptly evaluate their performances for a specific matrix/analyte combination [15]. An example of this kind of chart, related to PTs on milk and infant formula, is reported in Figure 2. It is evident that the participant has never had problems to analyse arsenic (open triangle) in these matrices whilst its performance for cadmium and lead (open diamond and open square, respectively) has been improved over the exercises.

The improvement of the network is not only proved by the increased percentage of satisfactory z-scores but it is also demonstrated by the harmonization of the submitted results. In fact, standard deviation of the mean (SD) has decreased over the PTs confirming that the data dispersion has become more and more narrow. In particular, since 2010 this indicator of data dispersion has resulted lower than the σ_{pt} and close to the robust standard deviation (s*). This trend is reported in Figure 3 for Cd (PTs on milk and infant formula) where the following ratio are reported as percentage: SD/x_{pt} (open square); σ_{pt}/x_{pt} (open diamond) and s^*/x_{pt} (open triangle).

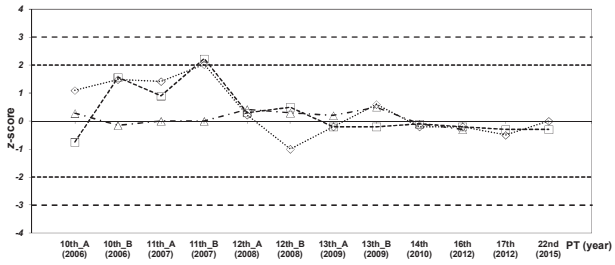

Fig. 2. Shewhart control chart of z-scores for As (open triangle), Cd (open diamond) and Pb (open square) in milk and infant formula from a single participant.

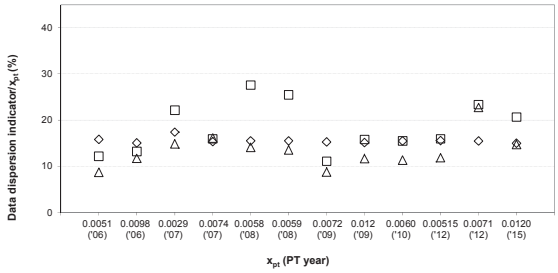

Fig. 3. Trend of data dispersion indicators for Cd over PTs on milk and infant formula: SD/x_{pt} (open square); σ_{pt}/x_{pt} (open diamond) and s^*/x_{pt} (open triangle).

3. CONCLUSIONS

Participation in PT is regarded as an useful way to assess laboratory's performance as well as to guarantee the reliability of its analytical results. As the EURL-CEFAO is well aware of the importance of this means, it has boosted its activity as accredited PTP over the years. Many efforts have been made to produce materials adequate to the PT purpose, and the procedure to derive the assigned values has been improved more and more. In particular, the high level of competence of the participants in the exercises and the effectiveness of the procedures used by the EURL to prepare the PT material and to treat the results, allow it to release assigned reliable values.

Furthermore, the organization of recurrent PTs on the same matrix/analyte combination enables participants to monitor the performance of their analytical methods on a regular basis and the PTP to constantly check the quality of the network. A thorough activity of follow-up (e.g., control charts of z-scores) performed by the EURL-CEFAO has resulted in an improvement of the network's general performance as well as in the harmonization of the results submitted by participants.

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