The French interlaboratory quality assessment programme for copper, zinc and selenium in blood serum

François BARUTHIO (a), Josiane ARNAUD (b) and Francis PIERRE (a)

(a) Institut National de Recherche et de Sécurité, Vandoeuvre-les-Nancy, France
(b) Laboratoire de Biochimie C, Centre Hospitalier Universitaire de Grenoble, Grenoble, France

Summary. - The group for quality assurance for trace elements of the Société Française de Biologie Clinique (SFBC) has operated an interlaboratory quality assessment programme for copper, zinc and selenium determinations in blood serum since 1988. The primary objective is to enable participants to maintain or improve the accuracy of their analytical performance by comparing their results with other laboratories, every two months, on the basis of three quality criteria: comparison to the mean value, recovery of added copper, zinc and selenium, between-run reproducibility for identical samples. A further aim of this scheme is to evaluate interlaboratory transferability of the results. The procedure for individual and overall evaluation is reported. For each participant a performance score is calculated for each quality criteria, and a global score is attributed. The analytical performances of the participants were considered "good", "acceptable" or "inadequate" according to their global scores. Some of the results observed in this scheme are interpreted on the basis of differences between the notions of quality control and total quality assurance.

Key words: quality control, blood, serum, copper, zinc, selenium, France.

Riassunto (Il programma interlaboratoriale francese per la valutazione esterna di qualità per la determinazione di rame, zinco e selenio nel siero). - Il gruppo per l'assicurazione di qualità per le analisi di elementi traccia della Società Française de Biologie Clinique (SFBC) organizza un programma interlaboratoriale di controllo di qualità per la determinazione del rame, dello zinco e del selenio nel siero dal 1988. Il principale obiettivo è quello di mettere i partecipanti a grado di mantenere o migliorare l'accuratezza delle proprie prestazioni analitiche valutando con frequenza bimestrale i propri risultati, in confronto a quelli di altri laboratori, in base alle tre criteri di qualità: confronto con il valore medio ottenuto dopo esclusione degli outlier (dati che cadono al di fuori dell'intervallo media ± 3 DS), recupero delle quantità di rame, zinco e selenio aggiunte, riproducibilità tra giorni per campioni identici distribuiti in diverse occasioni. Un ulteriore obiettivo di questo schema è quello di valutare la trasferibilità dei risultati tra laboratori. Viene descritto il metodo usato per valutare le prestazioni individuali e globali dei laboratori. Per ogni partecipante viene calcolato un punteggio per ciascun criterio di qualità e un punteggio globale, in base al quale le prestazioni di ciascun laboratorio sono definite "buone", "accettabili" o "inadeguate". Alcuni dei risultati osservati in questo schema sono discorsi in base alla differenza tra i concetti di controllo di qualità e sicurezza totale di qualità.

Parole chiave: controllo di qualità, sangue, siero, rame, zinco, selenio, Francia.

Introduction

Since 1988, the group for quality assurance for trace elements of the Société Française de Biologie Clinique (SFBC) has operated an interlaboratory quality assessment programme for copper, zinc and selenium determinations in blood serum, for European biochemists on a voluntary basis.

The main objective of the scheme is to enable participants to maintain or improve the accuracy of their analytical performance by comparing their results to those of other laboratories every two months on the basis of three quality criteria. After elimination of results lying outside the interval: mean ± 3 standard deviations, the analysis of results allows:
- comparison of the results of the participants to the mean value;
- comparison of the recovery of the added amount (difference between the two samples) to the target value of the added amount of copper, zinc and selenium;
- reproducibility of the measurement for identical samples sent at different times (evaluated at the end of the year).

Another aim of this programme is to evaluate interlaboratory transferability of the results.

This type of evaluation of analytical performances is useful to the participants only if they are respectful of the rules, namely routine and not preferential treatment of the samples.

