EUROPEAN COMMISSION
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BCR-636

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Report EUR 21066

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REFERENCE MATERIALS

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ABSTRACT

This report documents the preparation, homogeneity and stability study as well as the certification campaign of three lyophilised human whole blood materials, i.e. BCR-634, BCR-635 and BCR-636 with certified concentrations of lead and cadmium. The homogeneity and stability studies demonstrated that the materials are homogeneous and stable with respect to storage and transportation. The materials were subjected to a certification campaign for which the analytical work is described.

The concentration and uncertainty (expanded uncertainty with a coverage factor of k=2) of lead and cadmium are

- for BCR-634: (46 ± 5) µg/L Pb and (1.4 ± 0.4) µg/L Cd;
- for BCR-635: (210 ± 24) µg/L Pb and (6.6 ± 0.6) µg/L Cd;
- for BCR-636: $(0.52 \pm 0.05) \cdot 10^3$ µg/L Pb and (11.6 ± 0.6) µg/L Cd.

ABBREVIATIONS AND SYMBOLS

ASV	Anodic Stripping Voltammetry
BCR	Community Bureau of Reference
CRM	Certified Reference Material
CI	Confidence interval
CV	Coefficient of Variation
DPASV	Differential Pulse Anodic Stripping Voltammetry
ETAAS	Electrothermal Atomic Absorption Spectrometry
FAAS	Flame Atomic Absorption Spectrometry
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ISO	International Organization for Standardization
SM&T	Standards, Measurements and Testing
SD	Standard deviation
U	Uncertainty value
u_{bb}	Uncertainty component from homogeneity
u^*_{bb}	Upper limit of inhomogeneity that can be hidden by the method repeatability
u_{char}	Uncertainty component from batch characterisation
U_{CRM}	Expanded uncertainty of CRM
u_{lts}	Uncertainty component from long-term stability
u_{sts}	Uncertainty component from short-term stability

CONTENTS

1. INTRODUCTION

1.1 Justification and scope of the project

Health and safety at work is an important issue within the European Union. Exposures to toxic and carcinogenic elements such as lead (Pb) and cadmium (Cd) are considered a major environmental and occupational problem. Such exposure may pose both a short and a long term health risk to workers and populations at large living in industrial regions.

The EC Directive 80/1107/EEC [1] has been introduced to protect workers from risks related to exposure to chemical, biological and physical agents. More specific Directives have been introduced for lead (77/312/EEC and 82/605/EEC [2-3]), for carcinogenic substances (90/394/EEC [4]), and most recently a Directive (98/24/EC [5]) on chemical agents.

In occupational health, legislation often stipulates threshold limit values for concentrations of chemical agents in workplace air (i.e. Directive 91/322/EEC on recommended threshold limit values [6]). However, biological monitoring is a very important tool to assess exposure to chemicals where air monitoring alone may not be a reliable indicator of exposure, and to assess overall exposure, occupational and non-occupational, by being a more accurate assessment of the internal dose. When health risk has to be assessed and actions have to be taken to reduce exposure, reliable data from measurements for toxic and carcinogenic elements in human tissues and biological fluids are essential. In this context, the traceability of measurements needs to be documented by the use of certified reference materials (CRMs). Therefore, the availability of CRMs for trace elements in biological fluids is of great importance.

The Standards, Measurements and Testing Programme (SM&T, formerly BCR) of the European Commission has the task to assist in improving the quality of measurements. Therefore a project dealing with the production of three lyophilised human whole blood reference materials certified for the contents of Pb and Cd was launched.

The concentrations of Pb and Cd in BCR-634 (low level), BCR-635 (medium level) and BCR-636 (high level) were selected on the basis of the currently accepted values for non- occupationally exposed individuals and on *American Biological Exposure Indices* (BEI values) [7] and *German Biologische Arbeitsstoff-Toleranz-Werte* (BAT values) [8]. Where no BEI or BAT values exist, the elevated concentrations were based on data from occupationally exposed workers.

