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Quality assurance in the determination of metals in clinical chemistry and toxicology: the METOS project

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Summary. - National external quality assessment schemes (EQAS) for the determination of trace elements in blood (Al, Cd, Cu, Pb, Zn) have been promoted in Italy since 1983. They were organized by a working group of the Istituto Superiore di Sanità and known as "METOS (MEtalli TOSsici, toxic metals) project". The organization of the schemes included the preparation of suitable control materials by the promoting centre and the elaboration of valuable strategies of sample distribution, treatment of data and evaluation of results, that could be applied even to a small number of participants. The procedures used and the results obtained in ten years of activity of the METOS project are reported. Within the framework of the programme some information has been obtained, confirming the validity of the procedures used for sample preparation, sample distribution and evaluation of laboratories performance.

Key words: blood, trace elements, interlaboratory comparisons, analytical performance.

Riassunto *(Garanzia di qualità nella determinazione dei metalli in traccia nel laboratorio di chimica clinica e tossicologia: il progetto METOS).* - A partire dal 1983, un gruppo di lavoro dell'Istituto Superiore di Sanità ha promosso programmi di valutazione esterna della qualità per le determinazioni di metalli in traccia nel sangue (Al, Cd, Cu, Pb, Zn), con il nome di "progetto METOS (MEtalli TOSsici)". L'organizzazione dei programmi comprendeva la preparazione di idonei materiali di controllo da parte del centro promotore e l'elaborazione di valide strategie per la distribuzione dei campioni, il trattamento dei dati e la valutazione dei risultati, che potessero essere applicate anche ad un numero limitato di partecipanti. Vengono descritte le procedure usate e i risultati ottenuti in dieci anni di attività del progetto METOS. La validità delle strategie usate per la preparazione dei materiali di controllo, la loro distribuzione ai laboratori e la valutazione delle prestazioni dei laboratori è stata confermata dai risultati di alcuni esperimenti effettuati nel corso del programma.

Parole chiave: sangue, elementi in traccia, confronti interlaboratoriali, prestazioni analitiche.

Introduction

Determinations of trace metals in body fluids are performed in clinical chemistry and toxicology for diagnostic purposes, biological monitoring of occupational or environmental exposure and in both biochemical and epidemiological studies. According to the results of these determinations, decisions must be made, such as the removal of workers from their work-place, the acceptance or rejection of a clinical diagnosis and the implementation of measures of environmental protection.

The reliability of analytical data and their comparability among different laboratories can only be guaranteed through the constant adoption of quality assessment procedures including intra- and inter-laboratory activities. This need was also recognized by the Italian Decree of the President of the Council of Ministers of 10 June 1984 [1] which stated the implementation of quality assurance measures for various analytes in clinical pathology and occupational medicine, including lead in blood and urine and copper in serum.

External quality assessment schemes (EQAS) for trace element analysis should be organized at least on a national basis, due to the limited number of laboratories performing these determinations. The promoting centre provides for the preparation of suitable control samples and the planning of appropriate procedures for the distribution of the samples, the treatment of data and the evaluation of laboratories' performances.

In Italy, national EQAS for the determination of metals in blood have been promoted on a voluntary basis since 1983. They were organized by researchers of the Istituto Superiore di Sanità (ISS, Italian National Institute of Health), Laboratory of Clinical Biochemistry, and known as METOS (MEtalli TOSsici, toxic metals) project. This activity started with an EQAS for blood lead (B-Pb) and cadmium determination (B-Cd) [2], as a support to the first Italian campaign for the surveillance of the general population against the risk of lead intoxication [3, 4], and it was afterwards extended to serum aluminium analysis (S-Al) in 1985 [5]. A pilot EQAS for copper and zinc analysis in serum (S-Cu, S-Zn) was also carried out between 1989 and 1990 [6].

In this paper we will briefly outline the organization of the schemes, reported in detail elsewhere [6-10], highlighting the most recent developments, and summarize the main results obtained in ten years of activity.

Materials and methods

The general scheme of the programmes included both internal quality assurance (IQA) and external quality assessment (EQA). Control materials with assigned target values of Al, Cd and Pb, to be used for IQA, were provided to all laboratories. Interlaboratory comparisons for EQA were carried out periodically on samples which concentration was unknown to the operators. Laboratories' performance was evaluated in terms of imprecision, inaccuracy and acceptability of provided results according to pre-set limits.

The organizing centre provided for: the preparation of all control materials; their distribution to the laboratories, according to a randomized procedure; the elaboration of the results; the evaluation of laboratories' performances in each trial and the evaluation of global laboratories' performances over a period of time (phases of one or two years).