At the end of the first year the results showed a continuous improvement of the analytical performance for the 30 participating laboratories, with a significant diminution of the interlaboratory coefficients of variation [1]. This first observation evidenced the beneficial effect.
Table 1 - Analytical methods used by participants (1995)

<table>
<thead>
<tr>
<th>Analytical methods</th>
<th>Copper</th>
<th>Zinc</th>
<th>Selenium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flame atomic absorption spectrometry (FAAS)</td>
<td>41</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>Electrothermal atomic absorption spectrometry (ETAAS)</td>
<td>9</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Inductively coupled plasma atomic emission spectrometry (ICP-AES)</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>ICP-AES hydride generation (ICP-AES-HG)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Colorimetry</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Fluorimetry</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Laser ablation mass microspectrometry (LAMMA)</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Gas-chromatography mass spectrometry (GC-MS)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>X-Ray fluorimetry</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>59</td>
<td>54</td>
<td>26</td>
</tr>
</tbody>
</table>

of the scheme and the adherence of the participants to the effort for quality improvement.

Three years after the programme began, we drew conclusions on the basis of differences between the notions of quality control and total quality assurance [2]. The main finding was a surprising change in the annual score of the laboratories. Laboratories often showed a significant change in their individual performance from one year to the next. This could be interpreted as a modification of vigilance in laboratory practice in relation to the publication of the annual report. The second finding was the inversion of results which reveals a failure in the procedures of reception and treatment of the samples, or transcription of the results.

**Materials and methods**

Each participant receives two bovine serum specimens (prepared in-house), sent from Grenoble by express mail every 2 months, i.e., 6 times per year. One sample is spiked with known amounts of copper, zinc and selenium. The samples are sent in liquid form in polystyrene vials (each sample in duplicate: two tubes for the unspiked sample and two tubes for the spiked sample). The management of the scheme, and the treatment of the results are carried out in Vandoeuvre-lès-Nancy. The reports are transmitted to each participant before the subsequent sample distribution in order to allow them to take corrective action if needed.

**Participants**

At the beginning of the programme, in 1988, 30 laboratories took part in the scheme. In 1995, 64 clinical chemistry and biology laboratories are involved in this interlaboratory comparison.

They were from France (49), Spain (9), Belgium (3), Switzerland (3) and The Netherlands (1). The distribution by type of laboratory is the following: hospital (38), private (18), veterinary (4), research centres (2) and university (2).

Analytical methods

The analytical methods used by the 64 laboratories are listed in Table 1.

**Sample preparation and distribution**

A pool of bovine serum is divided in two fractions after homogenization by magnetic stirring for one hour:
- unspiked sample;
- spiked sample with known amounts of copper, zinc and selenium; the homogeneity is achieved by magnetic stirring for one hour.

Unspiked samples and the amounts added vary at each round: new samples are prepared for each occasion of testing except for identical samples sent at different times. No supplementary testing is organized to test the homogeneity of the batch. Two tubes of each control sample are sent to each participant, to provide sufficient sample volume for further use as internal quality control and in case of problems arising during sample dispatch (i.e. tube breakage or serum leakage).

The concentrations of samples distributed and added amounts of copper, zinc and selenium are reported in Table 2.

### Table 2 - Ranges of concentrations for test samples and added amounts

<table>
<thead>
<tr>
<th>Element</th>
<th>Unspiked sample</th>
<th>Spiked sample</th>
<th>Added amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/l</td>
<td>μmol/l</td>
<td>μmol/l</td>
</tr>
<tr>
<td>Copper</td>
<td>10.18 - 25.48</td>
<td>12.67 - 25.93</td>
<td>2.30 - 9.00</td>
</tr>
<tr>
<td>Zinc</td>
<td>7.91 - 23.98</td>
<td>8.06 - 27.07</td>
<td>2.10 - 9.00</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.36 - 1.11</td>
<td>0.38 - 1.62</td>
<td>0.63 - 1.28</td>
</tr>
</tbody>
</table>
Reporting results

Six weeks are allowed for reporting results. The participants are requested to report one result per unspiked sample and one result per spiked sample. The two vials of the same sample are labelled with the same number, for example "vial 53, round 42" for the two vials of unspiked sample and "vial 84, round 42" for the two vials of spiked sample. Laboratories are requested to give control samples a routine treatment and not a preferential one.

