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Trace elements in body fluids: external quality assessment scheme in The Netherlands

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Summary. - External quality assessment schemes (EQAS) for trace elements in body fluids are organized in The Netherlands by the SKZL (Foundation for Quality Assessment in Clinical Laboratories). Medical laboratories participate on a voluntary base. The EQAS is designed to evaluate laboratory performance in terms of accuracy, precision and linearity. Laboratory-made frozen samples of whole blood, serum and urine, enriched with the elements of interest, i.e. Tl, Cd, Co, Hg, Se, Pb, Mg, Li, Al, Cu, Zn and As, are used. The present state of art shows an acceptable mean recovery, intra-laboratory variation and linearity. Interlaboratory variation, comparability of reference ranges and criteria of interpretation used in different laboratories are less satisfying and require improvement.

Key words: External quality assessment schemes (EQAS), trace elements, accuracy, precision, linearity, The Netherlands.

Riassunto *(Elementi in traccia nei fluidi biologici: programmi di valutazione esterna di qualità nei Paesi Bassi)*. - Programmi di valutazione esterna di qualità (EQAS) per gli elementi in traccia nei fluidi biologici sono organizzati nei Paesi Bassi dalla Foundation for Quality Assessment in Clinical Laboratories (SKZL). I laboratori di analisi cliniche vi partecipano volontariamente. Gli EQAS sono finalizzati alla valutazione delle prestazioni dei laboratori in termini di accuratezza, precisione e linearità. I campioni utilizzati sono campioni congelati di sangue intero, siero e urine, addizionati degli elementi di interesse (Tl, Cd, Co, Hg, Se, Pb, Mg, Li, Al, Cu, Zn e As) preparati in laboratorio. Lo stato dell'arte attuale indica che il recupero medio, la variabilità intra-laboratorio e la linearità sono accettabili. La variabilità tra laboratori e la confrontabilà degli intervalli di riferimento e dei criteri interpretativi dei risultati delle analisi usati in laboratori diversi è meno soddisfacente e necessita di miglioramento.

Parole chiave: programmi di valutazione esterna di qualità, elementi in traccia, Paesi Bassi, accuratezza, precisione, linearità.

Introduction

External quality assessment schemes (EQAS) for the assays performed by medical laboratories in The Netherlands are organized by the SKZL (Foundation for Quality Assessment in Clinical Laboratories), an independent organization for quality assessment covered by professional colleagues. Although it is not mandatory in The Netherlands to participate in EQAS, almost all (99%) laboratories take part in the SKZL quality control surveys. A growing number of foreign participants has been welcomed during recent years. Different sections of the SKZL cover the whole field of clinical chemistry. External quality assessment schemes to improve the quality of less routinely analysed parameters (including trace elements) have been developed by the SKZL-section multi component analysis. Reports deal with issues like accuracy and precision and have options for testing linearity, pattern recognition, recovery and interfering substances. All samples used in the programme are laboratory-made. Additional services include meetings and the preparation of reference materials for the participating laboratories. External quality assessment schemes are developed for three body fluids (whole blood, urine and serum) and comprise trace elements of physiological and toxicological interest. Participants are either clinical chemistry or toxicology laboratories.

Materials and methods

Participation is open to all medical laboratories in The Netherlands and foreign laboratories who are interested. Participants pay an annual fee to cover the costs of the EQAS organization.

The design of EQAS is illustrated in Table 1. Pools of human whole blood, urine and serum are enriched with the trace elements of interest, dispensed in plastic tubes, in 10 ml portions and frozen. Sets of 12 samples, consisting of 6 unidentifiable pairs, are distributed to the participating laboratories once a year and stored frozen. Every month one sample of the series is assayed. Forms are sent along with the samples and laboratories are asked to fill results and the analytical method used. Results are reported per set of 3 samples and participants are supplied with provisional reports after the closing-date of the first,

Table 1. - Design of the EQAS for trace elements in body fluids in The Netherlands

Sample pair	Added quantity of analyte	Results (example)	Difference between duplicates
a and f	0	1 - 0	1
b and d	10	11 - 12	1
c and m	20	22 - 17	5
e and k	30	30 - 30	0
g and l	40	41 - 43	2
j and h	50	51 - 56	5

second and third set of 3 samples, respectively. Data from the fourth set complete the results of the whole series of 12 samples and then the final report is prepared in terms of accuracy, precision and linearity. Transmission is performed conventionally by post. Trace elements included in the EQAS are: a) Cd, Co, Hg, Se and Pb in whole blood; b) Co, Se, Mg, Li, Al, Cu and Zn in serum; c) Tl, Cd, Co, Hg, Pb, Mg, Cu, Zn and As in urine.

