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External quality assessment scheme in Belgium. Heavy metal analyses

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Summary. - An external quality assessment scheme for heavy metal analyses has been implemented in Belgium (quality control in Belgium, QCB) by the Epidemiology Unit of the Institute of Hygiene and Epidemiology since 1993. Aims of the scheme are to improve laboratories performances by means of interlaboratory comparisons and to provide national authority with information about the dispersion of results for heavy metal analyses in Belgium. The analytes included in the QCB are lead, cadmium and selenium in blood. Control samples are in-house prepared from human blood, spiked with appropriate amounts of Pb, Cd and Se. Four exercises are performed every year and participants receive three samples to analyze. Target values are calculated for each element from the regression lines obtained by plotting the results of a reference laboratory against the added amounts. The QCB does not yet include acceptability criteria of the results and no measures are taken against poor performers at this stage, although advice is available by personal contacts with the organisers.

Key words: external quality assessment, Belgium, blood, lead, cadmium, selenium.

Riassunto *(Lo schema di valutazione esterna di qualità in Belgio. Analisi di metalli pesanti).* - Uno schema di valutazione esterna della qualità per le analisi di metalli pesanti viene organizzato in Belgio (QCB) dall'Unità di Epidemiologia dell'Istituto di Igiene e Epidemiologia a partire dal 1993. Scopi dello schema sono di migliorare le prestazioni analitiche dei laboratori mediante confronti interlaboratoriali e di fornire alle autorità nazionali alcune informazioni riguardanti la dispersione dei risultati delle analisi di metalli pesanti in Belgio. Gli analiti inclusi nel QCB sono piombo, cadmio e selenio nel sangue. I materiali di controllo sono preparati in laboratorio da sangue umano addizionato con opportune quantità di piombo, cadmio e selenio. Vengono effettuati quattro esercizi ogni anno e ciascun laboratorio riceve tre campioni da analizzare. I valori attesi sono calcolati per ogni elemento dalle rette di regressione ottenute tra i risultati di un laboratorio di riferimento e le quantità aggiunte. A questo stadio del programma, il QCB non ha adottato criteri di accettabilità dei risultati, né vengono presi provvedimenti nei confronti dei laboratori che dimostrano prestazioni di scarso livello, ma è possibile discutere i problemi incontrati con gli organizzatori.

Parole chiave: valutazione esterna di qualità, Belgio, sangue, piombo, cadmio, selenio.

Introduction

The laboratory implementing the external quality assessment scheme (EQAS) in Belgium (quality control in Belgium, QCB) is also responsible for the programme of surveillance of the general population to determine exposure to heavy metals.

Since 1973, the authors have worked in the field of trace metal analyses and the acquired experience in analytical management has been useful to help the participating laboratories to improve their analytical performance.

To reach this objective, the QCB organisers carried out research on the best approach to treat outliers and define target values and acceptability criteria of results. This included research on deviation from consensus values, correlation between measured and target value, recovery of added analyte and elimination of systematic bias.

All this was put into practice during a five year epidemiological study "Cadmibel" conducted in Belgium by scientific institutions and universities to assess the effects of environmental exposure to cadmium and lead on the blood levels of these metals in the general population. In support of that study, internal and external QC programmes were implemented together with intercomparisons on 10% of all samples between the participating laboratories. To be able to assess possible risks to the population at the low levels of exposure in the general environment, special attention was paid to the evaluation of accuracy, the day-to-day precision, time trend or other possible sources of bias [1].

Belgian round-robin

The Epidemiology Unit laboratory of the Institute of Hygiene and Epidemiology is in charge of the organization of an EQAS for Belgian laboratories involved in trace element analyses. The laboratories participate on a voluntary basis since no legislation has been implemented to compel external quality control in this analytical field.

The aim of the first years, in 1993 and 1994, was to allow each laboratory to evaluate its own performance in terms of accuracy and precision.

In 1993, 37 laboratories participated in the EQAS for lead in blood and 28 for cadmium. Every three months, each laboratory received 3 blood samples spiked with different amounts of lead and cadmium.