Bi-monthly analysis of results

The analysis is performed in two steps for each analyte. First, the mean value (m1) and standard deviation (SD1) are calculated for each sample. Results lying outside the interval: m1 ± 3 SD1 are eliminated. Results showing an inversion between unspiked and spiked samples are also discarded. A second mean value and standard deviation are then calculated (m2 ± SD 2), as well as median value (md2) and a coefficient of variation from the remaining data (CV2%).

The reports sent to the participants include 9 frequency histograms (3 for each element: unspiked and spiked sample and recovery of added amount), individual values and the statistical terms m2 ± SD 2, md2, CV%, number of results retained, and target value of added amount. An example is given in Fig. 1. This report is provided before the subsequent distribution of specimens, to allow participants to take corrective measures if necessary.

Evaluation of laboratory performance for each criterion

The evaluation procedure used in this scheme was developed by Yeoman [3] and described by Vahter [4]. This procedure was adopted by the Commission of the European Communities for monitoring the analytical performances of laboratories in the determination of cadmium and lead in blood [5]. Taylor and Briggs [6] and Weber [7] applied this methodology to other elements such as copper, zinc and selenium. The performance of each participant is evaluated by comparing the proximity of his results to the mean or target value with limits (inner and outer limits) defined by the authors of the method [3-7]. These limits take into account the relation between analytical performance and the concentration of an analyte, and also the clinical needs.

These limits are defined as follows:
- for copper and zinc (Fig. 2): at 4 µmol/l level, ± 0.5 µmol/l for inner limit and ± 1.0 µmol/l for outer limit; at 20 µmol/l level, ± 0.75 µmol/l for inner limit and ± 1.5 µmol/l for outer limit;
- for selenium (Fig. 3): at 0.75 µmol/l level, ± 0.060 µmol/l for inner limit and ± 0.120 µmol/l for outer limit; at 2.00 µmol/l level, ± 0.100 µmol/l for inner limit and ± 0.200 µmol/l for outer limit.

Limits at other levels are obtained by extrapolation of these values by linear regression.

Comparison to mean value. - As regards to the proximity of the results to the mean value, individual results are qualified to be "good" within the inner limits

![Copper or Zinc](image1.png)

**Fig. 2.** - Acceptability criterion for proximity to mean for copper and zinc analysis and example of the results obtained in one year by one participant.

![Selenium](image2.png)

**Fig. 3.** - Acceptability criterion for proximity to mean for selenium analysis and example of the results obtained in one year by one participant.
Table 3. - Evolution of the percentages of participants achieving "good", "acceptable" or "inadequate" global scores

<table>
<thead>
<tr>
<th>Element and duration</th>
<th>Line title and year of best score</th>
<th>&quot;Good&quot; laboratories scores &gt; 70%</th>
<th>&quot;Acceptable&quot; laboratories scores 70 &gt; scores &gt; 50%</th>
<th>&quot;Inadequate&quot; laboratories scores &lt; 50 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper 1988 to 1994</td>
<td>Evolution best scores: 1992</td>
<td>from 20 to 26%</td>
<td>from 30 to 35%</td>
<td>from 50 to 39%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35%</td>
<td>47%</td>
<td>18%</td>
</tr>
<tr>
<td>Zinc 1988 to 1994</td>
<td>Evolution best scores: 1993</td>
<td>from 18.5 to 17%</td>
<td>from 44.5 to 35.7%</td>
<td>from 44.5 to 47.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.3%</td>
<td>34.9%</td>
<td>41.8%</td>
</tr>
<tr>
<td>Selenium 1992 to 1994</td>
<td>Evolution best scores: 1994</td>
<td>from 21.4 to 40%</td>
<td>from 21.4 to 20%</td>
<td>from 57.2 to 41%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.3%</td>
<td>34.9%</td>
<td>41.8%</td>
</tr>
</tbody>
</table>

(score = 2), "acceptable" between inner and outer limits (score = 1) and "inadequate" outside the outer limits (score = 0) (Figs 2 and 3).

