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Quality Specifications for the Determination of Copper, Zinc, and Selenium in Human Serum or Plasma: Evaluation of an Approach Based on Biological and Analytical Variation

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BACKGROUND: Trace element external quality assessment schemes monitor laboratory performance and provide a stimulus for improvement in accuracy. However, monitoring of participant performance varies according to the scheme and can lead to conflicting conclusions.

METHODS: Quality specifications based on biological intra- and interindividual variability were calculated and compared to those currently used by various trace element external quality assessment schemes for plasma or serum copper, zinc, and selenium concentrations. For this purpose, we evaluated results reported by participating laboratories in different schemes, at key concentrations, using *z* scores.

RESULTS: Minimal quality specifications developed from the biological intra- and interindividual variability were, for Cu, ±0.84 μmol/L or 12% of the assigned target concentration, whichever is greater; for Zn, ±1.20 μmol/L or 15% of the assigned target concentration, whichever is greater; and for Se, ±0.072 μmol/L or 12% of the assigned target concentration, whichever is greater. Reported performance of the participating laboratories depended on analyte, concentration, and the selected quality specification. In addition, the most commonly used methods for the determination of Cu, Zn, and Se may give different results.

CONCLUSIONS: The proposed minimal quality specifications based on biological variation are generally slightly less stringent than those currently in use, although they do not drastically change the performance evaluation in the different schemes. These specifications are a first step in the harmonization of practices among the schemes and remain to be evaluated.

The trace elements copper (Cu), zinc (Zn), and selenium (Se) are essential for life but can also be toxic in humans *(1)*. As components of proteins, enzymes, and hormones, they regulate biological functions, particularly redox balance and inflammatory and immune responses. Pathological disorders such as infections, cancers, and cardiovascular and neurological diseases may be seen when their homeostasis is modified *(2–9)*, and interactions between essential and toxic minerals may have some role in determining the susceptibility of individuals to disease *(10)*. Genetic disorders of Cu and Zn transport are known to have profound clinical consequences *(5, 11)*.

Serum concentrations of Cu, Zn, or Se provide useful information in the clinical categorization of deficiency and toxicity states. Serum selenium values <0.25 μmol/L, reported in China (Keshan province), correspond to severe selenium deficiency. Concentrations around 1.25 μmol/L provide for optimal glutathione peroxidase activity, whereas concentrations >1.50 μmol/L afford protection against at least some cancers *(9, 12)*. Toxic values (>4 μmol/L) have been

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found in Venezuela and China *(13)*. Serum zinc values <6 μmol/L are associated with severe zinc deficiencies, whereas zinc concentrations generally fall within the range 12–17 μmol/L in healthy adults and values >25 μmol/L are infrequent in clinical practice even after long-term zinc supplementation *(5, 6, 14–16)*. Serum copper concentrations fall within the range 13–22 μmol/L in healthy adults and increase up to 40 μmol/L in inflammation *(14, 15)*. Therefore, accurate determinations in plasma or serum are crucial for the diagnosis and follow-up of patients suffering from various diseases *(5–9, 11, 17, 18)*.

Imprecision, bias, and uncertainty in the measured concentrations of serum Cu, Zn, or Se are related to the analytical methods and more generally to the expertise of individual laboratories. The most commonly reported methods for Cu and Zn determination are colorimetry, flame atomic absorption spectrometry (FAAS),[11] inductively coupled plasma atomic/optical emission spectrometry (ICP-AES/OES), electrothermal atomic absorption spectrometry (ETAAS), and inductively coupled plasma–mass spectrometry (ICP-MS), whereas for Se, ETAAS and ICP-MS are the most commonly used methods.

Trace element external quality assessment schemes (TE-EQASs) are used in addition to internal quality control as tools for monitoring laboratory performance. However, preparation of samples, monitoring of participant performance, and the associated assessment of competence, vary between the TE-EQASs *(19–21)*, a problem that can lead to conflicting conclusions.

The aims of the present work were to use European and North American TE-EQAS results to

- evaluate analytical quality specifications (QSs) based on human biological intra- and interindividual variability from published data for monitoring the performance of laboratories measuring Cu, Zn, and Se in serum or plasma;
- compare these QSs with those currently used by existing quality assessment schemes; and
- use the proposed QS to evaluate the reliability of the most commonly used methods.

[11] Nonstandard abbreviations: FAAS, flame atomic absorption spectrometry; ICP-AES/OES, inductively coupled plasma atomic/optical emission spectrometry; ETAAS, electrothermal atomic absorption spectrometry; ICP-MS, inductively coupled plasma–mass spectrometry; TE-EQAS, trace element external quality assessment scheme; QS, quality specification; BE, Belgium; CA, Canada; FR, France; DE, Germany; IT, Italy; NL, the Netherlands; x_i, individual value or participant value; X, target value; Abs, absolute value.

