Blood Cadmium Determination—Results of an External Quality Assessment Scheme

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Laboratory performance with respect to blood cadmium determination in the Guildford Trace Elements External Quality Assessment Scheme has been examined. Between March 1982 and September 1985 over 20 laboratories participated in the scheme and more than half submitted results regularly. A methodology survey showed that, whilst more laboratories used electrothermal atomic absorption spectrometry (ETA-AAS), sample pre-treatment, standardisation procedures and furnace temperature programmes varied enormously. Mean values for recovery of cadmium added to blood during each 6-monthly cycle were between 91 and 102% but varied more widely for individual samples (81–117%). Inter-laboratory coefficients of variation usually exceeded 20% at cadmium concentrations below 100 nmol l^{-1} (11.2 μg l^{-1}) but were lower (12–20%) at higher concentrations. No demonstrable changes in these parameters during the period of study were evident and it is concluded that laboratory performance with respect to cadmium analysis requires considerable improvement.

Keywords: *Blood cadmium determination; quality assessment scheme*

The health hazards of increased cadmium absorption from industrial processes and environmental pollution are well recognised.[1,2] Absorbed cadmium is excreted very slowly[3] and continued exposure is associated with pulmonary, renal and hepatic damage. Measurement of Cd in blood has been shown to be of value in the assessment of occupational cadmium exposure (*e.g.*, battery manufacture, welding and metal refining)[4,5] and environmental cadmium exposure (*e.g.*, tobacco smoke, vegetables from cadmium-rich soils and shellfish).[6,7]

Many methods for blood cadmium determination have been described. They include those dependent upon chelation of cadmium by pyrrolidines[8] with subsequent organic extraction and measurement by either flame atomic absorption spectrometry (FAAS)[9] or ETA-AAS.[8] Others[9] have used the microsampling technique of Delves[10] to avoid laborious sample preparation. Alternative techniques, including fluorescence[11] and anodic-stripping voltammetry,[12] have been used, but most of the methods reported more recently have been based on ETA-AAS.[13–17]

The multiplicity of methods in use for blood cadmium determination has led to a great diversity of results, as evidenced by the wide range of reference intervals quoted.[2] The lack of suitable reference material with assigned values for blood cadmium concentration renders the assessment of analytical performance difficult. This led to inclusion of blood cadmium in the Guildford Trace Elements External Quality Assessment Scheme. The results from this scheme are presented here, and the performance of laboratories is discussed.

Experimental

Apparatus

All glass- and plastic-ware used for preparation of control material was immersed in hydrochloric acid (10% *V/V*) for 24 h, rinsed in de-ionised, reverse osmosis treated water and dried in a dust-free cabinet prior to use. Trace metal free polycarbonate tubes for the distribution of samples were obtained from Teklab, Durham and each batch was checked for Cd contamination prior to use. Cadmium measurements were performed using a PU9000 furnace atomic absorption spectrometer with autosampler (Pye Unicam, Cambridge).

Reagents

Nitric acid (Aristar), saponin and cadmium nitrate (1 mg ml^{-1}) standard solutions were purchased from BDH Chemicals, Poole, Dorset. Blood was drawn from healthy volunteers and EDTA (dipotassium salt) was used as an anticoagulant (final concentration 2 mg ml^{-1}). Water was purified before use by de-ionisation and reverse osmosis (Elgastat Spectrum System, Elga, High Wycombe, Bucks).

Operation of the Quality Assessment Scheme

The preparation and distribution of samples, the statistical treatment of the results and the assessment of laboratory performance have been described in detail elsewhere.[18] The general outline of operation is summarised in Fig. 1.

Methodology Survey

Laboratories were asked, by means of a questionnaire, for details of their method for blood cadmium determination. Information was requested concerning sample pre-treatment, assay standardisation, instrumentation and, where applicable, furnace conditions in use.

Other Investigations

To determine the contribution of inter-sample variability to the range of results returned by scheme participants, 15 samples from each of three distributed batches of material were assayed in the authors' laboratory.

Results and Discussion

Data on the mean recoveries of added cadmium (Table 1) demonstrated that, for each 6-monthly period, the mean recovery of cadmium from blood approximated to 100% (91.3–101.8%), implying that the additions of cadmium to blood were accurate. Mean recoveries found for individual samples distributed during each 6-monthly period were, however, much more variable (81–117%). This was particularly evident where the additions of cadmium were small and

probably reflects the greater difficulty experienced by laboratories in measuring accurately low [less than 50 nmol l^{-1} (5.6 μg l^{-1})] cadmium concentrations.