Preparation of control materials for the determination of trace elements in blood

All control materials were prepared in the organizers' laboratory, from animal blood or serum. These matrices were chosen because of their easy availability, low cost and lack of biological hazards from hepatitis B and HTLV III viruses, in comparison with the corresponding human products, whereas their analytical behaviour did not differ from human blood or serum [11]. Various levels of concentration (two for IQA and from five to eight for EQA) were obtained by addition of inorganic salts of the element of interest, except for Cu and Zn control samples. The concentrations of the samples for EQA were chosen in order to evaluate the analytical performance over the whole range of concentrations that may be expected in human specimens, including unusually high and low values. The concentrations of the two control materials for IQA were chosen at a medium-low and a medium-high level within the interval of concentrations expected for human specimens. All control materials were divided in 2 ml aliquots (4 ml for Cu and Zn samples) in plastic vials. Precautions to avoid contamination were taken during all steps of sample preparation and all glass and plastic-ware was either acid-washed or checked for metal content. For long term stability, samples were either sterilized by gamma irradiation or lyophilized, then stored at 4°C. Homogeneity was tested by analyzing at least 5% of the samples. Target values for the materials for IQA were established by "consensus" from the results obtained by all participants analyzing from two to four vials of each material.

Lyophilization was first introduced for serum samples for Al determination, to improve the stability of the products and safety of despatch. In this occasion, six control materials for EQA were divided into 2 ml aliquots and half of the samples obtained for each material were lyophilized using facilities at the Sclavo Institute (Siena, Italy). This experiment was successful and freeze-dried serum samples were then routinely utilized in the EQAS for S-Al. At present, the lyophilization of both serum and blood samples is carried out in our laboratory.

Since Cu and Zn are endogenous elements, normally present in human and animal serum at concentrations of 1 mg/l, the production of control materials for their determination required a somewhat different procedure. Control materials for both elements were prepared from bovine serum. Serum fractions with different levels of Cu and Zn, were obtained using a procedure based on the formation of gradients of concentration for these elements. This occurs spontaneously in serum after deep freezing (-80 °C) and thawing without shaking. Six serum aliquots with increasing Cu and Zn concentrations, ranging from 0.5 to 2.0 mg/l, were obtained by gently withdrawing them from the top to the bottom of the container. Each aliquot was subdivided into 4 ml aliquots in plastic tubes. All samples were sterilized by gamma rays, stored at 4 °C and tested for homogeneity as described above.

Procedures for sample distribution and collection of results

Participants received a batch of samples for IQA once a year. Two IQA samples (one for each concentration level) were required to be analyzed together with EQA samples.

Trials for EQA were carried out every three or four months. The eight samples distributed to each laboratory (including a variable number of duplicates), were randomly chosen and codified to avoid identification. Over the years, an effort has been made, to introduce automatic procedures whenever possible. At present, on the occasion of every new trial, a computer programme - which requires name of element (and matrix), codes of the control materials to be distributed and codes of all laboratories participating in that trial as input - assigns the EQA samples to each laboratory and generates random conventional reference codes for the assigned samples. Input data and conventional reference codes are used by the program to: a) print sample labels b) print data sheets to be used by the laboratories for reporting results; c) add records to a data base system, that will be ready to accept the results provided by each laboratory in that trial for both IQA and EQA samples. The only operations that remain completely manual are sample labelling and the delivery of materials to the participants.

Results sent by the laboratories by mail or fax within two months from the date of samples dispatch are entered into the data base. An experiment for the automatic implementation of the data base using data transmission by modem is now in progress.

Elaboration of results and evaluation of the performance of the laboratories at the end of each trial

At the end of each trial, data provided by the participants were elaborated. No result was excluded from the elaboration and, for each analyzed sample, the median of all provided data was chosen as target value. Information sent to participants included statistics for each analyzed sample and specific information on their performance, provided individually to each laboratory, i.e. the pooled standard deviation (PSD) for the duplicate samples examined in the trial as a measure of imprecision and the comparison of its results with acceptability criteria, defined by the organizers [6, 9]. The acceptability limits for B-Pb determination are represented in graphic form in Fig. 1, as an example, and the correspondent parameters for other elements are reported in Table 1.

With the implementation of a system of data transmission via modem, participants will be able to receive immediately preliminary information on the accuracy of their results, because, after every input of data, an estimate of the target value for each EQA sample will be automatically computed from the data available.

Evaluation of the overall performance of participants at the end of each phase

The overall performance of participants was evaluated at the end of each phase (1 or 2 year of activity).