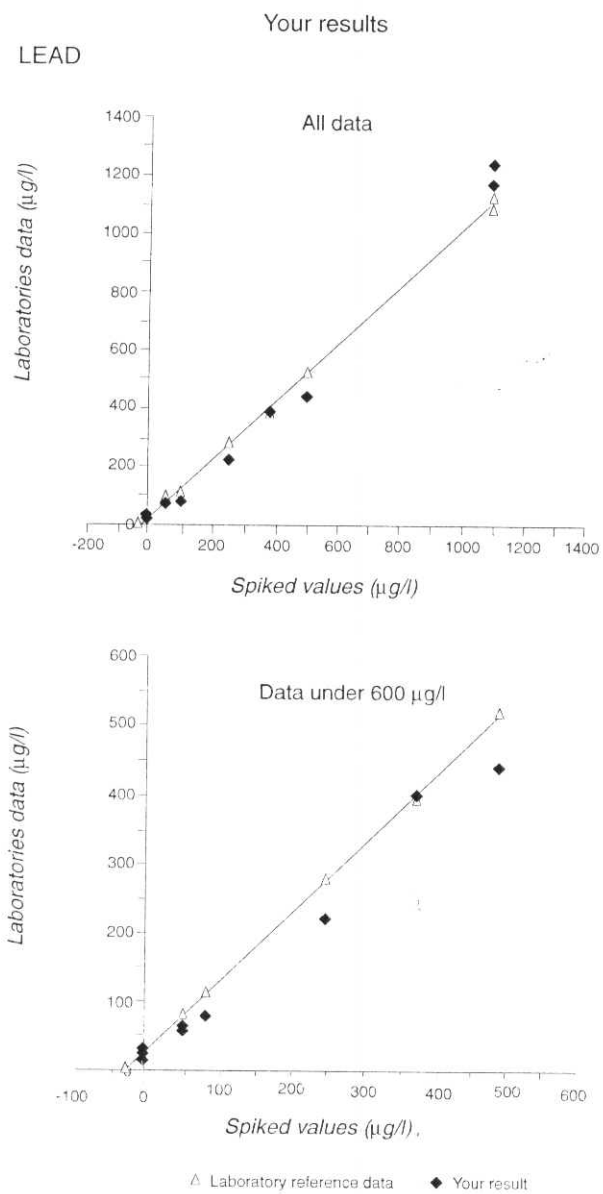

Fig. 1. - Reference regression line together with individual laboratory values (1993).

Control samples were prepared in January for the whole year with blood supplied by the Red Cross Transfusion Centre, containing EDTA as anticoagulant. To ensure haemolysis and homogeneity, the blood samples were frozen, thawed, sonicated and then pooled. The blood pool was divided in twelve batches, some were used as such, while others were spiked with various amounts of cadmium and lead. The final concentrations covered the range of values found in the general population as well as among subjects exposed at work to heavy metals.

In 1993, the range of values for lead was: from 30 µg/l (unspiked blood level) to 1130 µg/l, and, for cadmium: from 0.6 µg/l (unspiked blood level) to 15.6 µg/l. In 1994, the values were: from 56 µg/l (unspiked blood level) to 1006 µg/l for lead, from 0.97 µg/l (unspiked blood level) to 25.97 µg/l for cadmium, and from 72 µg/l (unspiked blood level) to 152 µg/l for selenium, respectively.

One university laboratory, not taking part in the assessment, was asked to analyze the 12 samples with special care and without knowledge of the protocol for preparation, by means of inductively coupled plasma - mass spectrometry. The university laboratory results were plotted against the spiked amounts and the regression line was calculated, from which the best estimate of the true values was obtained.

In the quarterly reports, the analytical performances of individual laboratories were given in terms of descriptive statistics (number, median, mean, standard deviation, range with and without outliers) together with the graphs of the distribution of the general results.

Outliers were excluded taking into account the four following criteria:

- personal appreciation of the cut-off;
- a t-test applied on extreme values to determine whether they were part of the distribution;
- two Dikson's tests based on an acceptable deviation from: a) the median value and b) the concentration estimated from the recovery of the added amounts.

Hereby any values eliminated by three out of the four criteria were considered as outliers and excluded from statistical treatment.

In the annual report, sent to each laboratory, its individual results and the results obtained by the reference laboratory are plotted against the spiked amount.