The inaccuracies of the laboratory analysis of low cadmium concentrations are further exemplified in Fig. 2, which shows the inter-laboratory variation of results for cadmium determination in different concentration ranges during consecutive time periods. The inter-laboratory coefficients of variation at Cd concentrations of less than 50 nmol l^{-1} (5.6 μg l^{-1}) varied considerably (17–46%) but usually approximated to 40%. At higher cadmium concentrations (100–300 nmol l^{-1}) these coefficients of variation were in the range 12–20%.

The contributions of sample-to-sample variation, sample quality and sample stability to the range of results found by participants were investigated by assaying samples from three distributions covering a wide concentration range (Table 2). To simulate distribution within the scheme, these samples were stored initially at −20 °C, and then for 15 d at 4 °C prior to analysis. Samples were analysed within the same analytical batch. The coefficients of variation found were similar to those for within-batch precision data generated by repeated analysis of a single sample, implying that samples can be considered as identical. Inter-laboratory coefficients of variation were higher by a factor of 2.6–7.7. Whilst it could be argued that samples assayed in this manner would be expected to give much better precision than that found in an inter-laboratory comparison, the discrepancy found is large. Between-batch precision data from the authors' laboratory demonstrate that the between-batch coefficient of variation is normally less than twice that found within-batch at the same concentration.

The results from the methodology survey are summarised in Tables 3 and 4. To maintain anonymity the laboratory codes shown are not those used in the Quality Assessment Scheme. Pre-treatment of samples prior to analysis (Table 3) varied enormously; from no treatment as in the Delves cup procedure, to protein precipitation or dilution in complicated solutions. Despite the use of matrix modifiers (*e.g.*, Triton), ashing agents (*e.g.*, ammonium nitrate) or matrix removal (*e.g.*, by precipitation with nitric acid), no laboratory used aqueous standards. All laboratories used either matrix-matched standards or standard additions techniques, presumably because the effects of the blood matrix on assay procedures could not be completely eliminated. All but one laboratory used ETA-AAS for the analysis, reflecting the greater sensitivity achievable by this technique compared with flame AAS. No laboratories in the group used the older chelation-based methodologies. Furnace instruments from five different manufacturers were in use and the wide diversity of furnace cuvettes, temperature programmes and modes of background correction used are summarised in Table 4. Six laboratories used furnace cuvettes of pyrolytically coated graphite rather than uncoated tubes, presumably to confer lower detection limits to their assays. No laboratory, however,