A graph is provided in the annual report (e.g. Figs 2 and 3), showing the proximity of each result to the mean for each participant and for each analyte. In the example for copper and zinc (Fig. 2), 11 results are within the inner limits and one result is within the outer limits. In Fig. 3 (example for selenium) the laboratory has 2 results outside the outer limits, 6 results within the outer limits and 4 results within the inner limits. In this instance a good quality assurance procedure could certainly improve the analytical performance.

Recall of the added amount. - The recovery of added amounts for each analyte is evaluated by the proximity of the difference between results for the unspiked and spiked samples to the target value (amount of added analyte). The individual scores for this criterion are attributed by comparing this proximity, at the concentration level of the target value, with the inner and outer limits of acceptability.

Between-run precision for identical samples. - The individual precision criteria is evaluated by the proximity to zero of the difference between results obtained for identical samples distributed in two or three rounds. Scores are attributed by comparing this value (at the concentration level of the individual results) to inner and outer limits of acceptability.

Annual global performance of the laboratories

The annual global score is calculated as the sum of the scores for the three criteria evaluated, expressed in percentage of the maximum possible score (100% if all results are within the inner limits of acceptability). The laboratories are qualified "good" if their global score is greater or equal to 70%, "acceptable" if their global score is between 70% and 50% and "inadequate" if their global score is lower than 50%.

Fig. 4. - Observed trends for outliers (percentage) in the distribution of the results provided for the determination of copper in serum samples and the recovery of the added amount.

Fig. 5. - Observed trends for outliers (percentage) in the distribution of the results provided for the determination of zinc in serum samples and the recovery of the added amount.
Results and discussion

Dispersion of results

Copper and zinc (7 years of operation). - The dispersion of results, expressed as coefficient of variation (CV%) showed a continuous and significant improvement during the first year (1988), followed by a relative stability up to the 6th year (1993). At the 7th year (1994) the CV increased again. Some new participants, and low amounts of additions to the native sample could partially explain this increase. In 1994, mean interlaboratory variability was 10.9% and 11.2% at concentration levels of 14.4 and 16.3 μmol/l for copper and zinc respectively. These results are quite acceptable for an interlaboratory comparison. For Italian laboratories, Patriarca et al. [8] observed CV% of 16.9% and 16.4% at concentrations of 14.79 and 16.52 μmol/l for copper and zinc, respectively.

Selenium (3 years of operation). - Selenium was included in the scheme in 1992 with 15 participants. This number was too low to yield reliable statistics. High, and irregular coefficients of variation were observed for spiked samples. The CV for spiked samples showed a relative stability at around 15%. These higher CVs could be explained by the low concentration level and differences in analytical techniques, and need to be reduced. These statistics will hopefully be better in 1995 with 26 participants.

Global scores

The evolution of global scores of the participants, for copper, zinc and selenium is reported in Table 3. The best scores are also reported in this table.

At present, the percentage of inadequate laboratories remains important. This suggests that quality assurance procedures need to be revised in 40% of the laboratories.

Rejected results

Another finding of the scheme was the evolution of the number of outliers, inverted results and the use of incorrect units in the expression of results. Figs 4 to 7 show the variation of these rejected results.

Number of outliers. - A regular decrease in the number of outliers was observed for copper (Fig. 4) up to the 6th year (1993) for the two unspiked and spiked samples, and for recovery of additions. 1994 showed an increase which could be attributed to new participants, and perhaps to a decrease of vigilance.

For zinc the decrease was regular (Fig. 5) up to the 7th year (1994), except for the fourth year (1991). We have no explanation for this last observation.