In 1993, the general equation of the regression line giving estimated values was:

for lead: $[Pb] = 0.9678\ X + 28.7046 \quad r = 0.9997$
and for cadmium: $[Cd] = 0.9250\ X + 0.6158 \quad r = 0.9985$

X being the concentration corresponding to the added amount and r the correlation coefficient.

In 1994, the general equation of the regression line giving estimated values was:

for lead: $[Pb] = 0.9857\ X + 56.18 \quad r = 0.9973$
and for cadmium: $[Cd] = 0.9737\ X + 1.0227 \quad r = 0.9994$.

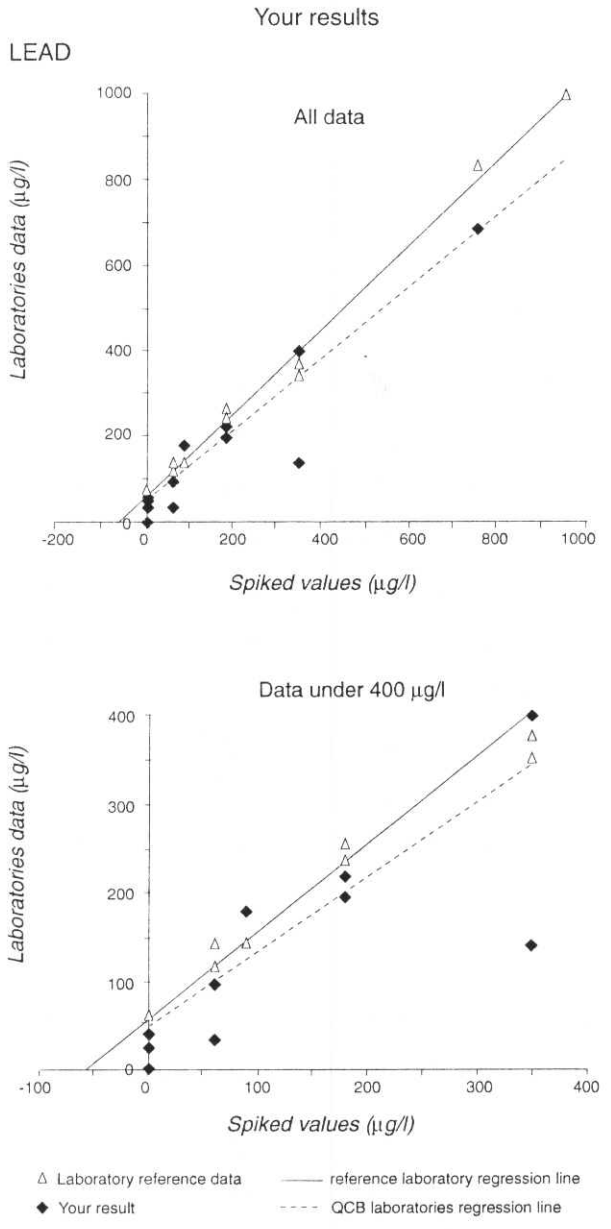

Fig. 2. - Reference and QCB laboratories regression lines together with individual laboratory values (1994).

Both graphical and numerical evaluations of their own results are provided to participants. An example of this evaluation is presented for the results obtained by one laboratory for lead in blood in 1993 (Fig. 1 and Table 1) and in 1994 (Fig. 2 and Table 2). The reference regression line together with individual values found by the laboratory is presented in Fig. 1. The reference regression line, the regression line for all the QCB laboratories together with individual values found by one laboratory are presented in Fig. 2.

Tables 1 and 2 gather for each element and sample the following data: the spiked amount, the consensus mean, median and standard deviation, the estimated values derived from the regression line, the laboratory's own results and their deviations from the estimated values.

From Table 1 it can be seen that each laboratory received 12 samples coded 1 to 12, some of which had the same concentration. For each concentration level (duplicate or triplicate samples differently coded) a graph is obtained reporting all individual results.

Fig. 3 gives an example of the graph obtained for the four identical unspiked samples distributed in 1993, containing an estimated cadmium concentration of 0.62 µg/l. To help reading the graph, the estimated value (most likely true value) is indicated by the vertical line. In collecting the data corresponding to the same concentration level, this graph gives an idea of the overall precision.