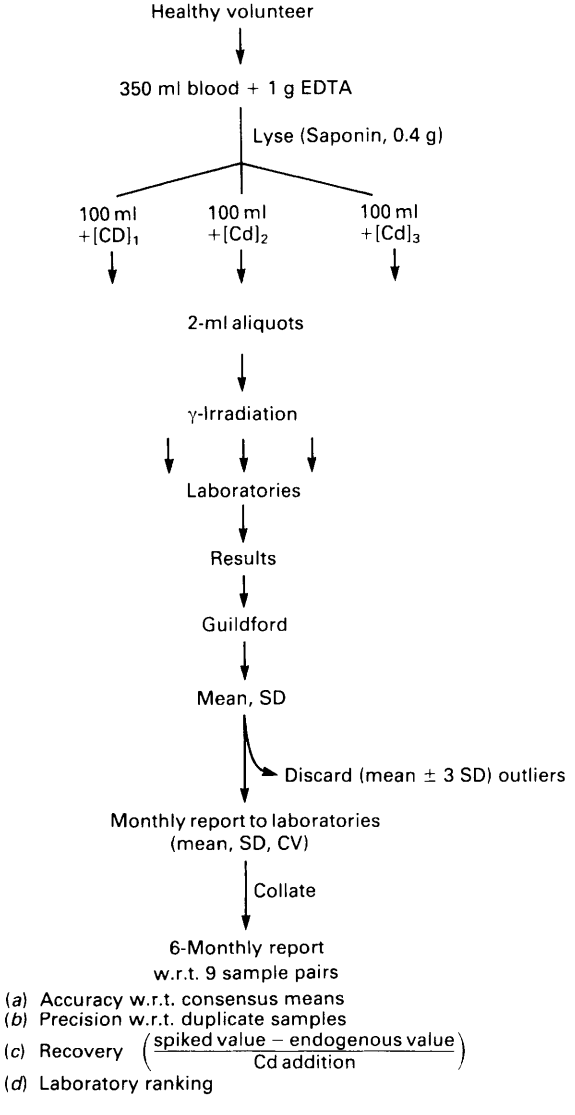

Fig. 1. Operation of the scheme

Table 1. Recovery of added cadmium from blood

Period	No. of participants	*N**	Mean recovery, %	Range,† %
April '82–Sept. '82 . .	21	24	100.6	88–115
Oct. '82–March '83 . .	20	18	98.2	85–115
April '83–Sept. '83 . .	17	20	101.8	94–117
Oct. '83–March '84 . .	21	20	93.4	82–108
April '84–Sept. '84 . .	22	21	97.3	86–107
Oct. '84–March '85 . .	18	18	91.3	82–100
April '85–Sept. '85 . .	17	19	94.0	81–112

* *N* is the mean number of results per sample pair. Cadmium added to samples varied from zero to 300 nmol l^{-1}.

† Range of mean cadmium recovery from each sample distributed.

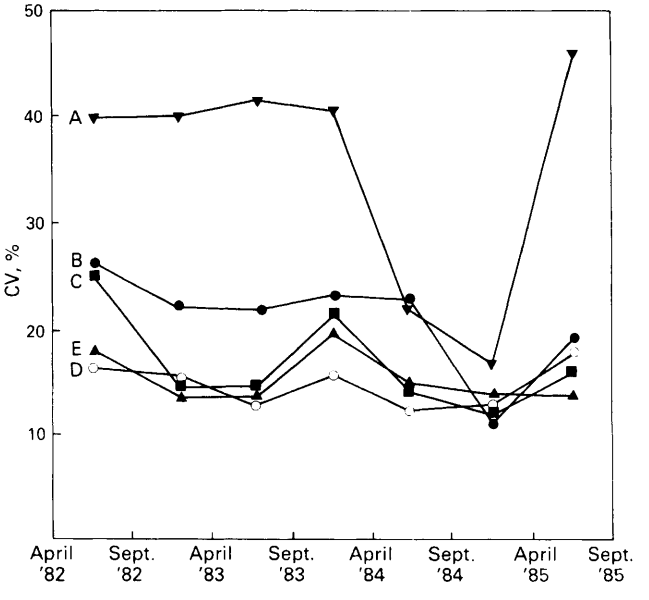

Fig. 2. Inter-laboratory coefficients of variation at different cadmium concentrations. The results represent the mean inter-laboratory coefficients of variation (%) during consecutive 6-monthly periods for different ranges of blood cadmium concentration: A, 0–50; B, 50–100; C, 100–150; D, 150–200; and E, 200–300 nmol l^{-1}

Table 2. Comparison of intra- and inter-laboratory precision

Intra-laboratory*			Inter-laboratory		
N	Mean/ nmol l^{-1}	CV, %	*N*†	Mean/ nmol l^{-1}	CV, %
15	8.2	10.5	6	6.6	17.5
15	135.1	2.2	9	134.4	17.1
15	302.0	2.2	9	299.8	17.0

* Analyses were performed within the same analytical batch in the intra-laboratory study.

† In the inter-laboratory study, *N* is the number of laboratories each returning a result.

used a stabilised temperature platform. The use of such a technique[19] may help circumvent some of the matrix interferences incumbent in blood cadmium measurement. The great diversity of temperature programmes reflect, in part, the different instruments in use. In blood cadmium determination the ashing phase is critical. Cadmium may be lost at temperatures as low as 400 °C, which is too low to ensure complete removal of organic matter. Several laboratories in the group used addition of phosphate salts to stabilise cadmium and prevent its co-volatilisation with organic material, enabling ashing temperatures of up 650 °C to be used. Great care is essential, however, to ensure that minor variations in the ashing temperatures chosen do not lead to

