Journal of Trace Elements in Medicine and Biology 59 (2020) 126414

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Biochemistry

External quality assessment schemes for inorganic elements in the clinical laboratory: Lessons from the OELM scheme

Josiane Arnaud[a,*], Marina Patriarca[b], Béatrice Ma'atem Fofou-Caillierez[c], Montserrat González-Estecha[d], MªCarmen González Gómez[e], Irene De Graaf[f], Valeria Patriarca[b], Martine Ropert-Bouchet[g], Liesbeth Schröer-Janssen[f], Carla Siebelder[f], Marieke Te Winkel[f], Montserrat Ventura Alemany[e], Cas Weykamp[f]

[a] Unité de biochimie hormonale et nutritionnelle, Institut de Biologie et Pathologie, CHU Grenoble Alpes, CS 10217, 38043, Grenoble cedex 9, France
[b] Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Roma (I), Italy
[c] Biochimie et Biologie Moléculaire, Nutrition et Métabolisme, Hôpital Brabois, CHRU Nancy, 5 rue du Morvan, 54511 Vandoeuvre-les-Nancy Cedex, France
[d] Servicio de Análisis Clínicos, IML. IdISSC, Hospital Clínico San Carlos, Calle Profesor Martín Lagos 1, 28040, Madrid, Spain
[e] Programas de Garantía Externa de la Calidad para Laboratorios Clínicos, Sociedad Española de Medicina de Laboratorio (SEQC-ML), Padilla 323, despacho 68, 08025 Barcelona, Spain
[f] MCA Laboratorium, Streekziekenhuis Koningin Beatrix, Beatrixpark 1, Postbus 9005, 7100 GG Winterswijk, the Netherlands
[g] Laboratoire de Biochimie Toxicologie, CHU de Rennes, 2 rue Henri Le Guilloux, 35033 Rennes cedex 9, France

ARTICLE INFO

Keywords:
External Quality Assessment Schemes
Inorganic elements
Clinical laboratories
Blood
Urine

ABSTRACT

Measurements of inorganic elements in clinical laboratories produce results used for the diagnosis, the treatment and the monitoring of deficiencies or overloads. The main objective of External Quality Assessment Schemes is to verify, on a regular frequency, that clinical laboratory results correspond to the quality requirement for patient care. Therefore, External Quality Assessment Schemes represent an essential component of a laboratory's quality management system. However, External Quality Assessment Schemes within the same analytical field remain heterogeneous for different reasons such as samples, determination of assigned value, acceptable limits, content of the reports. The aim of this review was to describe and illustrate some major critical aspects of External Quality Assessment Schemes based on Occupational and Environmental Laboratory Medicine external quality assessment scheme experience.

Abbreviations: x, Assigned value; CRM, Certified reference material; CV, Coefficient of variation; EEE-PT, WG, EA-Eurolab-Eurachem; EAAS, Electrothermal atomic absorption spectrometry; EFLM, European Federation of Clinical Chemistry and Laboratory Medicine; EQALM, European Organisation for External Quality Assurance Programmes in Laboratory Medicine; EQA, External Quality Assessment scheme; FAAS, Flame atomic absorption spectrometry; GUM, Guide to the expression of the Uncertainty Measurement; ICP-AES/OES, Inductively coupled plasma atomic / optical emission spectrometry; ICP-MS, Inductively coupled plasma coupled to mass spectrometry; ILC, Interlaboratory comparison; IQC, Internal quality control; IFCC, International Federation of Clinical Chemistry and laboratory medicine; ISO, International Organisation for Standardisation; IDMS, Isotope dilution mass spectrometry; LOD, Limit of detection; LOQ, Limit of quantification; m, Mean; n, Number; OELM, Occupational and Environmental Laboratory Medicine; PA, Proficiency assessment; PT, Proficiency testing programs; QS, Quality specifications; SD, Standard deviation; u, Standard uncertainty

All the authors are members of the scientific advisory board of "Occupational and environmental laboratory medicine (OELM) external quality assessment/ proficiency testing schemes". The MCA laboratory team prepares the samples (responsibles: Marieke Te Winkel and Cas Weykamp) according to the decision of the board, is responsible of the website management (Irene de Graaf) and of the quality assurance management (Liesbeth Schröer-Janssen). The authors are also members of the network "Organizers of external quality assessment / proficiency testing schemes related to occupational and environmental medicine". This network includes organisers of inorganic element external quality assessment schemes from different countries in Europe, North America and Asia and its aim is focused on the harmonization and improvement of trace element external quality assessment schemes.