Statistical treatment includes removal of outliers (data lying outside the interval mean ± 3 SD) and subsequent calculation of the parameters of interest, notably accuracy, interlaboratory variation, intralaboratory variation, linearity, and recovery of added quantities. The accuracy is derived from the comparison of results of an individual laboratory with both the added quantity and the observed mean of the results of all laboratories. The interlaboratory variation, expressed as CV, indicates the degree of standardization throughout the group of laboratories. The precision is expressed as the intralaboratory CV, calculated from the differences observed for the 6 sample-pairs and compared with CVs of the other participants. Linearity is derived from linear regression analysis between added quantities (x) and measured amounts (y). The resulting correlation coefficient and slope indicate the linearity and recovery, respectively. The intercept

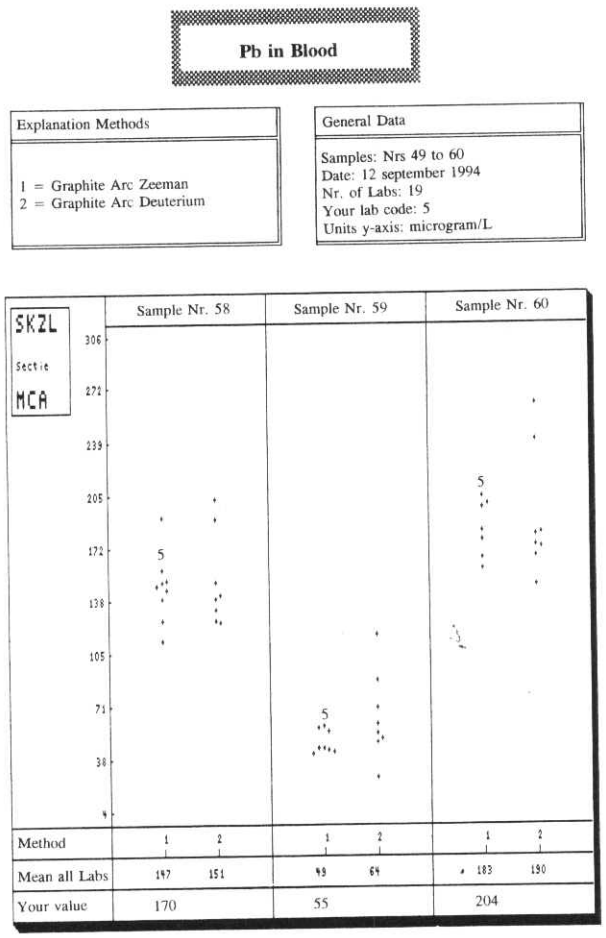

Fig. 1. - Lay-out of report for individual samples (example for Pb in whole blood).