Materials and Methods

As a database for this study, we used TE-EQAS results from Belgium (BE), Canada (CA), France (FR), Germany (DE), Italy (IT), the Netherlands (NL), New York (NY), the United Kingdom (UK) (see Data). Data handling varies considerably among these schemes. Fixed, concentration-related QSs are used in most schemes to demonstrate acceptable results as shown in Fig. 1. Schemes from DE and NL apply limits that are associated with each test sample—either the calculated uncertainty from the reference laboratory (DE) or the consensus CV obtained by the participants after exclusion of outliers—and, therefore, cannot be extrapolated to other samples or other concentrations. Further differences regarding these TE-EQASs, including material collection (serum or plasma, number and selection of donors, collection vials), preparation, range of concentration, number of mailings per year, number of samples per mailing, exclusion of outliers, and target value assignment have been published elsewhere *(21–23)* and are beyond the scope of this study.

CALCULATION OF THE PROPOSED QUALITY SPECIFICATIONS

We defined QSs for each of the 3 assays as proposed by Fraser *(24)*, using the few studies that reported human biological intra- and interindividual variability of serum or plasma Cu, Zn, and Se (Fig. 2). We used the average data to calculate optimal, desirable, and minimal QS *(19, 24)*.

The QSs were applied to the following formula to calculate *z* scores:

$$z = (x_i - X)/(\mathrm{QS}/2),$$

where x_i refers to the participant's result and X to the target value. The QS was divided by 2 to be consistent with the International Organization for Standardization (ISO) guide, which states that performance is satisfactory when the absolute value (Abs) of the *z* score is <2 *(25)*.

Our previous work with assays for aluminum in serum and lead in whole blood showed that the QSs need to be modified at low concentrations, when analytical imprecision increases rapidly *(19, 24)*. The threshold concentrations, at which the modification should be introduced, were found by further analysis of those data sets with low concentrations of Cu (<10 μmol/L), Se (<0.75 μmol/L) *(9, 26)*, or Zn (10.7 μmol/L) *(27)*. For each of these TE-EQAS samples, we calculated *z* scores for all of the reported results in 2 ways, by using *a*) the minimal QS and *b*) the minimal QS found at the concentrations used to indicate a low value (i.e., 1.2 μmol/L at 10 μmol/L for Cu, 0.09 μmol/L at 0.75 μmol/L for Se, and 1.6 μmol/L at 10.7 μmol/L for Zn).

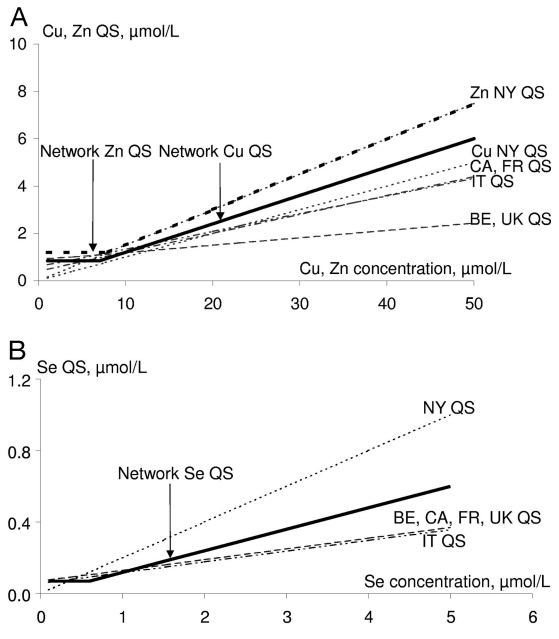

Fig. 1. QSs used by trace element external quality assessment schemes and the proposed minimal QS for Cu, Zn, and Se.

(A), QSs used by trace element external quality assessment schemes and the proposed minimal QS for Cu and Zn. *x* Axis, target concentrations in μmol/L; *y* axis, QS in μmol/L. For BE and UK schemes, QS = 0.03*x* + 0.9. For CA and FR schemes, QS = 0.08*x* + 0.4. For IT scheme, QS = 0.075*x* + 0.6. For NY scheme, Cu QS = 0.10*x* and Zn QS = 0.15*x*. QSs proposed by the network are for Cu, QS = 0.12*x* if $x > 7$ μmol/L, and for Zn, QS = 0.15*x* if $x > 8$ μmol/L. (B), QSs used by trace element external quality assessment schemes and the proposed minimal QS for Se. *x* Axis, target concentrations in μmol/L; *y* axis, QS in μmol/L. For BE, CA, FR, and UK schemes, QS = 0.06*x* + 0.07. For IT scheme, QS = 0.06*x* + 0.06. For NY scheme, QS = 0.20*x*. Quality specifications proposed by the network, QS = 0.12*x* if $x > 0.6$ μmol/L.