* Corresponding author.
E-mail addresses: jos-arnaud@hotmail.fr (J. Arnaud), marina.patriarca@iss.it (M. Patriarca), b.caillierez@chru-nancy.fr (B.M. Fofou-Caillierez), montse@cmpx.net (M. González-Estecha), mcgonzalez@seqc.es (M.G. Gómez), I.deGraaf@skbwinterswijk.nl (I. De Graaf), valeria.patriarca@iss.it (V. Patriarca), Martine.ROPERT-BOUCHET@chu-rennes.fr (M. Ropert-Bouchet), L.Janssen@skbwinterswijk.nl (L. Schröer-Janssen), C.Siebelder@skbwinterswijk.nl (C. Siebelder), M.teWinkel@skbwinterswijk.nl (M. Te Winkel), mventura@seqc.es (M. Ventura Alemany), c.w.weykamp@skbwinterswijk.nl (C. Weykamp).

https://doi.org/10.1016/j.jtemb.2019.126414
Received 3 June 2019
0946-672X/

1. Introduction

Inorganic trace element results produced by clinical laboratories are used to diagnose and treat deficiencies or overloads, and to monitor patients or exposed workers. The main objectives of External Quality Assessment schemes (EQA), also call Proficiency Testing programs (PT) or interlaboratory comparisons (ILC) are to verify, on a regular frequency, that laboratory results correspond to the quality requirement for patient care, to improve result transferability among laboratories whatever the analytical method used and to detect analytical errors [1–4]. Consequently, participation in EQA allows laboratories to implement corrective actions, when necessary, to improve analytical data, and represents an essential component of a laboratory's quality management system. Participation in EQA is a requirement for laboratory accreditation according to International Organisation for Standardisation (ISO) 15189 [1,3–6]. Two ISO standards give information on how to conduct these schemes and interpret their results. ISO/IEC 17043 describes the technical and managerial requirements to be fulfilled by competent providers whereas ISO 13528 describes statistical methods to analyse the data obtained as well as recommendations for the interpretation of results by participants and by accreditation bodies [7,8]. However, EQA schemes within the same analytical field remain heterogeneous as demonstrated for lead in whole blood [9]. Many factors explain this heterogeneity, particularly sample commutability, determination of assigned value and acceptable limits, analytical factors accessed in the reports, number of participants [1,3,4,6,9–12]. Consequently, participation in EQA does not guarantee its effectiveness.

The aims of this review were to describe some major critical aspects of EQA which allow to evaluate the value of EQA and to give clues on how the EQA result should be interpreted by participating laboratories. Examples based on occupational and environmental laboratory medicine (OELM) EQA experience illustrate these different aspects [13]. More detailed information is found in the different cited references.

2. EQA description

The different steps of EQA are summarized in Fig. 1. Briefly, EQA samples are periodically sent by a provider to a group of laboratories for a given set of analyses. The participating laboratories do not know the concentrations of the analytes. Each laboratory treats the samples according to instructions for participants and performs measurements theoretically as for patient samples [4]. However, it is known that some laboratories treat EQA samples as special [6,14,15]. Then, participants submit their results to the organizer for evaluation according to a precise schedule. The organizer analyses the participant results and delivers a challenge report to each participant containing at least the number of received results, the assigned value and the acceptable limits, the standard deviation and/or coefficient of variation for all participants as well as for analytical method groups, the histogram of the distribution of the results from all participants, the deviation of laboratory result relative to the assigned value and the performance of the laboratory [1–3,10]. Additional information such as long-term bias, precision, participant uncertainty may appear in end of cycle reports [2,6,16,17]. Finally, the participant reviews carefully the reports and takes corrective actions when necessary.

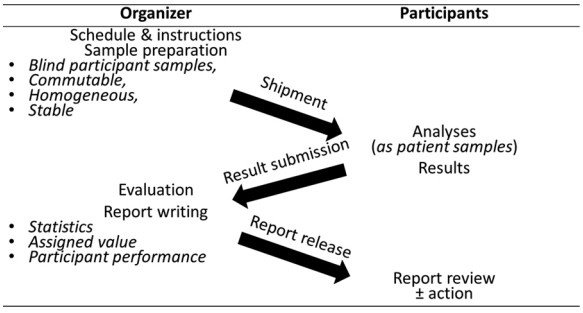

Fig. 1. Schematic description of the different steps of External Quality Assessment scheme.

Currently the OELM EQA includes Al, Co, Cr, Cu, Li, Mg, Mo, Se, Tl, V, Zn in serum, As, Cd, Co, Cr, Hg, Mg, Mn, Pb, Se, Tl, Zn in whole blood and Al, As, Be, Cd, Co, Cr, Cu, Fe, Hg, I, Mg, Mn, Ni, Pb, Sb, Se, Tl, V, Zn in urine. The OELM EQA has defined 6 groups of analytical methods: inductively coupled plasma coupled to mass spectrometry (ICP-MS), inductively coupled plasma atomic / optical emission spectrometry (ICP-AES/OES), flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (EAAS), colorimetry and other analytical methods. The monthly reports contain consensus robust assigned value, robust standard deviation and coefficient of variation for all the participants and for each group of method, the histogram of the distribution of the results from all participants centred on the assigned value as well as the results reported by laboratories using the participant's method, analytical performance of the participant laboratory expressed as z score and bias, participant laboratory cumulative score and median cumulative score of all the participating laboratories and a figure that shows the z scores for the past 12 months. Participating laboratories cover all the inorganic element fields i.e. clinical and veterinary medicine, public health, environmental and occupational exposure and forensic medicine [13].