Laboratories were divided into classes of performance according to the percentages of acceptable results provided (from 100% to ≥ 90%, from 90% to ≥ 80%,

Table 1. - Maximum acceptable deviations to the target values established for various elements at low and high concentrations, for the definition of the acceptability limits over the range of concentrations

Element	Concentration µg/l	Acceptable bias µg/l
Pb	100	± 20 (20%)
	800	± 80 (10%)
Cd	1	± 0.6 (60%)
	15	± 1.5 (10%)
Al	10	± 3 (30%)
	120	± 12 (10%)
Cu, Zn	100	± 20 (20%)
	1500	± 150 (10%)

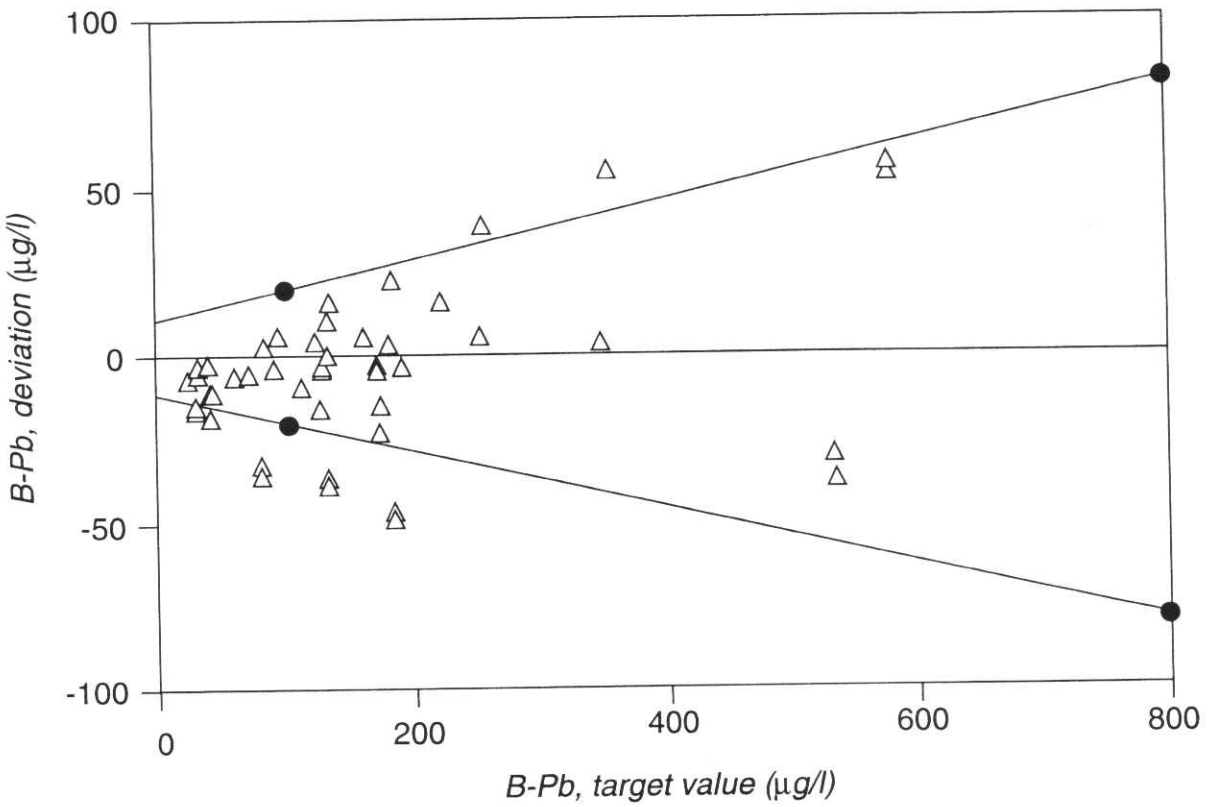

Fig. 1. - Acceptability criterion for B-Pb results. The maximum acceptable deviations to the target value at low and high concentration are established by the organizers (full circles). Acceptable deviations at any other concentration are obtained from the straight lines joining the full circles. Laboratory results are considered acceptable if their deviation to the target value (triangles) falls inside the zone defined by the two straight lines.

from 80% to ≥ 70%, from 70% to ≥ 50% and less than 50%). Laboratories achieving acceptable results for at least 80% of the examined samples were considered "good performers". Those providing less than 50% of results within the acceptability limits were considered "poor performers".

Overall inaccuracy was evaluated from the coefficients of variation of the results provided for each sample by all laboratories, except the batches with no addition of metals or lowest concentration, computed after exclusion of results outside the interval mean ± 3 SD. For each phase, the mean of the coefficients of variation (CVm%) was chosen as an index of the overall inaccuracy of the group of laboratories.

Results

Participants

All participants were enrolled on a voluntary basis and full anonymity was ensured. No restrictive measure was taken against poor performers although advice was available. Environmental and occupational health units, hospital laboratories, commercial laboratories, universities and research centres were among the participants. The average number of participants in the various phases of the schemes for B-Pb, B-Cd and S-Al is shown in Fig. 2. Compliance was satisfactory and ranged from 79% to 96%, for B-Pb; from 76% to 93% for B-Cd and from 63% to 89% for S-Al. The number of laboratories participating in the pilot EQAS for the determination of S-Cu and S-Zn was 40 for the first year, with an average compliance of 82%, and 33 for the second year, with an average compliance of 91%.