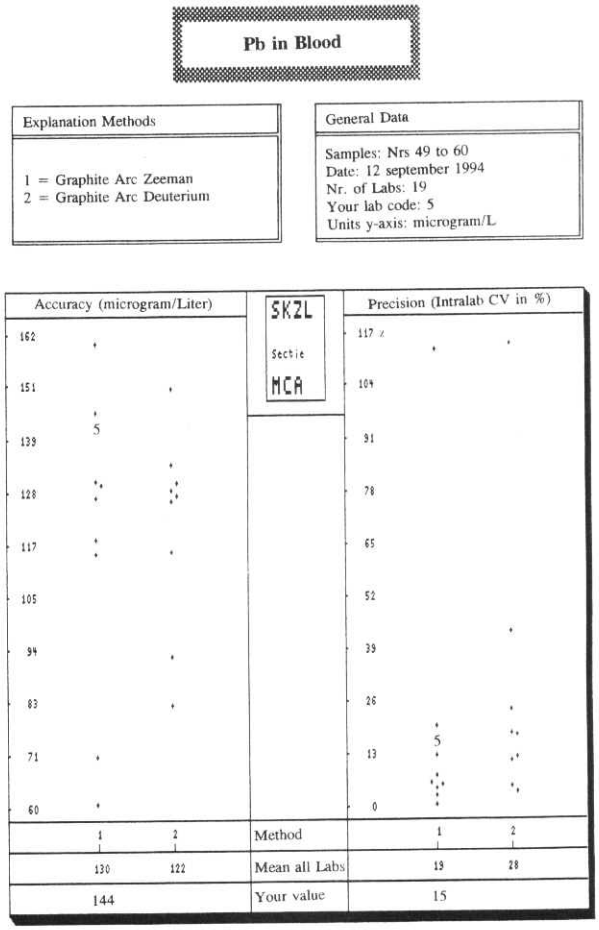

Fig. 2. - Lay-out of report for accuracy and precision as calculated from the series of 12 samples (example for Pb in whole blood).

gives the endogenous concentration of the element in the original body fluid (especially important in case of physiologically occurring trace elements). The lay-out of the report combines a numerical and graphical representation of accuracy, precision and linearity. As participation in the EQAS is voluntary, we do not include scores or certification procedures in our statistical treatment. Fig. 1 shows a typical lay-out of a report for individual samples. Accuracy, precision and linearity are presented in Figs 2 and 3.

Because of the voluntary basis of the EQAS, we do not take restrictive measures against poor performers. To stimulate the improvement of the performance of the participants, we organize annual meetings to discuss results and specific problems. In addition we make spare samples from the EQAS available to the laboratories to check up their performance.

Results and discussion

It is for each of the participants to judge their individual results. In the letter accompanying the final report we evaluate the general performance. Table 2 gives an example of the results obtained in 1994 for assays of Pb in blood. The mean recovery of 95% is good. The mean interlaboratory CV of 18% is less satisfying and shows a serious dispersion of assay results among individual laboratories: recoveries range from about 50 to 150%. The precision with a mean intralaboratory CV of 5% is good, as is the linearity with a correlation coefficient of 0.998. Similar results were obtained for other trace elements in whole blood, urine and serum, which are not discussed in detail here. Table 3 shows an overview of the general performances of the participating laboratories (recovery and interlaboratory CVs) for the various trace elements and body fluids. In general an acceptable recovery is seen. The best interlaboratory CVs are obtained for well-settled assays like Mg and Li in serum. Worse results are obtained for Se and Co, but it should be taken in account that the number of laboratories performing these assays is quite small.

Table 2. - General evaluation of the EQAS for Pb in blood in 1994

No. of laboratories	19
Accuracy	
Mean recovery	95%
Interlaboratory CV	18%
Precision	
Intralaboratory CV	5%
Linearity	
Correlation coefficient	0.998
Interpretation criteria	
(Reference ranges for non-toxic concentrations)	< 0.3 µmol/l (1 lab) < 0.5 µmol/l (3 labs) < 1.0 µmol/l (7 labs) < 1.5 µmol/l (3 labs) < 1.9 µmol/l (1 lab) no answer (3 labs)