The laboratories are classified according to the analytical technique used. Most laboratories use electrothermal atomization atomic absorption spectrometry. The background corrector facility used (Zeeman or Deuterium) divided laboratories into two major groups. The six remaining laboratories use different techniques (flameless atomic absorption spectrometry with Delves cup and anodic stripping voltametry), or did not specify their analytical procedures.

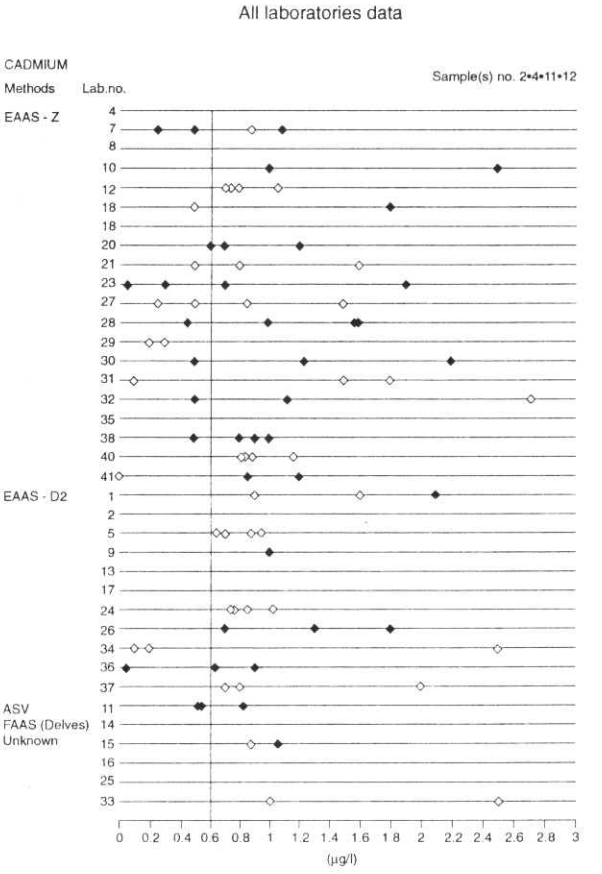

Fig. 3. - Replicate results for samples differently coded (1993).

Table 1. - Quality control in Belgium. Annual report 1993

Lead (µg/l)

Sample no.	Your results	Spiking (*)	Estimated value	Consensus			Your deviation
				Median	Mean	SD	
1	221	b + 250	270.67	261	279.8	63	- 49.67
2	31	b	28.70	42	48.7	19.2	2.30
3	436	b + 500	512.23	481	512.4	112	- 76.3
4	1155	b + 1100	1093.34	946	978.8	124.2	61.66
5	19	b	28.70	40	45.2	14.7	- 9.70
6	78	b + 80	106.13	111	111.9	25.8	- 28.13
7	66	b + 50	77.10	84.2	96.2	41.2	- 11.10
8	24	b	28.70	35	40.1	15.7	- 4.70
9	63	b + 50	77.10	82.8	90.9	39.3	- 14.10
10	389	b + 380	396.49	386	371.7	67	- 7.49
11	1227	b + 1100	1093.34	907	929.5	172.1	133.66
12	29	b	28.70	40	43	19.6	0.30

Cadmium (µg/l)

Sample no.	Your results	Spiking (*)	Estimated value	Consensus			Your deviation
				Median	Mean	SD	
1	2.7	b + 0.5	1.08	1.38	1.83	1.36	1.62
2	1.9	b	0.62	0.85	1.13	0.87	1.28
3	7.9	b + 9	8.94	8.2	8.24	3.13	- 1.04
4	0.7	b	0.62	0.79	0.86	0.41	0.08
5	2.6	b + 2	2.47	2.6	3.09	1.34	0.13
6	11.1	b + 15	14.49	12.15	13.29	5.61	- 3.39
7	4.1	b + 5.5	5.70	5.34	5.17	2.45	- 1.60
8	2	b + 2	2.47	2.1	2.32	1.2	- 0.47
9	4.2	b + 5.5	5.70	5	4.69	2.33	- 1.50
10	5.5	b + 7	7.09	6.5	6.35	2.12	- 1.59
11	0.05	b	0.62	0.84	0.86	0.54	- 0.57
12	0.3	b	0.62	0.88	1.02	0.69	- 0.32

(*) b = unspiked blood sample.