The CRMs will contribute to the harmonisation and comparability of methodological approaches and measurements of Pb and Cd between different member states, thus enabling economic and political decisions.

1.2 The certification procedure

The work of certification was co-ordinated by the National Institute of Occupational Health, Denmark on behalf on the Standards, Measurements and Testing Programme of the European Commission, Brussels.

After a feasibility study and a preparatory technical meeting in which all the technical requirements were carefully discussed, the candidate reference materials were produced. The homogeneity and stability of the materials were documented and the materials were shipped to the laboratories participating in the certification campaign (see chapter 2). The participants in the certification campaign all demonstrated satisfactory analytical performance in a preliminary intercomparison study.

In the certification campaign each participant was requested to analyse the candidate CRMs under reproducibility conditions, i.e. analyses performed on different days with different preparations of calibrants. Furthermore, the participants were requested to submit information on the pre-treatment of the samples (e.g. digestion procedures etc.), the detection principle (e.g. graphite furnace atomic absorption spectrometry, inductively coupled plasma mass spectrometry etc.) and detailed information on the calibration, e.g. the type of calibration (standard additions, matrix-matching calibrants etc.), the calibrant (reference material, spectroscopic standard etc.), the producer of the calibrant and the purity of the calibrant.

The different analytical techniques applied in the certification campaign were electrothermal atomic absorption spectrometry (Zeeman background correction), electrothermal atomic absorption spectrometry (deuterium background correction), flame atomic absorption spectrometry (microsampling cup), high resolution inductively coupled plasma mass spectrometry and differential pulse anodic stripping voltammetry.

The results from the certification campaign were scrutinised at a technical discussion meeting with the participants and then subjected to a statistical evaluation. The certification is based upon agreement between the results of the range of different methods applied in the different participating laboratories.

2. PARTICIPANTS

2.1 Co-ordination, sample preparation, homogeneity and stability studies

- National Institute of Occupational Health, Copenhagen DK

2.2 Analyses

- Analytisch Biochemisch Laboratorium, Assen NL
- Centre for Analytical Sciences, University of Southampton UK
- GSF – National Research Center for Environment and Health Oberschleissheim DE
- Health & Safety Laboratory, Sheffield UK
- Institut für Arbeits- und Umweltmedizin, Universität München DE
- Institut National de Recherche et de Sécurité, Vandoeuvre FR
- Institute of Occupational Health, University of Brescia IT
- Instituto Nacional de Seguridad e Higiene en el Trabajo, Barakaldo ES
- Istitute Superiore di Sanitá, Rome IT
- National Institute of Occupational Health, Copenhagen DK
- Robens Institute, University of Surrey UK
- Scientific Institute of Public Health, Brussels BE
- Sporstoflaboratoriet, Odense Universitetshospital, Odense DK
- Statens arbeidsmiljøinstitutt, Oslo NO

2.3 Statistical evaluation

- National Institute of Occupational Health, Copenhagen DK
- European Commission, DG JRC, Institute for Reference Materials and Measurements, Geel BE

3. PRELIMINARY INVESTIGATIONS

Pilot batches of the lyophilised blood materials were produced in 1996 and a feasibility study was organised with the participation of 22 laboratories for determination of Pb and/or Cd. The purpose of the study was to prove the suitability of the materials produced and to identify any sources of errors in the production and chemical analyses. Furthermore, the pilot batches should serve as test samples to improve the skills of the participating laboratories for the certification campaign. For each of three concentration levels of the blood material 300 samples were produced by the co-ordinator. The low concentration level was representative for an environmental exposure level, the medium and high levels were comparable to occupational exposure levels.

The results from all laboratories were analysed according to the procedure described in the BCR Guidelines [9]. Laboratories were excluded from the evaluation according to the following criteria:

- Those laboratories not present at the meeting.
- Those laboratories using an inadequate detection technique.
- Those laboratories using an inadequate calibration technique.