The value of EQA and how the EQA result should be interpreted depend on different key points listed on Table 1. Guidance on the selection, use and interpretation of PT and EQA can be found in a Eurachem Guide [18], produced by the EEE-PT Working Group (EA-Eurolab-Eurachem), with the involvement of EQALM (European Organisation for External Quality Assurance Programmes in Laboratory Medicine). The ideal EQA sample has three important properties: it behaves as a native patient sample whatever the laboratory method (is commutable), has an accurate assigned value and quality specifications that fit for clinical needs. If these three criteria are not entirely fulfilled, error in the evaluation of the laboratory performance may occur. When these criteria are fulfilled, EQA is a pillar in the overall process of laboratory quality assurance [4,6,12,19]. It provides evidence to confirm

Table 1
Principal factors to take into consideration for the choice of an External Quality Assessment scheme (EQA).

Samples	Nature (species, liquid or lyophilized…)
	Preparation and handling (chemical form of spikes, addition of stabilizers, number of freeze-thaw cycles, chelation or adsorption of trace element, matrix dilution or concentration, temperature and duration of shipment and storage…)
	Properties (commutability, homogeneity, stability, concentration range)
Assigned value	How is it determined?
	Is it traceable?
Quality specifications	How are they defined?
Reports	Total error
	Precision on duplicate samples
	Uncertainty
	Long term bias
Number of samples	Number per year
	Number per challenges
	Number of replicate samples
	Challenge frequency
Number of participants	
Peer group	Design of peer groups
	Number of participants per peer group
Service to customers	Treatment of claims and questions
	Satisfactory questionnaires
	Advices to poor performers….
Organizer	Does the organizer involve in a working group dealing with harmonisation between EQA within the same analytical field?

the best testing practices and therefore may help laboratories to select and validate their analytical method, monitor the method performance over time, improve their analytical performances, is generally included in the calculation of measurement uncertainty, and consequently increases the confidence of the laboratory [19].

3. Samples properties

The provider is responsible for the preparation and validation of commutable, homogeneous and stable samples with concentrations within the range observed in the clinical laboratories [3,19] which may represent a serious challenge. In the field of inorganic elements, the range of concentrations must ideally cover from severe deficient levels of essential trace elements to toxic levels of all elements as laboratories analyse samples from hospitalized patients, general population, environmental exposure, occupational exposure, and forensic samples as well as animal samples.

Sample commutability is a major factor that defines the quality of an EQA. A commutable EQA sample behaves as a native patient sample whatever the analytical method used. A non-commutable EQA sample includes matrix related bias that occurs only in EQA samples and never in authentic patient samples with at least one analytical method and therefore, does not give meaningful information to the participant(s) using the analytical method impacted by matrix bias [1,3,20]. However, the commutability of EQA samples is quite unpredictable as alteration of matrix may occur during all the steps of sample processing and handling. The main steps are mixing, centrifugation or filtration, freeze-thaw cycles, shipment, storage. Other steps may be included such as spiking, introduction of stabilizers, dialysis, matrix concentration or dilution, adsorption or chelation, lyophilization and are more likely to modify the matrix. Duration and temperature of shipment and storage also modify the commutability [1,3,12]. Therefore, the preparation of samples must be as simple as possible and preferably based on human samples. The international Federation of Clinical Chemistry and laboratory medicine (IFCC) has recently proposed recommendations for the evaluation of commutability [21–23]. However, it is quite difficult, time consuming and expensive to demonstrate commutability and most EQA, particularly small schemes, do not verify commutability [1,12]. In addition, the use of native clinical samples is limited, particularly in case of multiparameter samples, because they do not contain all the elements required over a wide range of concentration [1,20].

A case of non-commutability was observed in the OELM scheme regarding the determination of Se in serum. The performance of one participating laboratory out of 62 suddenly dropped from satisfactory (mean z score = 0.244) to unsatisfactory (mean z score = 7.86) without any change in its analytical technique. This change was simultaneous to a matrix modification from human to bovine serum. This participant determined Se by ICP-MS using the isotope ^{82}Se without collision or reaction cell. The apparent high concentrations of ^{82}Se were caused by the presence of bromide, at higher concentrations in bovine than in human serum, resulting in the formation of the polyatomic species at the same mass, $^{81}Br^{1}H^{+}$.

According the guideline ISO/IEC 17043, providers of EQA should demonstrate both the homogeneity and the stability of EQA samples [7,8]. The testing protocols are flexible, as long as they are statistically sound. When data are not readily available, it is recommended, to carry out studies before distribution to participants as all laboratories must receive similar samples. Homogeneity must be evaluated after the samples have been packaged in their final form. Stability should be demonstrated from the date of production to the final date for reporting results. This include the transport from the provider to the participants [3,7,8]. If the sample preparation is similar for all EQA sample batches, it is not necessary to test all the samples. Similarly, for multiparameter samples, it is not necessary to test all the analytes. The key analyte is generally the most vulnerable to mixing, contamination, time and/or temperature. It is also possible to rotate among the proposed analytes. Finally, when the organizer has significant historical data confirming homogeneity, it is not necessary to continue to undertake homogeneity testing as long as the sample preparation remain similar and participants' results do not show any unexpected variability [7,8].