Table 3. Methodology survey—sample pre-treatment

Laboratory code	Sample diluent	Pre-dilution (diluent : sample)	Source of standard*
A	30% 2,2′ diaminodiethylamine - 30% HNO_3 - 30% water	4 : 1	Merck
B	1% $NH_4H_2PO_4$	2 : 1	BDH
C	Triton X-100 - $(NH_4)_3PO_4$	6 : 1	BDH
D	5% HNO_3 (pptn.)	4 : 1	BDH
E	Triton X-100 - $(NH_4)_2HPO_4$	11 : 1	BDH
F	Triton X-100 - NH_4NO_3 - EDTA	10 : 1	BDH
G	0.02% Triton X-100 - 3% HNO_3	24 : 1	BDH
H	Triton X-100 - $(NH_4)_2SO_4$ - NH_4NO_3	5 : 1	BDH
I	5% HNO_3 (pptn.)	5 : 1	BDH
J	$NH_4H_2PO_4$ - HNO_3	NS†	BDH
K	Conc. HNO_3 digestion - $(NH_4)_3PO_4$ dilution	40 : 1	BDH
L	Triton X-100	5 : 1	BDH
M	0.5% Triton X-100 - 5% HNO_3	NS	BDH
N	0.2% Triton X-100 - 0.5% $(NH_4)_3PO_4$	12 : 1	Cd metal
O	None (Delves cup)	—	BDH
P	10% $(NH_4)_2HPO_4$	1 : 1	Merck
Q	20% HNO_3 (pptn.)	5 : 1	Merck

* All laboratories use standards in a blood matrix.

† NS = Not specified.

Table 4. Methodology survey—furnace conditions

			Dry		Ash		Atomise		
Laboratory Code	Instrument	Tube type	Temperature/ °C	Time/s	Temperature/ °C	Time/s	Temperature/ °C	Time/s	Background correction*
A	Hitachi	TaO_5 coated	120 150	60 20	300 600	20 30	1300	7 (GS)†	Z
B	Perkin-Elmer	Pyro-coated	110	20	600 (O_2)‡ 400	30 10 (cool)	2100	3	Z
C	Perkin-Elmer	Uncoated	85 100	10 30	500	30	2200	3	D_2
D	Perkin-Elmer	Uncoated	130	5	350	10	1900	6 (GS)	D_2
E	Perkin-Elmer	Pryo-coated	110 150	5 15	400 (O_2) 500	15 10	2200	6 (GS)	D_2
F	Varian	Uncoated	100	20	550	15	2000	1	D_2
G	IL	Uncoated	160	5	280	10	1850	5	S/H
I	Pye Unicam	Uncoated	80 120	2 10	400	30	2100	4	D_2
K	IL	Pyro-coated	150 (FASTAC)	—	300	—	1500	—	D_2
L	IL	Pyro-coated	Manual	Drying	450	20	1750	1	D_2
N	Perkin-Elmer	Pyro-coated	80 110	5 30	650 650	5 15	2300	8 (GS)	D_2
Q	IL	Pyro-coated	70	5	225 275 287	10 30 30	1400	5	D_2

* Z = Zeeman; D_2 = deuterium; S/H = Smith - Hieftje.

† GS = Gas stop.

‡ O_2 = Oxygen ashing.

cadmium loss. Deuterium background correction was used by most of the group. Zeeman correction, however, used by only two laboratories, has been advocated as a more accurate means of background correction for urinary cadmium determination[20] and may likewise improve the accuracy of the determination of cadmium in blood.

Examination of the methods used in the light of performance within the scheme allows no firm recommendations to be made with regard to the best analytical procedure for cadmium determination. Although each 6-monthly report ranks laboratories for analytical performance with respect to accuracy relative to concensus mean values, precision with respect to duplicate samples and recovery of added cadmium, no laboratory performed consistently well in all these respects, and no single laboratory performed better than the other laboratories in every 6-monthly cycle.

The great diversity of sample pre-treatment procedures, instrumentation and temperature programmes in use probably contributes to the wide variation in results which, from studies outlined here, is unlikely to be due to sample-to-sample variability, poor sample quality or sample instability. The poor laboratory performance may be attributed to contamination of samples within the laboratories, to procedural losses during sample pre-treatment or the use of inappropriate temperature programmes that may lead to cadmium loss during ashing or to inadequate background correction. There is a real need for reliable quality assurance material with agreed, assigned values for cadmium concentration. The use by laboratories of the same internal quality control material with well defined acceptance limits would do much to improve laboratory performance and reduce inter-laboratory variation.

In conclusion, current laboratory performance with respect to cadmium determination is poor and requires considerable improvement. This paper highlights the need for laboratories to pay strict attention to the quality of the results they produce.

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