For selenium, a relatively important increase in the number of outliers (Fig. 6) occurred during the second year (1993), followed in the third year (1994) by an important decrease. This was significant for an improvement in quality assurance. As for copper and zinc [2], this observation could be interpreted as a modification of vigilance during the second year. The results in the third year reveal the beneficial effect of the scheme and the adherence of the participants to the effort of quality improvement.

For these 3 elements the percentage of outliers remains under 6%.

Number of inverted results. - A decrease in the number of inverted results was observed, except in the third year (Fig. 7). Special advice was given to all participants after this observation. This advice was followed immediately.

![Fig. 6](image)

**Fig. 6.** Observed trends for outliers (percentage) in the distribution of the results provided for the determination of selenium in serum samples and the recovery of the added amount.

![Fig. 7](image)

**Fig. 7.** Observed trends for the percentage of inverted results reported during quality assessment exercises for serum copper and zinc analysis.
by an important decrease in the number of inverted results and a regular decrease up to the sixth year (1993). The number of inverted results increased again in the seventh year. This suggests that further advice from the organizers of the comparison is necessary when unexpected results are observed.

For selenium, four laboratories expressed their results in incorrect units on a single occasion and only one inversion of results was observed in three years.

Conclusions

An important improvement in the performance of the laboratories involved in this interlaboratory quality assessment programme was observed during the first year, followed by a slow, but regular improvement up to the sixth year for all criteria. During the seventh year the global performance level decreased. This observation could partially be explained by the presence of some new participants, and low levels of additions in two samples. The analysis of inverted results reveals failures in the procedures of reception and treatment of the samples, or in transcription of results. The classical quality control limited to analytical procedure should be replaced by a more complete quality assurance scheme, including all steps from sampling to the report of results.

Submitted on invitation.
Accepted on 5 September 1995.

REFERENCES

### Appendix. - Summary of the scheme

<table>
<thead>
<tr>
<th>Country</th>
<th>France.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of scheme</td>
<td>Interlaboratory quality assessment programme for copper, zinc and selenium in blood serum.</td>
</tr>
<tr>
<td>Scheme description</td>
<td>Control materials: two liquid, multielemental samples in polystyrene vials prepared in-house from bovine serum. One sample spiked with known amounts of copper, zinc and selenium. Target values are calculated as mean values after elimination of outliers lower or greater than mean ± 3 standard deviation. Organization of EQA exercises: two samples sent by express mail every 2 months, on a predetermined schedule. Each sample sent in two tubes. An anonymous number attributed to each laboratory warranties the confidentiality of the results. The time schedule for returning results is 6 weeks. Elaboration of results: reports are transmitted every two months before the subsequent distribution of samples and include histograms and statistical data (mean, SD, median, CV%, number of valid results, target values for additions and personal results). An annual report of overall and individual analytical performance is issued for each laboratory. Criteria for evaluation of laboratory performance: three quality criteria are evaluated: comparison to the mean value (after exclusion of outliers); recovery of the added amount (difference between the two samples where the target value is the added amount of copper, zinc and selenium); reproducibility of the measures for identical samples sent at different times (in the annual report of performance evaluation). Measures taken against poor performers: none (voluntary participation). Provision of advice and training: provided on individual request. Financial support: provided by a participation fee (350 FF, 550 FF or 650 FF respectively for 1, 2 or 3 elements). Participation is free of charge for the first year.</td>
</tr>
</tbody>
</table>
| Organization | J. Arnaud  
Laboratoire de Biochimie C  
CHRU Grenoble - B.P. 217  
38043 Grenoble Cedex 9, France  
Tel (33) 76 76 56 40. Fax (33) 76 78 58 21  
F. Baruthio and F. Pierre  
INRS - Service TMPC - B.P. 27  
54501 Vandoeuvre-les-Nancy, France  
Tel (33) 83 50 20 00. Fax (33) 83 50 20 19  
e-mail baruthio@inrs.fr |
| Analyte and matrices covered | Copper, zinc and selenium in serum. |