Most inorganic elements remain stable in biological fluids. However, urinary Hg is known to be unstable [24–26] whereas this element is stable in whole blood as suggested by the acceptable recovery of spike and the coefficient of variation (CV) between duplicate samples calculated according to the formula

$$CV_{duplicate\ samples} = 100\ (\frac{\sqrt{\sum_{1}^{n} \frac{\Delta^2}{2n}}}{m}) \quad (1)$$

where Δ is the difference between duplicate sample results measured in different challenges, n the number of duplicates and m the mean of all the results. Indeed, according to the last five OELM EQA cycles, spike recovery varied from 92 to 96% and $CV_{duplicate\ samples}$ from 7.8 to 9.2% in whole blood. On the contrary, in urine, a poor recovery of spike and rather high $CV_{duplicate\ samples}$ were noted. Spike recovery varied from 62.8 to 74.2% and $CV_{duplicate\ samples}$ from 14.7 to 18.6%. The stability was evaluated for 48 h at room temperature in order to mimic the sample transport and a huge decrease in Hg was noted in the two samples tested. The Hg loss was 39% for sample containing 0.31 µmol/l of Hg and 46% for sample at 0.73 µmol/l of Hg. As corrective action, two different stabilizers (nitric acid 1% and a mixture of nitric acid 1%, Triton X100 0.1% and sulfamic acid 0.2%) used by other EQAs were tested but no improvement was observed. As recommended by the standard [7], participating laboratories were informed and instructed to perform determinations of Hg in urine within four hours after thawing. However, still no improvement was seen. Some trace element EQAs use lyophilized samples, but this preparation carries the risk of commutability loss for other inorganic elements. Another solution may be to ship urine in dry ice but it is expensive and therefore necessitates the agreement of participants. Finally, as not all the participating laboratories determine Hg in urine, it may be useful to prepare samples dedicated to the determination of urinary Hg that could be shipped in dry-ice to the limited number of participants requesting them or, otherwise, stabilised specifically (lyophilisation, addition of chemicals), without affecting the commutability of the EQA samples for the determinations of other elements.

The concentrations of the samples should cover the range of values observed in patients. EQAs generally use native samples from donors. Therefore, inorganic element concentrations are mostly within the normal range. In order to simulate values observed in exposed subjects, native samples are spiked and then mixed [1,3]. However, the chemical species in the spikes usually do not match those naturally present in the sample, such as elements embedded into proteins and the isotopic signature may be different. In addition, in multielement samples such as in the OELM EQA, interferences may occur. So, there is a risk of losing commutability. For obtaining deficient values of essential inorganic elements, urgently needed by clinical medicine laboratories, it is necessary to remove them using treatments like adsorption or chelation and consequently there is a huge risk of losing commutability [1]. Therefore, currently, in the OELM EQA, reaching concentrations below the normal ranges for essential trace elements remains an unsolved problem.

4. Quality specification determination

One of the major issues for EQA organizers is the choice of quality specifications (QS), in other word the limits around the assigned value used for classifying laboratory results as satisfactory or unsatisfactory [2,7,10,11]. It also defines the standard deviation for proficiency assessment (SD_{PA}), determined as 0.5 QS. SD_{PA} is used to calculate participants' performance in term of z score and the acceptable standard uncertainty of the assigned value (u_x) determined as 0.3 SD_{PA}. QS

depends on the test and the scope of the EQA. Educational EQAs may propose more stringent QS than those with a regulatory scope [3,4,12]. Using fixed QS is highly recommended as it allows the evaluation of laboratory performances on similar bases over time and in different rounds [3,27]. However, the QS should be appropriate to support clinical decisions and modifying patient management [11,28].

Ideally, QS should be based on the interpretation of the results by physicians making the results clinically acceptable and reliable for clinical decisions and patient management. It is the outcome-based specification approach. However, this approach is not applicable for all tests, partly because very few clinical decisions are based on only one test result. The test result must be determinant for patient management and clinical decision, based on well characterized, standardized and accepted medical strategies. In addition, it necessitates to define cut-off values and key differences that generate a modification in the patient follow up or treatment with a limited risk of errors [1,11,12,28]. In the inorganic element field, it may be applied to the determination of Se in plasma. The normal range, defined as the optimal activities of glutathione peroxidases and selenoprotein P has been reported to be between 1.00 and 1.52 µmol/l [29,30]. The cut-off for sub-deficient level, which corresponds to non-optimal activity of desiodases has been reported to be between 0.76 and 0.82 µmol/l [29,30]. The cut-off for severe deficient level which corresponds to concentrations observed in Keshan disease has been reported to be between 0.25 and 0.50 µmol/l [29,30]. In contrast, the cut-off for side effects has been defined between 1.55 and 2.03 µmol/l and corresponds to an increased risk of diabetes and high blood pressure [30,31] whereas signs of Se toxicity such as hair loss, brittle, thickened and stratified nails, garlic breath and dermatitis appear at concentrations between 2.28 and 3.17 µmol/l [30,32]. The outcome-based approach has been documented for HbA1c [11,33]. The authors used the cut offs corresponding to poor (64 mmol/mol) and good (53 mmol/mol) glycemic control. Then, they estimated that, to properly classify an individual with an HbA1c value of 58.5 mmol/mol, the measurement error should not exceed ± 5.5 mmol/mol. Therefore, applying this strategy [11,33] to the smallest difference between the intervals of Se concentrations indicated above, the QSs vary from 1.6% to 21% (Table 2). The smallest QS corresponds to the gap between side effects and normal range whereas the highest corresponds to the gap between severe deficiency and sub-deficiency. A QS of 10% could be used for classifying the subjects between sub-deficient and normal and a QS of 5.8% could be used for classifying the patients as being at risk of side effects or toxicity. The mean CV% obtained in the OELM EQA for the last cycle (April 2018 – March 2019) that included 78 participating laboratories was 10%. Therefore, it is clear that QSs of 1.6% and 5.8% are not currently attainable.