The choice of the analytical method to be used was left to the operators. Direct electrothermal atomic absorption spectrometry (ETAAS) was the method of choice for B-Pb, B-Cd and S-Al. The percentage of laboratories using other procedures decreased over the years. Copper and zinc analysis were mainly performed by flame atomic absorption spectrometry. One third of the participants used ETAAS for S-Cu determinations.

Overall laboratory performances for B-Pb and B-Cd determination

The results of the EQAS for B-Pb and B-Cd have been recently reported [10] and will be only summarized here. The global inaccuracy, observed during various phases of the EQAS, is reported in Table 2. For B-Pb, the CVm% values showed little variation between the 1st and the 8th phase, but the average concentration of the sample analyzed decreased from 440 µg/l to 165 µg/l. This suggested an improvement of the laboratory performance, which was confirmed by other data. On the other hand, overall inaccuracy increased noticeably for B-Cd determinations in the last two phases, because the number of laboratories participating in this scheme almost doubled at the beginning of the 7th phase. These trends are confirmed by the variations in the percentages of "good" and "poor" performers over the various phases, represented in Figs 3 and 4, for B-Pb and B-Cd, respectively. In total, the percentage of "good performers" increased from 27.8% to 47.9%, for B-Pb analysis, and from 45.0% to 61.0%, for B-Cd analysis. The percentage of "poor performers" decreased in the EQAS for B-Pb, whereas, for B-Cd, this percentage showed a net increase in the last two phases, according to the above mentioned worsening of the overall inaccuracy.

Overall laboratory performances for S-Al determination

The overall inaccuracy observed for S-Al determination during the various phases are reported in Table 3. The CVm% decreased slowly but steadily from the 1st to the 8th phase of the programme, whereas the average concentration of the samples analyzed remained almost unchanged. The variations of inaccuracy in the determination of S-Al over the years have been studied by plotting the values of the CV% obtained for each sample (transformed in 1/CV% to obtain a linear relationship) *versus* the Al concentration of the samples. For each phase, the regression lines between 1/CV% values and sample concentrations have been plotted (Fig. 5). From this analysis, a trend can be pointed out toward lower CV% values (higher 1/CV%) over the whole range of concentrations, from the 1st to the 8th phase of the EQAS, except for the 2nd phase.

Overall imprecision for Al determination was computed at the end of each phase (Table 3), as PSD between the two values provided by every laboratory for the samples distributed in duplicate in the same trial, after the exclusion of data exceeding the interval mean ± 3SD. Laboratory imprecision worsened during the third phase, when duplicate samples were unknown to the participants, and showed a slow improvement during the 5th and 6th phase. Few data were available for the 7th phase, because duplicate samples were distributed only in one trial, and none for the 8th phase. The same trend can be observed over the whole range of concentration, from the regression lines, obtained, in each phase, between the values of the PSD for each control sample and their Al concentration (Fig. 6).

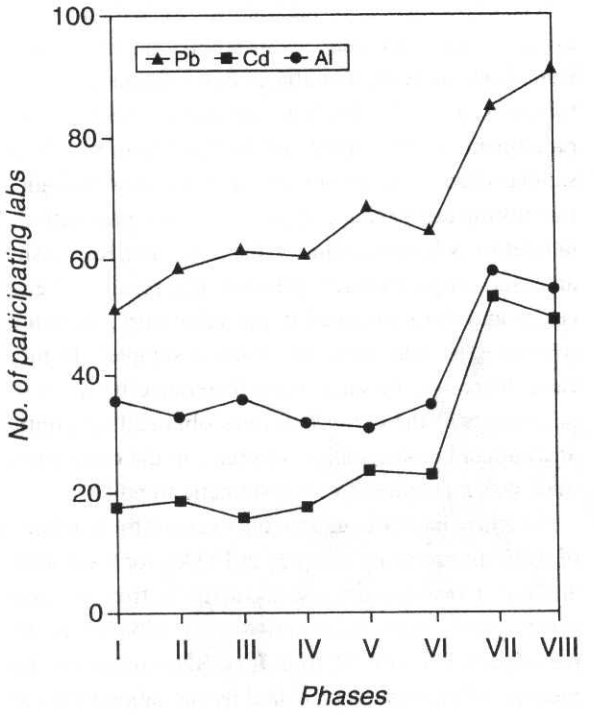

Fig. 2. - Number of participants in the various phases of the EQAS for B-Pb, B-Cd and S-Al.