The results were scrutinised for outliers, and technical reasons for suspected outliers were sought. If no technical reason for the suspect results could be found, the results were retained. No results were excluded from the evaluation due to statistical reasons only.

3.1 Results of the preliminary intercomparison study

3.1.1 Lead

Ten laboratories used graphite furnace atomic absorption spectrometry (GFAAS) while 5 laboratories used other methods. There was good consistency between the results obtained by GFAAS and other methods, although Delves cup atomic absorption spectrometry consistently revealed results a little lower. The calibration techniques were of different types and included standard additions, matrix matched standards and aqueous standards. When aqueous standards were used, the standards were checked for parallellity between aqueous and blood standard curves. The aqueous Pb solutions used for manufacturing the calibration standards were obtained from at least 9 companies. Some laboratories manufactured their calibration standards from certified aqueous Pb solutions. This should ensure that the bias in the consensus values due to calibration should be small.

A summary of the preliminary intercomparison study for Pb in whole blood is presented in table 3.a.

3.1.2 Cadmium

Nine of 12 laboratories with accepted results used GFAAS. Two laboratories using ICP-MS did not show mutual agreement, while the voltammetric method gave higher results at all three levels. Thus, it is very difficult to decide if there is any agreement between different techniques.

The variation in calibration techniques and sources of the Cd used for calibration were the same as for Pb. A summary of the preliminary intercomparison study for Cd in whole blood is presented in table 3.b.

Table 3.a Summary of the preliminary intercomparison study for Pb. Target value: The value to be achieved according to the protocol. Spike: The concentration due to the spike. Consensus value: The mean of laboratory means of accepted data. Statistical outlier evaluation has not been performed. 95 % CI : The 95 % confidence interval of the consensus value. Data sets: The total number of data sets received, and the number of data sets accepted for calculating the consensus value and confidence interval.

Level	*Target value (µg/L)*	*Spike (µg/L)*	*Consensus value, (µg/L)*	*95 % CI of consensus value, (µg/L)*	*Data sets*	
					Total	Accepted
Low	35	0	24	21.7 - 25.6	25	13
Medium	200	157	190	186 - 193	26	15
High	500	419	459	442 - 476	26	14

Table 3.b - Summary of the preliminary intercomparison study for Cd. Target value: The value to be achieved according to the protocol. Spike: The concentration due to the spike. Consensus value: The mean of laboratory means of accepted data. Statistical outlier evaluation has not been performed. 95 % CI : The 95 % confidence interval of the consensus value. Data sets: The total number of data sets received, and the number of data sets accepted for calculating the consensus value and confidence interval.

Level	*Target value (µg/L)*	*Spike (µg/L)*	*Consensus value, (µg/L)*	*95 % CI of consensus value, (µg/L)*	*Data sets*	
					Total	Accepted
Low	0.9	0	0.71	0.59 - 0.82	21	12
Medium	5	3.27	3.95	3.65 - 4.26	21	12
High	10	7.63	8.2	7.5 - 8.9	20	12

3.1.3 Conclusion

The outcome of this feasibility study was promising enough to justify a certification campaign on materials produced with similar concentrations. The laboratories that demonstrated satisfactory analytical performance in the feasibility study were invited to participate in the certification campaign. Furthermore, the feasibility study had proved that there was no risk of leaking of Pb and Cd from neither vials nor stoppers.

4. PREPARATION OF THE CANDIDATE CRMS

Thirty-five donations of fresh blood were obtained from normal (healthy) Danish blood donors in October 1996. The individual donations had been tested and found negative for hepatitis B antigen, and hepatitis C and HIV 1+2 antibodies. Sodiumethylene diaminetetra acetic acid (Na-EDTA) was used as an anticoagulant to ensure the stability of the liquid phase.