Another approach is based on individual variabilities. This approach necessitates a steady state status when people are healthy. For urinary tests for which the concentrations vary to maintain the plasmatic concentration stable, this approach is not applicable [11]. The desirable QS, calculated according the following formula, should be:

$$<0.25\sqrt{CV_{intra}^2 + CV_{inter}^2} + z\,(0.5\,CV_{intra}) \quad (2)$$

where CV_{intra} is the intra individual variability CV, CV_{inter} is the inter individual variability CV, and z = 1.65 for a 95% probability level. Adjustment can be made to take into account the analytical performances and three levels have been defined by the authors [34,35]: minimal, that is just sufficient for the test to be meaningful (when analytical precision is large), desirable, which corresponds theoretically to the best fitness for clinical purpose and optimal when analytical precision is good. Formulas for minimal and optimal levels are respectively:

$$\text{Minimal QS: } < 0.375\sqrt{CV^2{}_{intra} + CV^2{}_{inter}} + z\,(0.75\,CV_{intra}) \quad (3)$$

and

$$\text{Optimal QS: } < 0.125\sqrt{CV^2{}_{intra} + CV^2{}_{inter}} + z\,(0.25\,CV_{intra}) \quad (4)$$

Information on individual variability is obtained in literature reports which represents a serious limitation [11].

Regarding trace elements, only few papers have reported biological variability and, when different studies have been published, the results might be discrepant, as reported for zinc in plasma or serum. Depending on the study design, desirable QSs extended from 5.9 to 11% [36–39]. Data were more consistent for Se in serum or plasma (Table 2). Desirable QSs varied between 6.3 and 7.9% [39,40]. These differences can be explained by the study designs and particularly, the time span between the sample collections which may varied from hours to years as well as the number of time points per subjects and the characteristics of studied population: number of individuals, age, gender, geographical area, nutrition, healthy status and statistical treatment of individual results…. [41,42]. Consequently, studies with information about biological variation have to be critically appraised before they can be used to set QS. A check list has been proposed by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) to verify that studies deliver appropriate estimate of biological variability [42]. In addition, the population studied in these reports did not generally include volunteers with deficient or toxic values. Consequently, the relationship between concentrations and precision, illustrated on Fig. 2 for Se in serum, is not taken into account. In the deficient range of essential trace elements, the CV increased rapidly. Therefore, EQA organizers may add a fixed unit interval as QS in the deficient range of concentrations [1,3,43,44].

Another approach mixes normal range and analytical performances. The team of Haeckel [45] has updated this approach and proposed formulas according to the distribution of reference values.

$$QS = 4.68\sqrt{(CV_e\,(or\ CV_{eLn}) - 0.25)} \quad (5)$$

with

$$CV_e = [(\text{Upper range} - \text{Lower range})/3.92] \text{ x } (100 / \text{mean}) \quad (6)$$

when the distribution is normal, and

$$CV_{eLn} = 100\sqrt{(\exp([\text{Ln (Upper range)} - \text{Ln (Lower range)}]/3.92)^2 - 1)} \quad (7)$$

Table 2
Serum Se quality specifications according to different approaches.

Se,	Clinical	Biological variability[1]			Reference range		State of
µmol/l	Outcome	Minimal	Desirable	Optimal	Logarithm distribution	Normal distribution	the art[2]
0.63	21%	9.5	6.3	3.2	10%	15%	15%
0.91	10%	to	to	to			12%
1.54	1.6%	12%	8.0 %	4.0%			9.2%
2.16	5.8%						8.5%

[1] depending of published data on individual biological variability [39,40].
[2] corresponding to 2 SD_{PA}, SD_{PA} is calculated according to the equation 8. Conversion between molarity and mass fraction used 78.96 as Se atomic mass and 1.024 as density factor.

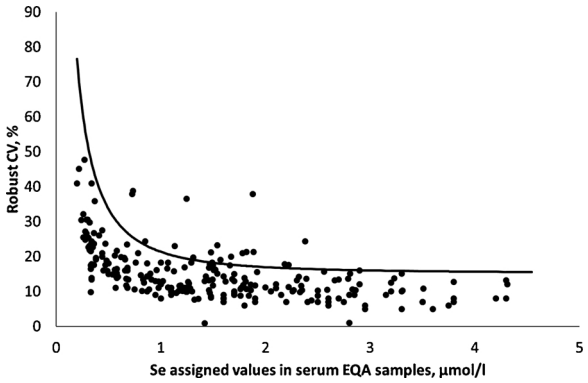

Fig. 2. Quality specifications (QS) based on External Quality Assessment scheme (EQA) performance [46]. Application to Se in serum.
Points represent EQA sample results from different EQA providers and the curve represents the QS. QS = 2 CV_{PA}, $CV_{PA} = \left(\frac{SD_{PA}}{C}\right)100$ where SD_{PA} is calculated according to the equation 8. Conversion between molarity and mass fraction used 78.96 as Se atomic mass and 1.024 as density factor.

in the other cases. Regarding Se in serum, the QS is equal to 15% using a normal distribution of the reference range and 10% when using logarithmic normal distribution (Table 2). As the previous approach, the low and high values are not taken into account.