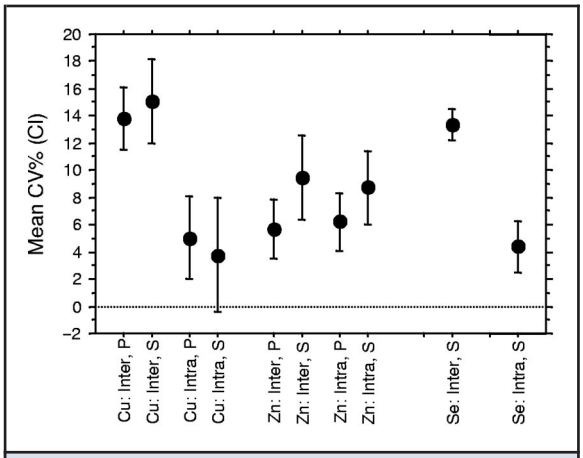

Fig. 2. Intra and interindividual biological variation of Cu, Zn, and Se in plasma *(36–39)*.

Results are expressed as the mean (points) and 95% CI (error bars) of the CV% reported in the literature *(36–39)*. Inter, interindividual variability; Intra, intraindividual variability; P, plasma; S, serum.

DATA

We used results reported by participants in each of the 8 schemes during a period of 3 consecutive years in this analysis. Participant values were obtained for plasma or serum samples with very low (around 3.5 μmol/L for Cu and 0.25 μmol/L for Se), low (9 μmol/L for Cu, 8 μmol/L for Zn, and 0.50 μmol/L for Se), median (15 and 20 μmol/L for Cu and Zn and 1.00 and 1.50 μmol/L for Se) and high (30 μmol/L for Cu and 3.00 μmol/L for Se) concentrations. We chose these concentrations to cover physiological and pathological ranges of values *(9, 12, 14–16, 26–28)*. For results associated with each set of target values, SD and CV were determined after elimination of outliers at ±3 SD. Participant values reported with qualifiers such as "less than" or "greater than" were also eliminated. These data sets were used to compare performance assessments determined from the QSs currently employed by the schemes and by the proposed QSs.

We also calculated *z* scores using 3 additional samples (1 native pool of human serum and 2 human serum pools spiked with known Cu, Zn, and Se concentrations), sent to all participants in each of the TE-EQAS for other purposes *(29)*, to provide comparisons of the analytical methods used. For this analysis, the analytical methods used by at least 15 participants were considered *(30)*.

STATISTICS

We used Kruskal–Wallis (or Mann–Whitney) test to compare *z* scores according to *a*) target concentrations and *b*) analytical methods, and Friedman tests and Spearman correlation coefficients for comparing *z* scores using the various QSs. We calculated simple linear regressions to evaluate the relationships between *a*) scheme SD and target values and *b*) scheme SD and QS.

Results

CALCULATION OF QS USING BIOLOGICAL VARIABILITY

Optimal, desirable, and minimal QSs for total error according to the Fraser formulae *(19, 24)* are shown in Table 1.

With decreasing target concentrations, the percentages of *z* scores with absolute values, abs(*z* scores) >2 found using the 2 calculations described above, remained similar down to threshold concentrations of 7 μmol/L for Cu, 8 μmol/L for Zn, and 0.6 μmol/L for Se. Below these thresholds a fixed concentration was taken for the QS. Therefore, the proposed minimal QSs are

- Cu in serum ±0.84 μmol/L or ±12%, whichever is greater;
- Zn in serum ±1.20 μmol/L or ±15%, whichever is greater; and
- Se in serum ±0.072 μmol/L or ±12%, whichever is greater.

COMPARISON OF EACH QS CURRENTLY USED BY THE VARIOUS TE-EQASs AND THE PROPOSED QS

The acceptable limits currently used for assessing laboratory performance in the various TE-EQASs are indicated in Fig. 1. Fig. 1 shows that BE and UK use the tightest tolerances for Cu and Zn at normal and high concentrations, whereas NY and the proposed minimal QS give the greatest tolerances. At the threshold values, most of the QSs are very similar for Cu and Zn. For Se, limits used by the NY TE-EQAS are greater than the minimal QS proposed herein, and the latter are greater than those used by the BE, CA, FR, IT, and UK TE-EQASs.