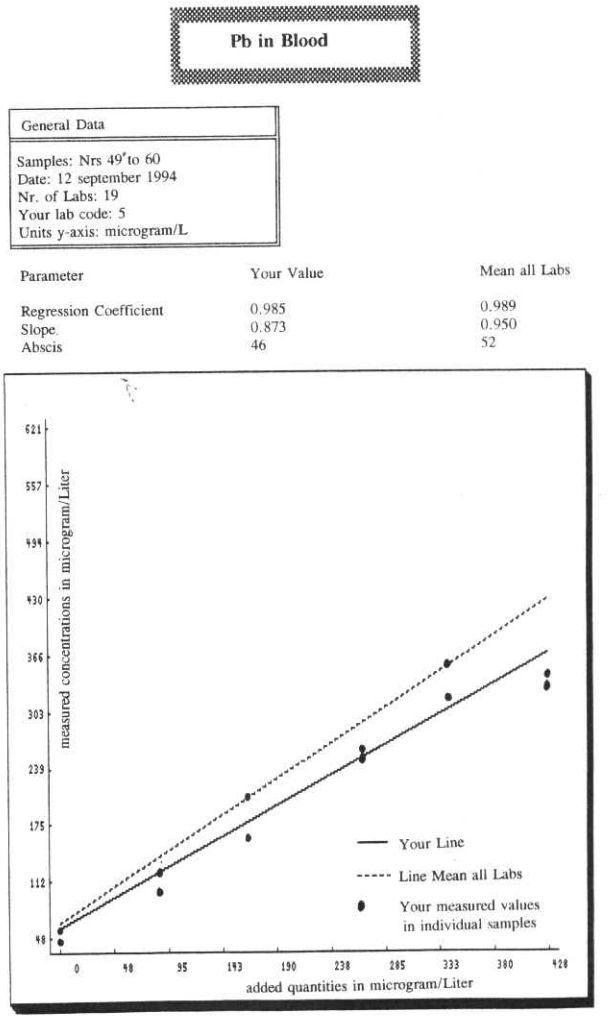

Fig. 3. - Lay-out of a report for linearity as calculated from the series of 12 samples (example for Pb in whole blood).

Whole blood seems to be a matrix that gives rise to a high interlaboratory CV (although Cd is an exception). In subsequent EQAS we observed a trend towards better interlaboratory CVs, especially when much attention in meetings was spent on it. In the most recent exercise for blood Pb (April 1995), the interlaboratory CV was 11% which is a clear improvement when compared to the 18% seen in 1994. We also made a survey of interpretation criteria. The reference ranges reported by the various

Table 3. - Number of participating laboratories (No.), recovery of added quantities (Rec.), and interlaboratory CV (Int. CV) for the various trace elements in whole blood, serum and urine (1994)

Trace element	Whole blood			Serum			Urine		
	No.	Rec. (%)	Int. CV (%)	No.	Rec. (%)	Int. CV (%)	No.	Rec. (%)	Int. CV (%)
Tl	4	95	23				6	97	20
Cd	8	85	4				6	81	15
Co	2	68	22	4	83	11	2	84	10
Hg	5	74	14				6	85	11
Se	3	86	23	9	100	21			
Pb	19	95	18				7	71	11
Mg				17	98	3	11	100	6
Li				15	97	6			
Al				16	101	13			
Cu				21	91	6	14	102	10
Zn				23	96	9	14	100	7
As							7	95	20

laboratories were highly variable: e.g. for blood Pb, non-toxic concentrations ranged from < 0.3 to < 1.9 µmol/l. Work is in progress to standardize the reference ranges used by laboratories.

We conclude that the present state of art shows that mean recovery linearity and precision are acceptable and do not need special attention. Interlaboratory standardization of both analytical results and interpretation criteria, require improvement and research focuses on these two items.

Submitted on invitation.
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Appendix. - Summary of the scheme

Country	The Netherlands.
Status of scheme	Essentially national with some participants from other countries. Participation on voluntary base. Run by SKZL (Foundation for Quality Assessment in Clinical Laboratories) major. *Aims*: quality improvement. *Participants:* 25 medical laboratories (clinical chemistry and toxicology laboratories).
Scheme description	*Control samples:* prepared in laboratory from a human matrix (whole blood, serum and urine). Sample enrichment by addition of salts of trace elements. Multi-elemental, frozen samples in plastic vials. Dual target values: weighed amounts and mean of all laboratories. *Internal quality control:* spare samples from the EQAS available as check samples. *Organization of EQA exercises:* twelve samples distributed frozen once a year, one sample analysed every month. Results returned 4 times a year. Transmission by conventional post. Three provisional reports (every three months) and a final report (once a year) provided to participants. *Elaboration of results:* results elaborated after exclusion of outliers (data outside the interval: mean ± 3 SD). The final report includes: evaluation of accuracy, in terms of recovery and proximity to the mean of all results; interlaboratory precision, linearity, evaluated by linear regression on the results provided in the year. *Criteria of evaluation of laboratory performance:* no score-system or certification; no criteria for evaluation of laboratory performance. *Measures against poor performers*: none. *Advice*: given in annual meetings of participants. *Financial support:* costs covered by participation fee of laboratories.
Organization	C.W. Weykamp and T.J. Penders SKZL, Section Multi Component Analysis Beatrix Park I NL-7101 BN Winterswijk, The Netherlands Tel + 31 543 544774. Fax + 31 543 524265
Analytes covered	Whole blood: Cd, Co, Hg, Se, Pb. Serum: Co, Se, Mg, Li, Al, Cu, Zn. Urine: Tl, Cd, Co, Hg, Pb, Mg, Cu, Zn, As.