The blood pool was left at room temperature for 10 days in order to ensure complete coagulation. After filtration through a 0.5 µm Millipore filter, the pool was stirred for 36 hours to obtain a homogeneous material.

4.1 Spiking

The blood pool was divided into 3 sub-batches each of 5 L. The base (unspiked) concentration levels of Pb and Cd were measured in order to calculate the amount of metal for the spiking. The material was spiked according to following scheme:

Table 4.a - Target values and spiking amounts for Pb and Cd content in BCR-634, BCR-635 and BCR-636. Values in brackets are expected endogenous concentrations.

Reference Material	*Lead (Pb)*		*Cadmium (Cd)*	
	Target value (µg Pb/L)	Spike (µg Pb/L)	Target value (µg Cd/L)	Spike (µg Cd/L)
BCR-634	(50)	0	(< 0.1)	0
BCR-635	200	154	5.0	4.9
BCR-636	500	462	10.0	9.9

Spiking solutions were prepared using NIST SRM 3128 (10.00 ± 0.03 mg Pb/mL) and NIST SRM 3108 (10.00 ± 0.03 mg Cd/mL).

After spiking and homogenisation, 3.0 mL blood was pipetted into each of 1300 brown glass vials. The variation (CV) of the filling was < 1 %.

4.2 Lyophilisation

Due to a limited volume of the freeze-drier equipment (Hetosicc CD 12 and Hetofrigg), the freeze drying of each concentration level was performed in two sub-batches in November 1996. In order to secure a more homogeneous material, each sub-batch was deep-frozen at - 50 °C overnight before lyophilisation. The frozen material was then transferred to the freeze- drying equipment and lyophilised for 48 hours under carefully controlled conditions. When the freeze-drying was completed the vials were sealed automatically under vacuum with butyl stoppers and encapsulated with an alumina cap.

The candidate CRMs were subsequently stored at -20 °C.

4.3 Residual moisture

The residual moisture content of 10 vials from each CRM was determined by the Joint

Research Centre, Institute for Reference Materials and Measurements, IRMM, in Geel, Belgium. The materials were analysed by Karl Fischer titration under dry atmospheric conditions in a glove box.

The residual moisture was < 2 % for all CRMs, which is sufficient to ensure a stable product without microbial activity. Therefore, the results (presented in table 4.b) confirm that the lyophilisation has been adequate.

Table 4.b - Residual moisture contents as mass fraction (%) determined by Karl Fischer titration

BCR-634		*BCR-635*		*BCR-636*	
1.50		0.74		1.75	
1.76		0.73		1.73	
1.89		0.81		1.71	
1.85		0.77		1.96	
1.98		0.72		1.82	
1.62		1.96		1.89	
1.61		1.86		1.83	
1.61		1.68		1.84	
1.4		1.93		1.87	
1.86		1.88		1.84	
Mean	1.71	Mean	1.31	Mean	1.82
SD	0.19	SD	0.59	SD	0.08

5. HOMOGENEITY STUDY

The homogeneity study was performed after the filling and lyophilisation process in order to determine the overall random variation originating from the potential variations during the filling and the production process.

The homogeneity was examined by analysing 10 vials from each candidate CRM. In order to detect any sub-batch-to-sub-batch variety originating from the lyophilisation, 5 vials from each sub-batch were selected randomly at each concentration level and analysed in random order.

5.1 Method

The lyophilised samples were allowed to reach ambient temperature and subsequently reconstituted by adding 3.000 ± 0.005 mL of Milli-Q water. The added volumes were controlled by weighing. The stoppers were replaced and the samples were homogenised at continuous agitation on a mixing apparatus for at least one hour at room temperature. Analysis of Pb and Cd content was performed by ETAAS (Perkin Elmer 5100-Z or Perkin Elmer SIMAA 6000 ZL). The analyses were performed as triplicate peak area measurements under repeatability conditions in order to reduce any analytical variation.