COMPARISON OF LABORATORY PERFORMANCE AND EACH TE-EQAS QS

A comparison analysis of target values and their SDs for all samples distributed for the various TE-EQASs during 3 consecutive years indicated that imprecision was significantly related to target concentrations for the 3 elements. Correlation coefficients were statistically significant for the 3 elements ($r > 0.56$, n = 279, $P < 0.0001$). The linear equations between SD and target values (X) were

- for Cu, SD = $0.06X + 0.49$;
- for Zn, SD = $0.06X + 0.87$; and
- for Se, SD = $0.09X + 0.07$.

We compared these equations relating SD to target values with those defining the relationships between QS and trace element concentration (Fig. 1). For Se, the observed slope was higher than those calculated with the QSs of the IT, BE, CA, FR, and UK schemes and lower than those proposed by NY and by the Network. The ordinate depicting the linear relationship between SDs and target values was similar to those proposed by BE, CA, FR, IT and UK TE-EQAS as well as the fixed limit proposed by the network "Organisers of External Quality Assessment/Proficiency Testing Schemes Related to Occupational and Environmental Medicine." For Zn and Cu, the slopes of the lines defining the relationship between SD and target value were higher than those of the BE and UK TE-EQASs but lower than those of other schemes. For Zn, the ordinate of this line was similar to those of the BE and UK TE-EQASs, and for Cu, the ordinate was similar to those of the CA and FR TE-EQASs.

Table 1. Quality specifications calculated according to Fraser formula *(24)* for the determination of serum or plasma Cu, Zn, and Se based on human biological variation.[a]

	Cu	Zn	Se
Minimal, %	12	15	12
Desirable, %	8	10	8
Optimal, %	4	5	4

[a] Desirable total error % = $0.25(CV^2_{intra} + CV^2_{inter})^{1/2} + z(0.5CV_{intra})$, where CV_{intra} = intraindividual variability, CV_{inter} = interindividual variability, and $z = 1.65$ for a 95% probability level. Optimal = 0.5 desirable; minimal = 1.5 desirable.

We also calculated the relationships between SD value and target concentration for the reference laboratories, although the number of samples was limited to the 25 samples from the DE and NY TE-EQASs. The equations were

- Cu, SD = $0.05X - 0.11$ ($r = 0.80$, n = 25, $P < 0.0001$);
- Zn, SD = $0.065X - 0.13$ ($r = 0.91$, n = 25, $P < 0.0001$); and
- Se, SD = $0.045X + 0.03$ ($r = 0.80$, n = 25, $P < 0.0001$).

For Se and to a lesser extent for Cu, the slopes of these relationships were found to be lower in the reference laboratories than the participant laboratories, but this was not the case for Zn. Although various methods were used across these laboratories, including FAAS, ICP-AES/OES, ETAAS, and ICP-MS, the differences between the reference laboratories and participant laboratories were not related to analytical method. In addition, the ordinates of the lines depicting the relationships between SDs and target values for the reference laboratories were lower than those obtained with the participant laboratories.

INDIVIDUAL PERFORMANCES EVALUATED USING EACH QS

Each of the participants' results was used to determine a *z* score according to the QS associated with each dif-

Table 2. Cu, Zn, and Se *z* scores (10th/50th/90th percentiles)[a] according to sample concentrations and quality specifications.[b]

Sample concentration, μmol/L	Number of participants	TE EQAS				Proposed minimal QS
		BE-UK	CA-FR	IT	NY	
Cu						
3.5	82	−2.60/−0.42/2.91	−3.85/−0.62/4.32	−3.03/−0.49/3.41	−7.46/−1.20/8.38	−3.21/−0.50/3.50
9.0	128	−1.99/−0.01/1.85	−2.07/−0.01/1.95	−1.83/−0.01/1.71	−2.55/−0.01/2.41	−2.13/−0.01/2.01
15.0	184	−2.19/0.04/1.86	−1.81/0.04/1.57	−1.70/0.03/1.46	−1.89/−0.04/1.66	−1.58/0.03/1.38
20.0	239	−3.02/0.18/2.88	−2.27/0.14/2.18	−2.17/0.13/2.08	−2.27/0.14/2.17	−1.90/0.12/1.81
30.0	168	−2.61/0.00/2.66	−1.69/0.00/1.73	−1.66/0.00/1.70	−1.57/0.00/1.62	−1.31/0.00/1.35
Zn						
8	144	−2.00/−0.03/3.95	−2.22/−0.04/4.38	−1.92/−0.03/3.79	−1.93/−0.03/3.71	−1.91/−0.03/3.71
15	156	−2.08/0.07/2.85	−1.78/0.06/2.43	−1.65/0.05/2.25	−1.29/0.04/1.72	−1.29/0.04/1.72
20	228	−4.38/0.07/4.23	−3.33/0.05/3.18	−3.17/0.05/3.03	−2.22/0.04/2.11	−2.22/0.04/2.11
Se						
0.25	69	−2.34/0.22/3.22		−2.85/0.27/3.92	−3.66/0.34/5.03	−2.94/0.28/4.06
0.50	101	−2.43/0.00/3.17		−2.91/0.00/3.81	−2.64/0.00/3.57	−3.44/0.00/4.44
1.00	95	−2.14/0.28/2.67		−2.49/0.32/3.09	−1.41/0.18/1.73	−2.36/0.30/2.88
1.50	143	−2.21/0.20/2.48		−2.53/0.23/2.83	−1.25/0.11/1.35	−2.08/0.18/2.25
3.00	97	−2.97/−0.31/3.33		−3.31/−0.35/3.71	−1.32/−0.14/1.48	−2.20/−0.23/2.47