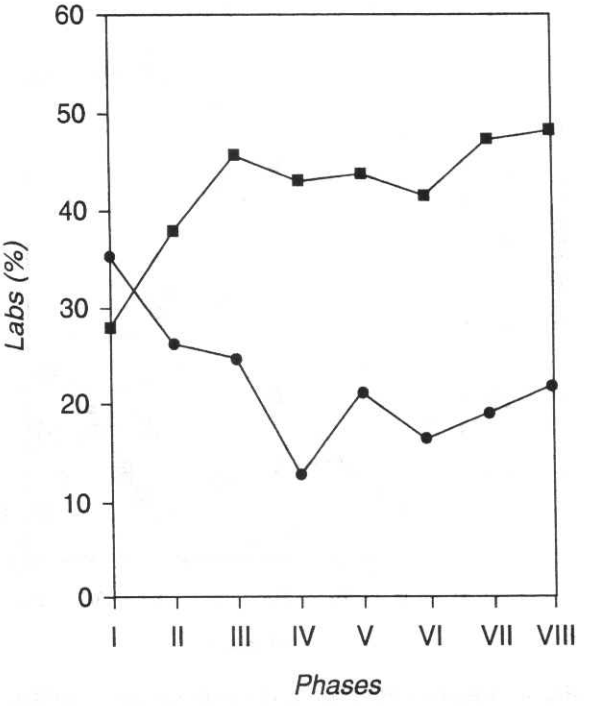

Fig. 3. - Percentages of good (squares) and poor (circles) performers in the various phases of the EQAS for B-Pb.

Table 2. - Overall inaccuracy in various phases of the EQAS for B-Pb and B-Cd, expressed as mean coefficient of variation (CVm%). The average concentration (avg. conc.) and the range (min., max.) of concentration of the samples distributed to the laboratories are also reported

Phase	1	4	5	6	7	8
B-Pb						
CVm%	22.0	14.4	18.5	19.9	19.3	21.8
Avg. conc., µg/l	370	440	390	315	261	165
Min., µg/l	100	70	80	80	80	60
Max., µg/l	820	820	800	800	588	538
B-Cd						
CVm%	35.0	26.5	25.8	24.4	34.6	48.2
Avg. conc., µg/l	3.2	3.6	3.7	3.6	2.7	2.1
Min., µg/l	0.9	1.1	1.2	1.2	0.8	0.8
Max., µg/l	15.0	5.7	7.8	7.8	5.0	5.1

Table 3. - Overall inaccuracy and imprecision in various phases of the EQAS for S-Al expressed as mean coefficient of variation (CVm%) and pooled standard deviation (PSD), respectively. The average concentration (avg. conc.) and the range (min., max.) of concentration of the samples distributed to the laboratories are also reported

Phase	1	2	3	4	5	6	7	8
CVm%	24.6	32.5	24.9	25.4	21.1	21.2	19.6	18.3
PSD, µg/l	4.5	3.9	5.7	8.7	6.6	5.6	7.6	-
Avg. conc., µg/l	74.2	73.9	67.5	68.9	77.7	75.1	77.3	87.0
Min., µg/l	27.5	28.0	27.0	31.0	35.0	34.0	30.0	29.5
Max., µg/l	135.0	133.5	120.0	107.0	145.5	134.0	125.0	169.5

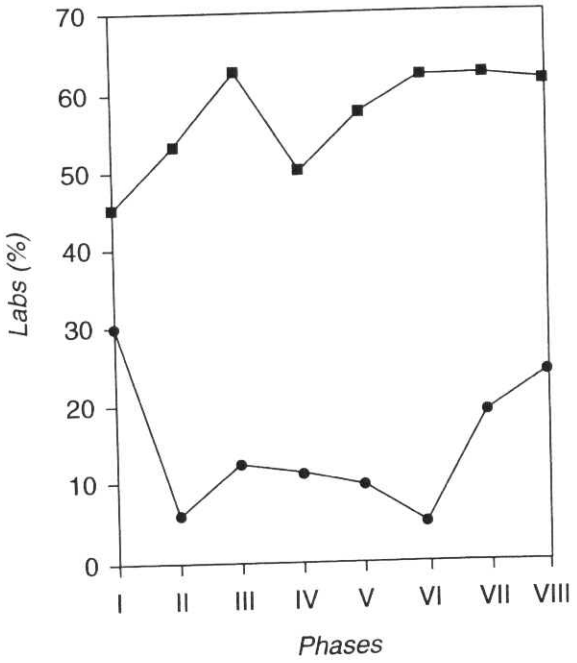

Fig. 4. - Percentages of good (squares) and poor (circles) performers in the various phases of the EQAS for B-Cd.

The percentage of laboratories providing acceptable results for at least 80% of the analyzed samples was rather low and variable in all the phases of the programme, but, at least, the percentage of "poor performers" showed a decreasing trend over the years (Fig. 7).