The last approach is only based on analytical performances. The relationship between concentration and CV has been modelled by different authors [46]. The following formula

$$SD_{PA} = \sqrt{\alpha^2 + \beta^2 C^2} \quad (8)$$

where β represents the CV at high concentrations, α describes SD at concentrations close to the detection limit and C the assigned value expressed in g/g seemed the most convenient for biological tests [46]. Fig. 2 represents the QS calculated according to this formula for Se in serum. However, it necessitates that the analytical performances fit for clinical needs [1].

As demonstrated above and in Table 2 for Se in serum, the QS largely vary according to the approach used.

5. Assigned value determination

The determination of assigned value is also a major issue, as the performance of individual laboratory is estimated by comparing their results with the assigned values. According to ISO 13528, assigned values can be determined by different ways [3,8,10] and examples have been previously reported for Cu, Se and Zn in serum [3,8,10,47]. The most accurate is to measure the concentrations of the inorganic elements using methods of higher metrological order, such as isotope dilution mass spectrometry (IDMS). However, it is very expensive and useful only if samples are commutable [1,3,12]. As stated previously, commutability is quite unpredictable, and EQA organizers generally use the robust consensus mean of participants obtained after exclusion of outlier values by different means such as Grubbs', Cochran's or Hampel's tests or algorithm A [8]. Algorithm A is currently the most employed. According to our experience [10,47], the assigned values for Cu, Se and Zn in serum were not significantly different when using IDMS, comparison with a certified reference material and consensus mean but the uncertainties were significantly larger when using consensus mean. However, there are some limitations to the use of consensus means. When the number of participants in each group is too small (n < 15) or when the variability of results is too high [1,3] as illustrated on Table 3 for zinc in serum. The assigned values were similar whatever the method group but the robust SDs, and therefore the uncertainties, were higher when the number of participants was small or/and for the groups using less reliable methods such as colorimetry or EAAS.

Table 3
Influence of method variability and number of participants on the accuracy of assigned value.

Robust mean ± Robust standard deviation	Zinc, µmol/l
• All (n = 130)	16.1 ± 1.23
• EAAS (n = 5)	16.1 ± 1.53
• FAAS (n = 50)	16.1 ± 1.17
• ICP-AES/OES (n = 4)	16.6 ± 2.22
• ICP-MS (n = 49)	16.1 ± 1.00
• Colorimetry (n = 21)	16.3 ± 1.57

EAAS: Electrothermal atomic absorption spectrometry.
FAAS: Flame atomic absorption spectrometry.
ICP-AES/OES: Inductively coupled plasma atomic / optical emission spectrometry.
ICP-MS: Inductively coupled plasma coupled to mass spectrometry.

6. Reporting concentrations outside the linear range

Another problem encountered by EQA organizers is the reporting of results for concentrations outside the linear range of the method. Indeed, statistics for determining assigned values are based on measured numerical values. Therefore, results outside the linear range of the method cannot be introduced properly whatever the solution used. When a concentration is higher than the linear range, the participant either does not submit its result or uses a higher dilution that may change the matrix. Therefore, the participant performance may be erroneous. There are many possibilities to submit concentration lower than the detection (LOD) or quantification (LOQ) limits such as zero, LOQ, LOQ/2, LOQ/$\sqrt{2}$, LOD, LOD/2, LOD/$\sqrt{2}$, (LOD + LOQ)/2 or not reported. Therefore, when a great number of participant results are lower than the LOD, the assigned value is at least not reliable or even impossible to evaluate. This was observed in the OELM EQA for Li determination in serum with a native sample. In the OELM EQA values lower than LOD are currently reported as zero. The analytical methods used by the participating laboratories for Li were colorimetry, FAAS and ICP-MS but none of them was sensitive enough to detect physiological level of Li. As a result, no value could be assigned. Li is mainly determined in patients under Li therapy which may explain the lack of sensitivity of the ICP-MS methods used by the participants. Consequently, in the following cycles all OELM serum samples were spiked with Li.

7. Interpretation of the results

As stated in EQA description paragraph, the organizer delivers a report to each participant at the end of each run. Most of EQA organizers estimate analytical performance by the determination of z score which is the distance from laboratory results to the assigned value divided by the SD_{PA}.

$$Z\ \text{score} = (\text{Laboratory result} - \text{Assigned value})\ /SD_{PA} \quad (9)$$

In cases where the uncertainty of the assigned value is not negligible (that is $u_x > 0.3\ SD_{PA}$), the organizer can take this into account using the z' score which introduces u_x.

$$Z'\ \text{score} = (\text{Laboratory result} - \text{Assigned value})\ /\sqrt{SD_{PA}^2 + u_x^2} \quad (10)$$

Using z score or z' score necessitates a normal distribution of results. A z score (or z' score) within the range - 2 ≤ z score ≤ 2 indicates that the laboratory result is satisfactory and within the 95% range of the distribution of all results. Results with a z score (or z' score) < - 3 or > 3 can be identified as unsatisfactory and correspond to an action signal, while results with a z score (or z'-score) between - 3 and -2 or 2 and 3 are questionable and correspond to a warning signal. This means