[a] 50th percentile corresponds to the median.
[b] z Score $= (x_i - X)/(QS/2)$, where x_i refers to the participant's result and X to the target value.

ferent scheme (Fig. 1) and the proposed minimal QS. For each set of scores, those at the 10th, 50th, and 90th percentiles are recorded in Table 2 according to concentration and QS. Spearman correlation coefficients between z scores obtained using the various QSs (Cons/NY, Cons/IT, Cons/CA-FR, Cons/UK-BE, NY/IT, NY/CA-FR, NY/UK-BE, IT/CA-FR, IT/UK-BE, CA-FR/UK-BE) were highly significant ($r > 0.989$, n = 801, $P < 0.0001$ for Cu; $r > 0.991$, n = 528, $P < 0.0001$ for Zn; $r > 0.981$, n = 505, $P < 0.0001$ for Se). No statistically significant difference was observed in the average value of z scores according to target concentration as indicated by the Kruskal–Wallis test, regardless of what QS was used in performing this analysis ($P > 0.79$ for Zn, $P > 0.10$ for Cu, $P > 0.24$ for Se). In addition, the z score values obtained using the various QSs were similar as indicated by the Friedman test ($P = 0.12$ for Cu, $P = 0.96$ for Zn, $P = 0.41$ for Se). When this analysis was performed at each concentration, however, the NY QS was significantly more stringent than the others ($P < 0.0001$) (Table 2) at a very low Cu concentration (3.5 μmol/L), whereas at a Se concentration around 1.0 μmol/L, the NY QS was less stringent than the other QSs ($P = 0.0009$) (Table 2).

The frequencies of unacceptable results [Abs(z score) >2], according to QS and target concentration, are indicated in Table 3. Proposed minimal QSs for Cu and Zn based on human biological variability are less stringent than those used by BE and UK TE-EQASs, whereas those used by IT, NY, CA, and FR TE-EQASs lead to similar results when concentrations are within the reference range or higher. When the target concentrations were low, the QS used by NY TE-EQAS tended to be the most stringent for Cu, whereas for Zn the most stringent were those used by CA and FR TE-EQASs. Achieving acceptable performance was easier for Cu than for Zn. For Cu, 43% to 97% (mean 85%) of the participants were within the acceptable range [Abs(z score) <2] (Table 3) depending on concentration and QS, whereas for Zn, 54% to 87% (mean 80%) of the participants were within the acceptable range. For Se, BE, CA, FR, IT, and UK, QSs were similar and tended to be more stringent than the proposed minimal QS based on human biological variation, whereas the QS used by NY TE-EQAS was the least stringent except at low Se concentrations. Using the different QSs, 57% to 92% (mean 73%) of the participants were within the acceptable range [Abs(z score) <2] (Table 3).

Table 3. Frequency of unacceptable results [Abs(*z* score) >2], expressed as percentage, according to quality specifications and concentrations.[a]

Sample concentration, μmol/L	Number of participants	TE EQAS BE-UK	CA-FR	IT	NY	Proposed minimal QS
Cu						
3.5	82	23	36	27	57	28
9.0	128	19	19	17	26	21
15.0	184	21	14	12	15	12
20.0	239	33	23	22	23	17
30.0	168	36	14	11	11	3
Zn						
8.0	144	27	35	26	25	25
15.0	156	26	21	19	13	13
20.0	228	46	35	33	21.5	21.5
Se						
0.25	69	26	26	36	43	36
0.50	101	27	27	35	32	40
1.00	95	26	26	29	16	28
1.50	143	29	29	30	9	25
3.00	97	31	31	34	8	23

[a] z Score = $(x_i - X)/(QS/2)$, where x_i refers to the participant's result and X to the target value.