Overall laboratory performances for S-Cu and S-Zn determination

The overall inaccuracy for the determination of S-Cu and S-Zn was 17% and 16%, respectively, in both phases of the pilot EQAS. For S-Cu analysis, the distribution of laboratories, according to the percentage of acceptable results provided, pointed out a net increase in the percentage of "good performers", from 15.4% in the first year to 43.8% in the second year, and a correspondent decrease of "poor performers", from 30.7% to 18.7%. On the contrary, for S-Zn analysis, no improvement could be observed between the two years of activity of the programme. The percentages of laboratories achieving at least 70% of acceptable results were 41.0% and 37.6%, respectively, and the percentages of "poor performers" 32.1% and 31.2%.

Validity of the procedures

The validity of the procedures used has been confirmed by some experiments carried out within the framework of the schemes.

The performances of laboratories for B-Pb analysis, evaluated from the analysis of control materials, have been shown to be comparable to those obtained on fresh human blood. On different occasions, some of the participants in the EQAS for B-Pb determined Pb in samples of fresh human blood for a campaign of biological monitoring and exchanged part of the samples with our laboratory, which coordinated the campaign. Regression analysis was performed between the results of each centre and those obtained in our laboratory, chosen as reference, on both fresh and control samples. In most cases there was no significant difference between the parameters of the regression lines obtained for control and human blood samples, whereas, in the other cases, there was no evidence of a systematic trend [11].

An experiment was carried out to assess the feasibility of using freeze-dried samples in EQAS for trace metal analysis. Lyophilized and liquid samples from the same serum pools were alternatively distributed to the participants in four EQA trials for Al determination. The medians of the results provided by the laboratories for lyophilized samples agreed with those obtained for liquid samples, the largest difference being 1.5 µg/l [9].

The need to assess laboratory performances using "blind" samples has been confirmed by the results obtained for the PSD between duplicate samples by 23 laboratories participating in two sequential trials for S-Al determination [9]. Operators were aware of the identity of duplicates in the former trial, whereas, in the latter, duplicate samples could not be identified. Almost all laboratories (15 out of 23) showed a much worse reproducibility in the latter trial, although the same batches of samples were analyzed. Mean imprecision, computed as the square root of the mean of the pooled variance of the results of each laboratory, was 2.6 µg/l in the former trial and 11.4 µg/l in the latter.

Conclusions

The implementation of EQAS for B-Pb, B-Cd, S-Al, S-Cu and S-Zn yielded an improvement of the quality of the data produced by the participants, except for Zn analysis. The worsening of overall performances in some of the phases of the programmes can be generally explained as an effect of the adhesion of new laboratories. Due to the difficulties of trace metal analysis and the lack of commercially available reference materials, participation in EQAS is essential to assess, improve and maintain adequate quality of the data produced. These programmes should be promoted by national organizations and harmonized among European countries.