5.2 Results

The variation of Pb and Cd peak area signals was evaluated by analysis of variance, ANOVA, and the outcome of the statistical evaluation is presented in tables 5a-c. A graphical survey of the results is presented in Annex I. The between-sample variance estimated in the ANOVA included both analytical variance and inhomogeneity variance. In case of perfect homogeneity, the ratio of the between-sample variance and the analytical variance equals 1. The observed ratio was tested at 5 % significance level.

5.3 Conclusion

For all three candidate CRMs, no statistical significant between-sample or between-sub batch variation was revealed at the 5 % significance level. The materials were consequently deemed homogeneous with respect to Pb and Cd contents and judged suitable for certification.

Table 5.a - Homogeneity test of Pb and Cd contents in BCR-634. The material is homogeneous with respect to Pb and Cd (P values ≥ 5 %)

	Element	*Source of variation*	*ANOVA estimated variance, s^2*	*F-test value*	*P-value*	*Mean*
BCR-634	Pb	Subbatch	0.13 (10.07)	0.48	49.3 %	
		Samples	0.22	0.72	67.0 %	
		Analytical method	0.30	-	-	10.07
	Cd	Subbatch	1.20	0.31	58.5 %	
		Samples	4.75	1.32	29.0 %	
		Analytical method	3.60	-	-	19.63

Table 5.b - Homogeneity test of Pb and Cd contents in BCR-635. The material is homogeneous with respect to Pb and Cd (P values ≥ 5 %)

	Element	*Source of variation*	*ANOVA estimated variance, s^2*	*F-test value*	*P-value*	*Mean*
BCR-635	Pb	Subbatch	0.30	0.41	52.6 %	
		Samples	0.38	0.44	88.1 %	
		Analytical method	0.87	-	-	36.05
	Cd	Subbatch	0.03	0.01	93.4 %	
		Samples	3.85	0.76	64.1 %	
		Analytical method	5.07	-	-	70.18

Table 5.c - Homogeneity test of Pb and Cd contents in BCR-636. The material is homogeneous with respect to Pb and Cd (P values ≥ 5 %)

	Element	*Source of variation*	*ANOVA estimated variance, s^2*	*F-test value*	*P-value*	*Mean*
BCR-636	Pb	Subbatch	0.03	0.01	91.2 %	
		Samples	1.83	0.60	76.3 %	
		Analytical method	3.03	-	-	91.23
	Cd	Subbatch	6.53	0.73	40.0 %	
		Samples	3.53	0.32	95.0 %	
		Analytical method	11.13	-	-	116.52

From these data uncertainty contributions for homogeneity issues were included in the overall uncertainties of the CRMs. For further details refer to chapter 8.

6. STABILITY STUDY

The stability of the candidate CRMs was evaluated in a long-term stability study and an accelerated degradation stability study at elevated temperatures.

6.1 Long-term stability study

The long-term stability was assessed by analysing 3 samples from each candidate CRM stored at – 20 °C at two occasions (July 1997 and November 1997).

6.1.1 Method

The samples were analysed by ETAAS (Perkin Elmer 5100-Z or Perkin Elmer SIMAA 6000 ZL) using calibration standards prepared by spiking human whole blood with NIST SRM 3128 (10.00 ± 0.03 mg Pb/mL) and NIST SRM 3108 (10.00 ± 0.03 mg Cd/mL) to appropriate concentrations. Statistical control of the analytical procedure was documented by the use of control charts. Analysis of BCR-194 and BCR-195 (Lead and Cadmium in Lyophilised Blood) and participation in proficiency testing schemes served to detect the emergence of bias problems.

6.1.2 Results

Any change of concentration with time indicates instability of the materials provided that a good analytical reproducibility has been achieved. Instability is detected by comparing the means of the measured contents of Pb and Cd at the two study occasions using ANOVA. Results from the ANOVAs are presented in the tables 6.a-c.