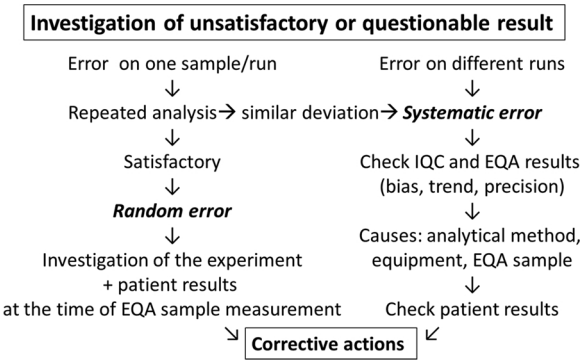

Fig. 3. Decision-making flow chart in case of questionable or unsatisfactory performance.
IQC: internal quality control.
EQA: external quality assessment scheme.

the laboratory should investigate whether there is a reason why the results tend to become an outlier [3]. According to ISO 15189, an accredited laboratory must investigate each unsatisfactory or questionable result and implement corrective actions, if needed [1,3,5]. A good procedure is to store the EQA samples under stable conditions until the report is received. A reanalysis of the sample may identify and eliminate many sources of error (Fig. 3). If the new result agrees with the assigned value within the QS, it is still necessary to review all the aspects of the analytical session leading to the erroneous result and all patient results produced at the time the EQA sample was measured. If the new result still deviates significantly after retesting, it indicates a systematic error. In case of systematic error, it is wise to check previous internal quality control (IQC) and EQA results to look for bias or trend or variability. A systematic error may have different sources related to materials, equipment, or technical environment (Table 4). It is important to make sure that the patient results were correct at the time the EQA samples were measured [1,3]. The potential cause should be investigated thoroughly, and appropriate actions should be undertaken and evaluated. The most common random errors are clerical errors as the process for reporting EQA results is different from reporting patient results. The participant should carefully review the process of result recording and take appropriate action to avoid this problem in future surveys. A double check of the result is highly recommended as for patient results [1,3,4,6]. Errors can be due to failure to follow the sample preparation and measurement procedures, to inappropriate reagents, standards or IQCs, to metrological problems with pipettes or oven, to the equipment or to the EQA samples (Table 4) [1,3,4,6]. If the problem is caused by external factors such as inadequate batch of reagent, standard stock solution or IQCs, the distributor or manufacturer must be contacted [1,3]. When the error is due to metrological problem, such as pipette or freezer malfunction, these materials must be checked, calibrated or changed and the metrological procedure carefully reviewed. Similarly, the temperature and hygrometry of the technical room must be carefully followed and adapted. Concerning IQCs, it may be useful to check the frequency of IQC testing and the limits of tolerance used [4]. The intra-laboratory CV should be largely lower than 0.5 EQA QS. For 4% of questionable or unsatisfactory EQA results, the participant did not reach any conclusion. In this case, it is recommended to carefully verify the later EQA challenges [3].

Many EQA organizer send a report at the end of each cycle which include the results of enough samples to evaluate bias and precision. Participating laboratory must carefully review these reports. Indeed,

Table 4
Causes of unsatisfactory or questionable performances.

	Random error	Systematic error
Technical Clerical	Transcription error Result in a wrong unit Result for a wrong sample Result for a wrong element Misplaced decimal point Result associated to a wrong method Result not properly saved or non-communicated	
Technical Procedure	Incorrect EQA sample storage Instruction to participant not followed properly Incorrect preparation of sample (including reconstitution of the sample), standards, reagent, internal quality controls Mislabelled test tube Use of expired reagent, standards, internal quality control Incorrect pipetting Calculation error Incorrect internal quality control result	Inappropriate EQA sample storage
Materials		Freezer or refrigerator malfunction Pipette malfunction Inappropriate batch of reagents, standard stock solution, internal quality controls EQA samples (non-commutable, small number of participants, non-homogeneous group of peers)
Method	Unidentified memory effect	Lack of sensibility Interference Internal quality control limits too wide Inadequate number of internal quality control Internal quality control not at relevant concentrations
Equipment	Instrument error misinterpreted Insufficient aspiration or partial obstruction Wrong settings Unidentified malfunction or software error Incorrect maintenance Water supply problem Gas supply problem	Equipment malfunction Software error Inappropriate maintenance
Technical Room	Inadequate room temperature or hygrometry	Inadequate or not followed room temperature or hygrometry

EQA: External Quality Assessment scheme.

even if all the results have been satisfactory during the cycle, a small positive or negative constant deviation suggests a problem of calibration, or of contamination (in case of positive bias), and should be investigated. In addition, when the deviations in EQA results are variable in magnitude and direction, it necessitates to review whether the deviation is caused by inappropriate EQA sample material (non-commutable) and/or the method itself. If the assigned value is calculated from a small number of results, the assigned value may be less reliable. These results must be interpreted carefully [3].