INFLUENCE OF METHOD ON THE LABORATORY PERFORMANCE AS ASSESSED USING THE PROPOSED MINIMAL QS

The most widely used analytical method for Cu and Zn was FAAS, and for Se, Zeeman background correction ETAAS (Fig. 3). However, the most popular method for Cu, Zn, and Se in the NY scheme was ICP-MS (41.7% for Cu; 43.7% for Zn; 36.8% for Se). In addition, for Se, 21.0% of the NY scheme's participants used dynamic reaction cell or collision cell ICP-MS. The analytical method significantly influenced Zn ($P = 0.05$), Cu ($P < 0.0001$), and Se ($P = 0.003$) z scores (Fig. 3). For Cu, ETAAS with background correction other than Zeeman effect exhibited the poorest performance, with 33% unacceptable z scores [Abs(z score) >2], whereas this percentage decreased to 8% with ICP-AES/OES and to 20% with the most commonly used FAAS method. For Zn, 11% of Abs(z score) values were >2 when FAAS, ICP-MS, or ICP-AES/OES were used. This percentage increased to 34% with colorimetric methods. For Se, the percentage of Abs(z score) values >2 was 20% with ICP-MS and 40% with ETAAS-Z.

Discussion

Reliable measurements are necessary to assist clinicians in the diagnosis of trace element deficiencies and excesses, as well as for monitoring patient follow-up. Participation in external quality assessment schemes or proficiency testing schemes allows laboratories to evaluate the quality of their results. It is also necessary to fulfill the requirements for accreditation *(31, 32)* and, in some jurisdictions, it may be mandated by law *(33, 34)*. Hitherto, definitions of acceptable performance largely have depended on the judgment of scheme organizers. Therefore, from the same set of results, the performance of a laboratory can be considered as adequate in one scheme and inadequate in another, as clearly demonstrated by our results. This observation is well known and has been previously reported *(19, 20)*. This conflict has occurred as the selection of QS can be a difficult task and should take into account both analytical performance and clinical needs *(35)*.

From the analytical point of view, the comparison of equations relating participant SD or reference laboratory SD to target values with those defining the relationships between the QS and trace element concentrations suggests that, for these 3 elements, the QSs in use by IT, BE, CA, FR, and UK schemes are too stringent, when the current interlaboratory analytical precision is taken into account. In addition, achieving acceptable performance was easier for Cu than for Zn, probably owing to the difficulties encountered for zinc determination, especially contamination, which is considered only by the NY QS.

For clinical purposes, the method proposed by Fraser *(24)* for the calculation of QS may afford a useful and objective way to assess laboratory performance. However, it suffers from 2 limitations. First, biological variability is rarely reported in pathological states. As test performance depends on concentration, QS derived exclusively from the healthy population could be insufficient. This is obvious at low concentrations *(19)*. Second, the intra- and interindividual biological variability of the analyte of interest must be evaluated with confidence. Reports dealing with intra- and interindividual variability show discrepant results *(36–39)*. Ricos et al. *(40)* evaluated the desirable QS for Cu, Zn, and Se and found 12%, 11%, and 14.5% in plasma and 8%, 13.5%, and 14% in serum, respectively. Taking into account more recent reports of inter- and intraindividual variability for Cu, Zn, and Se, the desirable QS obtained in the present work was evaluated at 8% for Cu and Se and 10% for Zn in both serum and plasma. Compared with the current typical analytical performance of laboratories, these desirable QS are unattainable by a large number of participants, especially for Se, but they are also unattainable by reference lab-

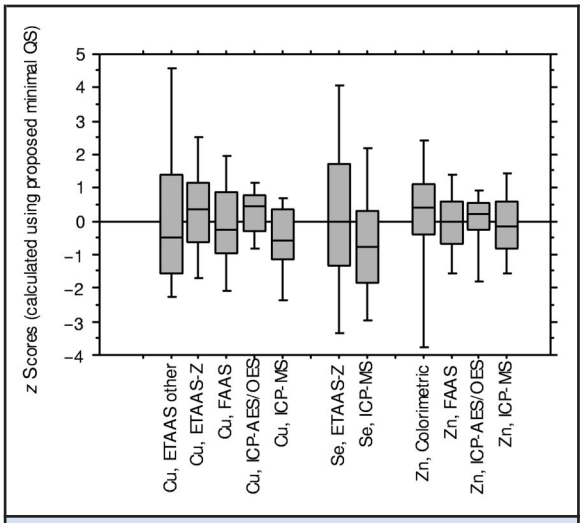

Fig. 3. Influence of the method used on Cu, Zn, and Se laboratory performances evaluated by the proposed minimal quality specification.