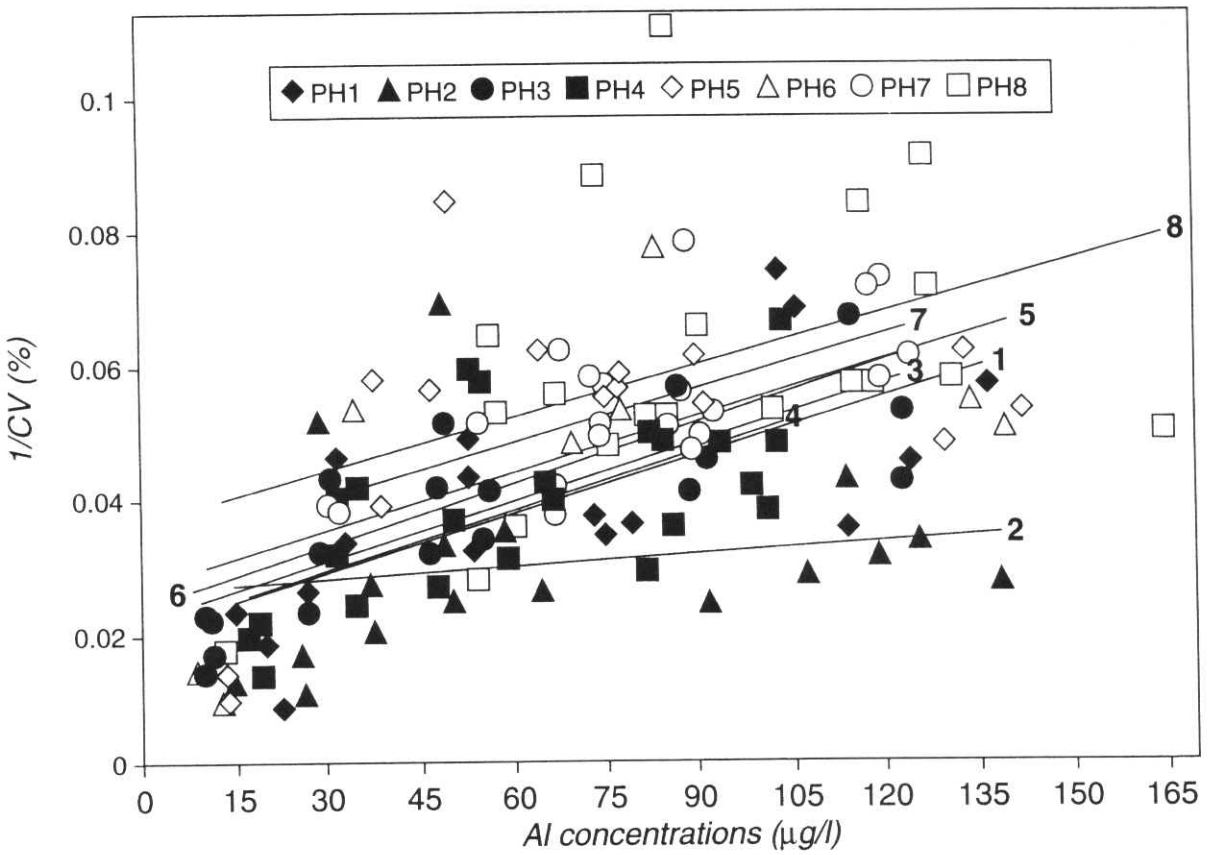

Fig. 5. - Variation of the regression lines computed, for each phase (PH) of the EQAS for S-Al, between the reciprocal of the CV's% (1/CV%), obtained for each sample in each trial, and the Al concentration of the samples.

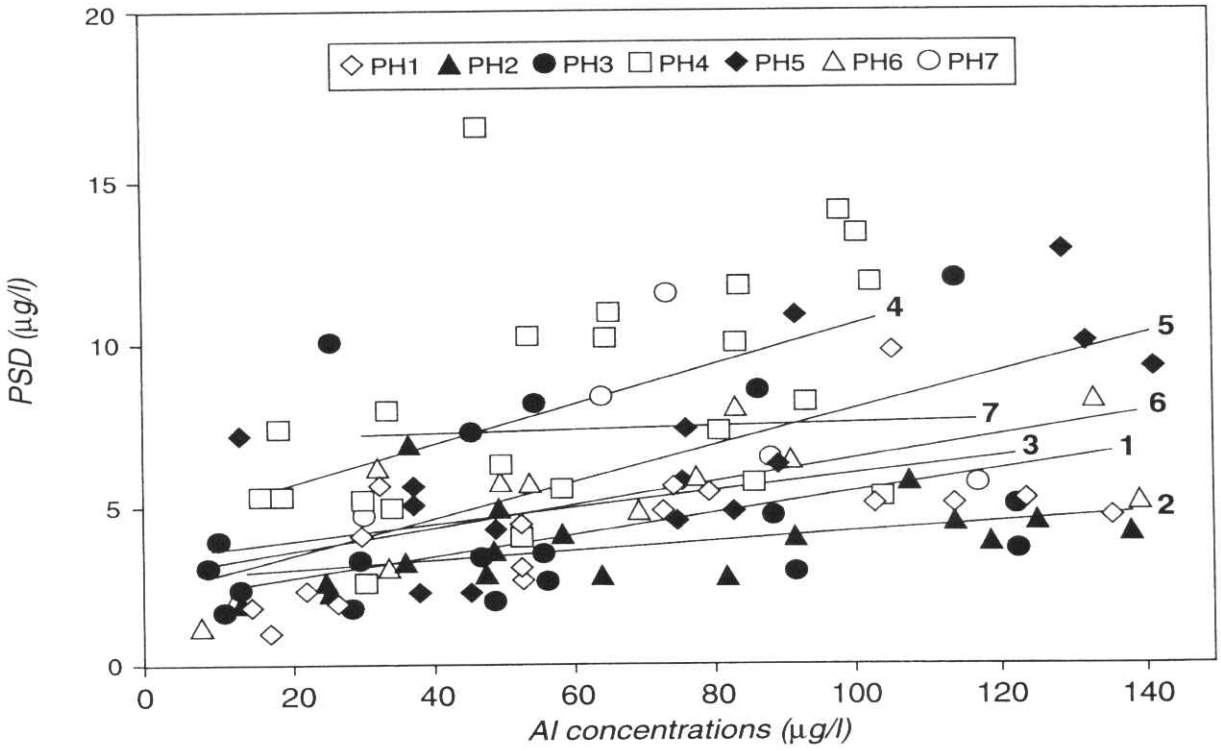

Fig. 6. - Variation of the regression lines computed, for each phase (PH) of the EQAS for S-Al, between the PSD of the couples of results obtained by every laboratory, for the samples distributed in duplicate in the same trial, and the Al concentration of the samples.