The participant must regularly evaluate the measurement uncertainty of its different tests [5]. The measurement uncertainty gives information about the quality of the measurement, is useful for comparing results obtained by several laboratories or methods, it helps in the interpretation of results, especially those close to critical values [27,48]. The evaluation of measurement uncertainty can be done via different approaches [49–51], based on the principles described in the Guide to the expression of the Uncertainty Measurement (GUM) [52] but all the calculations have advantages and limitations [16,53]. Laboratories generally used the results of IQCs coupled to standards or certified reference materials (CRM) or EQA samples. On the one hand, contributions related to analytical precision may be estimated as repeatability, or intra-laboratory reproducibility, thus leading to different estimates. On the other hand, contributions related to bias, estimated via CRMs or EQA samples or standards, may in turn suffer from limited concentration interval (CRMs or standards) or large uncertainties associated with assigned values (EQA samples). In addition, the matrix of IQCs, standards, CRMs and EQA samples may be different. Recently, some EQA organizers have proposed to evaluate measurement uncertainty of participants' results using data reported over a period of time in their periodic or long-term reports [16,17,48]. The Nordtest method [50] includes the laboratory imprecision calculated from duplicate sample results, the bias estimate variability and the uncertainty of the assigned values, using data collected over a period of time and covering the working range of the method. Another approach is the long-term evaluation of the measurement uncertainty [16,54] based on the linear regression between data obtained by the participant and the assigned values, provided that the EQA values fit a normal distribution and cover a large range of concentrations. The method evaluates both random error which is defined as the dispersion of results around regression line and systematic errors defined as the deviation of the regression line from the identity line both in term of slope and intercept. These approaches are very useful. However, these estimates of measurement uncertainty would be dramatically affected if an unsatisfactory result has been submitted.

8. Interaction between organizer and participant

The organizer must also reply to the questions and claims of the participants and send regularly satisfaction questionnaires in order to improve its EQA. Inappropriate shipment (i.e. delay, damage of the package) may affect the stability of the samples. It is important to check the appearance of sample quality and physical integrity at reception as well as to verify the sample labelling. If something is wrong, the EQA provider must be contacted for the replacement of the sample(s) [3]. Regarding shipment delay, this may be caused by wrong or insufficient address details or wrong distribution within the hospital or institution, or problem with delivery service, the EQA provider should be informed and when it is due to the delivery service, another way of delivery of the samples should be used. Problems of homogeneity, stability, instructions, leakage, error in sample labelling, error in presentation of results necessitate a note from the EQA provider [3].

9. Conclusion

In conclusion EQA represents an indispensable tool for monitoring laboratory performance and more generally for laboratory quality assurance management. But this statement is true only if samples are commutable, assigned values are accurate and quality specifications fit for clinical needs.

A strong relationship between providers of similar EQA samples is highly recommended for improving the standardization and harmonization of laboratory performance evaluation as well as for sharing samples and verify their commutability [1,4,10–12].

A strong relationship between provider and participants also allows to improve EQA by firstly the means of queries and claims that generate corrective actions and secondly satisfaction questionnaires that help to improve the EQA for example by addition of new elements or biological matrix, by identifying non commutability by comparison of the results obtained by different analytical methods, by adjusting QS, and improving information on the reports.

The regular exchanges with the members of the network "Organizers of external quality assessment / proficiency testing schemes related to occupational and environmental medicine", and the participating laboratories are contributing to the continuous improvement of OELM EQA scheme. However, problems remain unsolved such as urinary mercury stability, samples with low essential trace element concentrations and report of lower than the LOD or LOQ concentrations.

Funding sources

This review did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The authors are either employees from public institutions (J Arnaud, I De Graaf, MB Fofou-Caillierez, M González-Estecha, M Patriarca, V Patriarca, M Ropert-Bouchet, L Schröer-Janssen, C Siebelder, M Te Winkel, C Weykamp) or from non-governmental non-profit associations (MC González Gómez, M Ventura Alemany). They do not receive any financial support linked to the OELM scheme. The participating laboratory annual fees are managed by a public institution in Italy: Istituto Superiore di Sanita (ISS) or non-governmental non-profit associations in the other countries: Societé Francaise de Toxicologie Analytique (SFTA) for France, Stichting Kwaliteits Bewaking Medische Laboratoria (SKML) for the Netherlands and Sociedad Española de Medicina de Laboratorio (SEQC^{ML}) for Spain. The examples that illustrate this review are based on objective analyses of OELM data or on past published research reports.

Declaration of Competing Interest

The authors are either employees from public institutions (J Arnaud, I De Graaf, MB Fofou-Caillierez, M González-Estecha, M Patriarca, V Patriarca, M Ropert-Bouchet, L Schröer-Janssen, C Siebelder, M Te Winkel, C Weykamp) or from non-governmental non-profit associations (MC González Gómez, M Ventura Alemany). They do not receive any financial support linked to the OELM scheme. The participating laboratory annual fees are managed by a public institution in Italy: Istituto Superiore di Sanita (ISS) or non-governmental non-profit associations in the other countries: Societé Francaise de Toxicologie Analytique (SFTA) for France, Stichting Kwaliteits Bewaking Medische Laboratoria (SKML) for the Netherlands and Sociedad Española de Medicina de Laboratorio (SEQC^{ML}) for Spain. The examples that illustrate this review are based on objective analyses of OELM data or on past published research reports.

Acknowledgements

We are very grateful to the present and past members of the OELM board as well as to the present and past participants of the OELM EQA and of the "Organizers of external quality assessment / proficiency testing schemes related to occupational and environmental medicine" Network.

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