Table 6.a - Long-term stability test of Pb and Cd contents in BCR-634. The material is stable with respect to Pb and Cd (P values ≥ 5 %)

	Element	*Source of variation*	*ANOVA estimated variance, s^2*	*F-test value*	*P-value*
BCR-634	Pb	Time	4.00	1.26	32.5 %
		Samples	3.18	-	-
	Cd	Time	0.0096	2.11	22.0 %
		Samples	0.0045	-	-

Table 6.b - Long term stability test of Pb and Cd contents in BCR-635. The material is stable with respect to Pb and Cd (P values ≥ 5 %)

	Element	*Source of variation*	*ANOVA estimated variance, s^2*	*F-test value*	*P-value*
BCR-635	Pb	Time	134.43	5.40	8.1 %
		Samples	24.88	-	-
	Cd	Time	0.0504	2.80	17.0 %
		Samples	0.0180	-	-

Table 6.c - Long term stability test of Pb and Cd contents in BCR-636. The material is stable with respect to Pb and Cd (P values ≥ 5 %)

	Element	*Source of variation*	*ANOVA estimated variance, s^2*	*F-test value*	*P-value*
BCR- 636	Pb	Time	423.36	2.41	19.5 %
		Samples	175.47	-	-
	Cd	Time	0.0600	4.62	9.8 %
		Samples	0.0130	-	-

6.1.3 Conclusion

For all three candidate CRMs, no statistical significant variation was revealed between the two study occasions at the 5 % significance level. The materials were consequently deemed stable with respect to Pb and Cd contents and judged suitable for certification. An estimate for the uncertainty related to stability, u_{lts}, was included in the uncertainty budget. This allowance will be used to establish an expiry date of the certificate. For long-term storage a temperature of –20 °C was envisaged.

6.2 Accelerated degradation stability study

The accelerated degradation stability study started in February 1997 with a duration of 24 weeks. In the study, samples were exposed to various degrees of thermal stress by storage at 3 different temperatures: 20 °C, 37 °C and 45 °C. The reference temperature was -80 °C. At representative time intervals (4, 12 and 24 weeks, respectively, after the start of the study) samples were taken out and brought to the reference temperature. At the end of the study, all samples (3 samples from each of 3 concentration levels, exposed to 3 different temperatures, for 3 time intervals) were brought to room temperature and analysed together with 3 samples kept at the reference temperature during the whole study period.

6.2.1 Method

The samples were analysed by ETAAS (Perkin Elmer 5100-Z or Perkin Elmer SIMAA 6000 ZL). The analyses were performed as peak area measurements under repeatability conditions in order to reduce any analytical variation. The mean sample signal of the samples exposed to thermal stress was compared to the mean signal of the samples kept at the reference temperature. The target value of the ratio of the means is 1 (exactly). 95 % confidence intervals are calculated for the ratios, and it is considered as an indication of thermal instability if the target value is outside the confidence interval.

6.2.2 Results

Results from the accelerated degradation study are presented in figures 6.1-3 on the following pages. On the basis on the analyses of the reference samples and the samples subjected to thermal stress, no statistically significant difference could be detected for BCR- 634, BCR-635 or BCR-636

at the 5 % significance level. For Pb in BCR-636, a significant increase in the ratio (peak area at test temp./peak area at ref. temp.) for the samples stored at 20 °C for 12 weeks was observed. However, after 24 weeks the ratio has returned to the normal level with the CI including the value 1.

6.2.3 Conclusion

The results indicate that the materials have a sufficient resistance to thermal stress. Consequently, no special efforts with respect to temperature have to be taken at transportation of the materials.