ETAAS with Zeeman (ETAAS-Z) (n = 36 for Cu and 70 for Se) or other background correction (ETAAS-other) (n = 17); FAAS (n = 125 for Cu and 155 for Zn); ICP-MS (n = 17 for Cu, 34 for Zn, and 37 for Se); ICP-AES/OES (n = 30 for Cu and 19 for Zn). Results are expressed as median (line within the box), 25th and 75th percentiles (bottom and top lines of the box), and 10th and 90th percentiles (bottom and top error bars).

oratories. Consequently, we proposed using the minimal QS, which is generally slightly less stringent than those currently in use although they do not drastically change the performance evaluation in the different schemes. Nevertheless, desirable QS could be used as an analytical goal. In addition to analytical and clinical needs, the majority of scheme participants must exhibit acceptable results, particularly in sectors where acceptable performance is required for laboratory licensing. At the same time, an objective of external quality assessment is to promote continuous improvement in participant performance, and therefore the QS must not be too large as to give an impression that all is well and not to offer a stimulus toward improvement. The comparison of the participant and reference laboratory precision indicates a huge gap for Se, confirming the expertise of the latter and consequently the possibility of performance improvement by the former. This statement was also true for Cu but to a lesser extent. Unexpectedly, it was not the case for Zn. These observations suggest a high level of expertise of the Cu and Zn TE-EQAS participants and consequently a weaker potential for performance improvement. Based on the results of our analysis, TE-EQAS organizers may implement slightly different QS for Cu, Zn, and Se in serum or plasma depending on local circumstances (regulatory requirements, participant expertise).

The analytical method used, or perhaps its different implementation by various laboratories, is a complicating factor for evaluating performance, as confirmed by our results. Better performances were observed with ICP-OES for Cu and ICP-MS for Se. TE-EQAS organizers are responsible for informing their participants on the limitations of an analytical method and for promoting recommendations for the improvement of analytical performance, as suggested by the International Union of Pure and Applied Chemistry (IUPAC) *(30)*.

Taken as a whole, the proposed minimal QS represent a first step for harmonization of practice among the schemes. The biological view suggests the use of the more stringent desirable QS as a goal standard, whereas the participant results argue for the use of the less-stringent minimal QS. Other issues of TE-EQAS must also urgently be harmonized, such as the determination of target values and elimination of outliers, but also the concentration ranges of delivered samples and the number of duplicate samples per year. It will be equally important to achieve more consistency among schemes in the methods used for the preparation and control (stability and homogeneity) of delivered samples, as well as the measures taken against poor performers.

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References

1. Taylor A. Detection and monitoring of disorders of essential trace elements. Ann Clin Biochem 1996;33:486–510.
2. Boosalis MG. The role of selenium in chronic disease. Nutr Clin Pract 2008;23:152–60.
3. Stern BR, Solioz M, Krewski D, Aggett P, Aw TC, Baker S, et al. Copper and human health: biochemistry, genetics, and strategies for modeling dose-response relationships. J Toxicol Environ