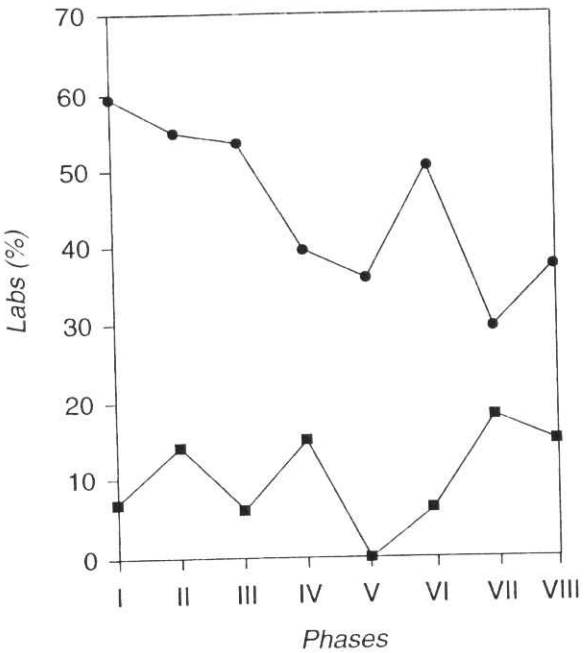

Fig. 7. - Percentages of good (squares) and poor (circles) performers in the various phases of the EQAS for S-Al.

Extension to other elements and further studies to validate the procedures for assessment of laboratory performance are also needed.

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REFERENCES

1. ITALIA. Decreto del Presidente del Consiglio dei Ministri, 10 giugno 1984. Indirizzo e coordinamento dell'attività amministrativa delle regioni in materia di requisiti minimi di strutturazione, di dotazione strumentale e di qualificazione funzionale del personale dei presidi che erogano presta-zioni di diagnostica di laboratorio. *GU* n. 55, 24 febbraio 1984. pp. 1559-1566.

2. MORISI, G., MACCHIA, T., PATRIARCA, M. & TAGGI, F. 1985. Articolazione del programma italiano per il controllo di qualità nella determinazione del piombo e del cadmio nel sangue. *Ann. Ist. Super. Sanità* **21**(1): 97-110.

3. EUROPEAN COMMUNITIES. 1977. Council Directive of 29 March 1977 on biological surveillance of the general population against the risk of saturnism (77/312/EEC). *Off. J. Eur. Communities* L 105 of 28 April 1977. pp. 10-17.

4. ITALIA. Decreto del Presidente della Repubblica n. 496 dell'8 giugno 1982. Attuazione della direttiva CEE n. 77/312 relativa alla sorveglianza della popolazione contro il rischio di saturnismo. *GU* n. 212, 4 agosto 1982. pp. 5492-5493.

5. MORISI, G. & PATRIARCA, M. 1989. Italian quality assurance programmes for biological monitoring of trace elements (Al, Cd, Cu, Pb, Zn). In: *Third Italo-Hungarian symposium on spectrochemistry. Proceedings*. Ispra, 8-12 June 1987. S. Caroli, G. Rossi, E. Sabbioni & K. Zimmer (Eds). Commission of the European Communities (EUR 11703 EN). pp. 177-186.

6. PATRIARCA, M., MENDITTO, A., MURAN, A. & MORISI, G. 1993. Quality of analytical determinations of Cu and Zn in serum. A collaborative study in a sample of Italian laboratories. *J. Trace Elem. Electrolytes Health Dis.* **7**: 257-259.

7. MORISI, G., PATRIARCA, M. & TAGGI, F. 1989. The interlaboratorial quality assurance program for blood lead determination. An evaluation of methods and results. *Ann. Ist. Super. Sanità* **25**(3): 405-416.

8. MORISI, G., PATRIARCA, M., & TAGGI, F. 1989. Evaluation of the performance of Italian laboratories in the determination of cadmium levels in blood. *Ann. Ist. Super. Sanità* **25**(3): 449-456.

9. MENDITTO, A., PATRIARCA, M. & MORISI, G. 1992. Organization of interlaboratorial quality assurance programmes for the analysis of trace elements in blood: evaluation of procedures and analysis of data. *Microchem. J.* **46**: 137-146.

10. PATRIARCA, M., MENDITTO, A. & MORISI, G. 1992. The performance of Italian laboratories in the determination of trace elements in blood. *Microchem. J.* **46**: 147-158.

11. MORISI, G., PATRIARCA, M. & TAGGI, F. 1989. Comparable laboratory performances in the analysis of lead in control samples and fresh human blood. *Ann. Ist. Super. Sanità* **25**(3): 417-422.