Table 2. - Quality control in Belgium. Annual report 1994

Lead (µg/l)

Sample no.	Your results	Spiking (*)	Estimated value	Consensus			Your deviation
				Median	Mean	SD	
1	196	b + 180	233.61	202	201.75	28.1	- 37.61
2	96	b + 60	115.32	98.25	101.18	21.08	- 19.32
3	43	b	56.18	41	41.52	17.38	- 13.18
4	180	b + 90	144.89	125	125.81	22.9	35.11
5	1020	b + 950	992.60	847	856.01	155.39	27.41
6	400	b + 350	401.18	344.5	349.53	55.23	- 1.18
7	220	b + 180	233.61	224.4	227.2	25.14	- 13.61
8	30	b	56.18	49	49.37	13.81	- 26.18
9	680	b + 750	795.46	666.5	661.89	97.87	- 115.46
10	140	b + 350	401.18	324	315.39	43.01	- 261.18
11	33	b + 60	115.32	100	98.81	17.54	- 82.32
12	0.5	b	56.18	42.45	45.47	12.05	- 55.68

Cadmium (µg/l)

Sample no.	Your results	Spiking (*)	Estimated value	Consensus			Your deviation
				Median	Mean	SD	
1	6.8	b + 9	9.74	7.91	7.84	2.82	- 2.94
2	0.25	b	0.97	0.6	0.77	0.68	- 0.72
3	25.6	b + 25	25.32	20.75	21.43	6.62	0.28
4	5.3	b + 6	6.82	5.84	6.01	0.92	- 1.52
5	0.1	b	0.97	0.5	0.64	0.57	- 0.87
6	3.2	b + 3.5	4.38	3.73	3.75	1.06	- 1.18
7	9.5	b + 9	9.74	9	8.64	2.32	- 0.24
8	30	b + 25	25.32	22.05	22.99	6.37	4.68
9	1.5	b + 1.2	2.14	1.56	1.9	0.92	- 0.64
10	2.6	b + 3.5	4.38	3.05	3.08	0.79	- 1.78
11	0.05	b	0.97	0.58	0.65	0.42	- 0.92
12	9.7	b + 12	12.66	9.7	9.73	2.21	- 2.96

(*) b = unspiked blood sample.

At present the distribution of the results is still rather wide. As soon as a sufficient number of results will be normally distributed, acceptability criteria will be elaborated and proposed to the participants.

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Appendix. - Summary of the scheme

Country	Belgium.
Name of scheme	Quality control Belgium (QCB).
Status of scheme	National. Voluntary. Run by the Epidemiological Unit of the Institute of Hygiene and Epidemiology. *Aims:* the aim is on one hand to help laboratories to improve the quality of their results and on the other hand to provide national authorities with information on the dispersion presently encountered among the Belgian laboratories. *Participants* in 1993: lead analysis no. = 37; cadmium analysis no. = 28. *Participants* in 1994: lead analysis no. = 27; cadmium analysis no. = 23; selenium analysis no. = 8.
Scheme description	*Control materials:* prepared in house, from a human matrix (blood from blood donors); multielemental; sample enrichment is obtained by addition of inorganic lead, cadmium and selenium; liquid samples are stored frozen in plastic vials, at -18 °C. The target values are obtained from the regression line calculated from the results of a reference laboratory plotted against the added amounts. *Internal quality control samples:* no provision of calibrators/internal quality control samples. *Organization of EQA exercises:* samples are sent every three months, three samples per batch. The results are returned within one month, by mail. *Elaboration of results*: reports include a frequency distribution graph together with: number of results (after trim), mean (µg/l), SD (µg/l), CV (%), median (µg/l), range before trim (µg/l), range after trim (µg/l), the laboratory results. *Criteria for evaluation of laboratory performance*: none. *Measures taken against poor performers*: none. *Advice and training:* given by personal contacts when asked for. *Financial support:* a financial contribution is requested from each laboratory and the program is partly supported by national financing (towards salary of a person involved in the program).
Organization	Institute of Hygiene and Epidemiology Rue Juliette Wytsman, 16 B-1050 Brussels, Belgium Contact person: Pierre De Plaen Tel 32-2/6425024. Fax 32-2/6425410 e-mail: pierre.de.plaen@ihe.be
Analytes and matrices covered	Blood lead, blood cadmium and blood selenium.