Health B Crit Rev 2007;10:157–222.
4. Stefanidou M, Maravelias C, Dona A, Spiliopoulou C. Zinc: a multipurpose trace element. Arch Toxicol 2006;80:1–9.
5. Maverakis E, Fung MA, Lynch PJ, Draznin M, Michael DJ, Ruben B, Fazel N. Acrodermatitis enteropathica and an overview of zinc metabolism. J Am Acad Dermatol 2007;56: 116–24.
6. Walsh CT, Sandstead HH, Prasad AS, Newberne PM, Fraker PJ. Zinc: health effects and research priorities for the 1990s. Environ Health Perspect 1994;102(Suppl 2):5–46.
7. Beshgetoor D, Hambidge M. Clinical conditions altering copper metabolism in humans. Am J Clin Nutr 1998;67:1017S–21S.
8. Brown KM, Arthur JR. Selenium, selenoproteins and human health: a review. Public Health Nutr 2001;4:593–9.
9. Thomson CD. Assessment of requirements for selenium and adequacy of selenium status: a review. Eur J Clin Nutr 2004;58:391–402.
10. Telisman S. Interactions of essential and/or toxic metals and metalloid regarding interindividual differences in susceptibility to various toxicants and chronic diseases in man. Arh Hig Rada Toksikol 1995;46:459–76.
11. Shim H, Harris ZL. Genetic defects in copper metabolism. J Nutr 2003;133:1527S–31S.
12. Neve J. New approaches to assess selenium status and requirement. Nutr Rev 2000;58: 363–9.
13. Neve J. Methods in determination of selenium states. J Trace Elem Electrolytes Health Dis 1991;5: 1–17.
14. Iyengar GV. Reference values for elemental concentrations in some human samples of clinical interest: a preliminary evaluation. Sci Total Environ 1984;38:125–31.
15. Iyengar GV. Reevaluation of the trace element content in reference man. Radiat Phys Chem 1998;51:545–60.
16. The effect of five-year zinc supplementation on serum zinc, serum cholesterol and hematocrit in persons randomly assigned to treatment group in the age-related eye disease study: AREDS Report No. 7. J Nutr 2002;132:697–702.
17. Cordano A. Clinical manifestations of nutritional copper deficiency in infants and children. Am J Clin Nutr 1998;67:1012S–6S.
18. Araya M, Koletzko B, Uauy R. Copper deficiency and excess in infancy: developing a research agenda. J Pediatr Gastroenterol Nutr 2003;37: 422–9.
19. Taylor A, Angerer J, Claeys F, Kristiansen J, Mazarrasa O, Menditto A, et al. Comparison of procedures for evaluating laboratory performance in external quality assessment schemes for lead in blood and aluminum in serum demonstrates the need for common quality specifications. Clin Chem 2002;48:2000–7.
20. Christensen JM, Olsen E. Comparison of some European external quality assessment schemes in the field of occupational and environmental medicine. Ann Ist Super Sanita 1996;32:285–94.
21. Morisi G, Menditto A, Patriarca M, Taylor A. European external quality assessment schemes in occupational and environmental medicine. Ann Ist Super Sanita 1996;32:191–307.
22. Taylor A, Angerer J, Arnaud J, Claeys F, Jones RL, Mazarrasa O, et al. Occupational and environmental laboratory medicine: a network of EQAS organisers. Accred Qual Assur 2006;11:435–9.
23. Occupational and Environmental Laboratory Medicine. http://www.occupational-environmental-laboratory.com (Accessed October 2008).
24. Fraser CG. General strategies to set quality specifications for reliability performance characteristics. Scand J Clin Lab Invest 1999;59:487–90.
25. ISO-Guide 43. Proficiency testing by interlaboratory comparison. Part 1: Development and operation of proficiency testing schemes. Geneva: International organization for standardization 1997.
26. Van Dael P, Deelstra H. Selenium. Int J Vitam Nutr Res 1993;63:312–6.
27. King JC. Assessment of zinc status. J Nutr 1990; 120(Suppl 11):1474–9.
28. Lockitch G, Halstead AC, Wadsworth L, Quigley G, Reston L, Jacobson B. Age- and sex-specific pediatric reference intervals and correlations for zinc, copper, selenium, iron, vitamins A and E, and related proteins. Clin Chem 1988;34:1625–8.
29. Patriarca M, Weykamp C, Taylor A. Use of reference materials in EQAS for traceability for measurement of trace elements and comparison of procedures for assigned values. Ann Clin Biochem 2007;44(Suppl 1):61.
30. Thompson M, Ellison SRL, Wood R. The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories (IUPAC Technical Report). Pure Appl Chem 2006; 78:145–96.
31. International Standards Organization. ISO-15189. Medical laboratories: particular requirements for quality and competence. Geneva: International Organization for Standardization; 2003.
32. International Standards Organization. ISO/IEC-17025. General requirements for the competence of testing and calibration laboratories. Geneva: International Organization for Standardization; 2005.
33. Medicare and Medicaid programs; Medicare, Medicaid, and Clinical Laboratories Improvement Act (CLIA) patient confidentiality rules: PHS and HCFA. Final rule. Fed Regist 1988;53:48645–8.
34. Medicare, Medicaid, and Clinical Laboratories Improvement Act (CLIA) patient confidentiality rules; proposed rule. Fed Regist 1988;53:10404–6.
35. Kenny DJ, Conway RM, Johnston DH. The development of ISO 9002 quality management standards for Canadian dental practices. J Can Dent Assoc 1999;65:105–8.
36. Gallagher SK, Johnson LK, Milne DB. Short-term and long-term variability of indices related to nutritional status. I: Ca, Cu, Fe, Mg, and Zn. Clin Chem 1989;35:369–73.
37. Gonzalez-Revalderia J, Garcia-Bermejo S, Menchen-Herreros A, Fernandez-Rodriguez E. Biological variation of Zn, Cu, and Mg in serum of healthy subjects. Clin Chem 1990;36:2140–1.
38. Sabban A. Quality specifications for copper, selenium and zinc in serum [MSc Thesis]. Guildford (UK): University of Surrey, 2005.
39. Lacher DA, Hughes JP, Carroll MD. Estimate of biological variation of laboratory analytes based on the 3rd National Health and Nutrition Examination Survey. Clin Chem 2005;51: 450–2.
40. Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, et al. Current databases on biological variation: pros, cons and progress. Scand J Clin Lab Invest 1999;59: 491–500.