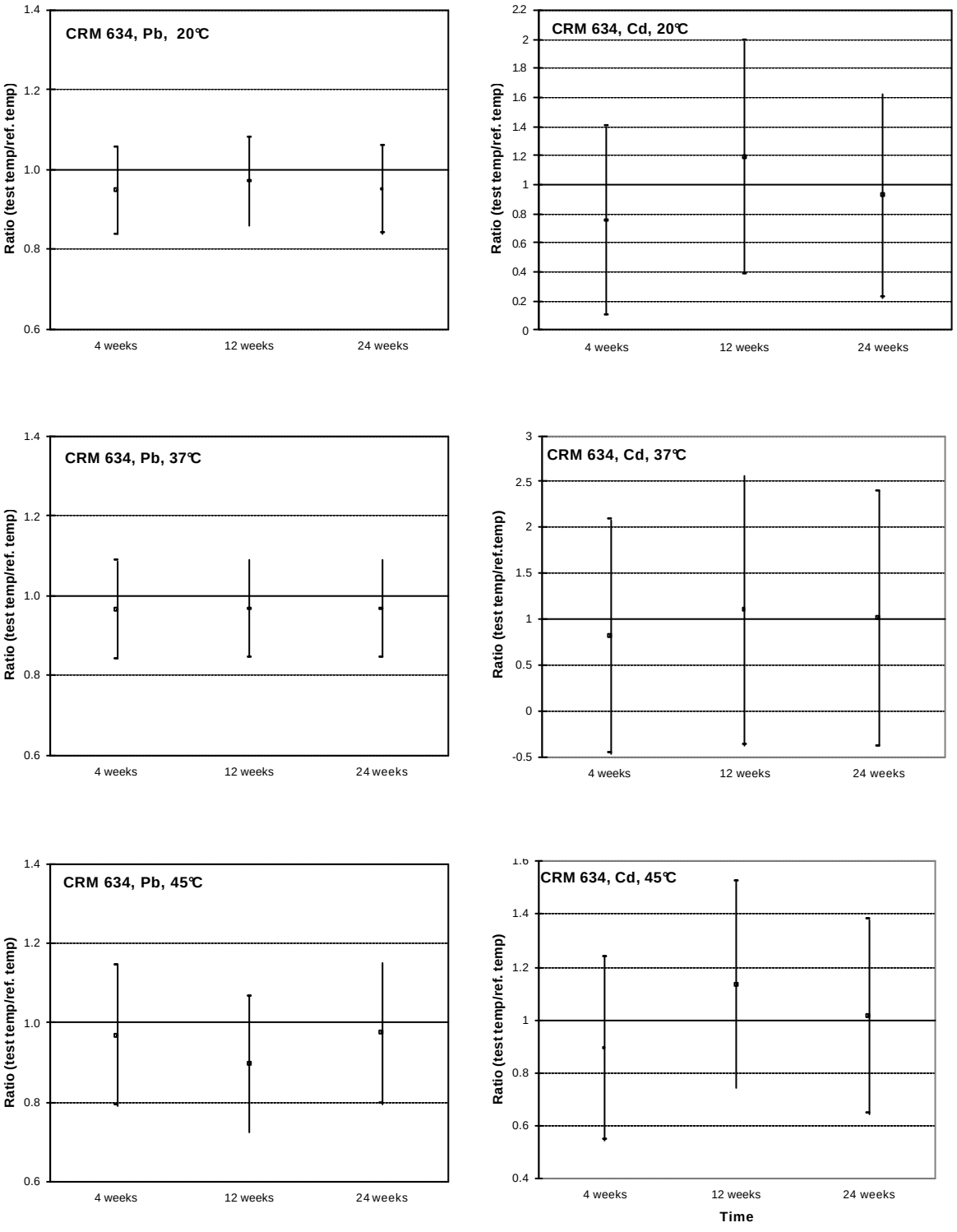

Figure 6.1 - Accelerated degradation study. Determinations of Pb and Cd in BCR-634 at three different time intervals. Ratio (test temp./ref. temp.) is the ratio of the peak area obtained at the test temperature and the peak area obtained at the reference temperature.

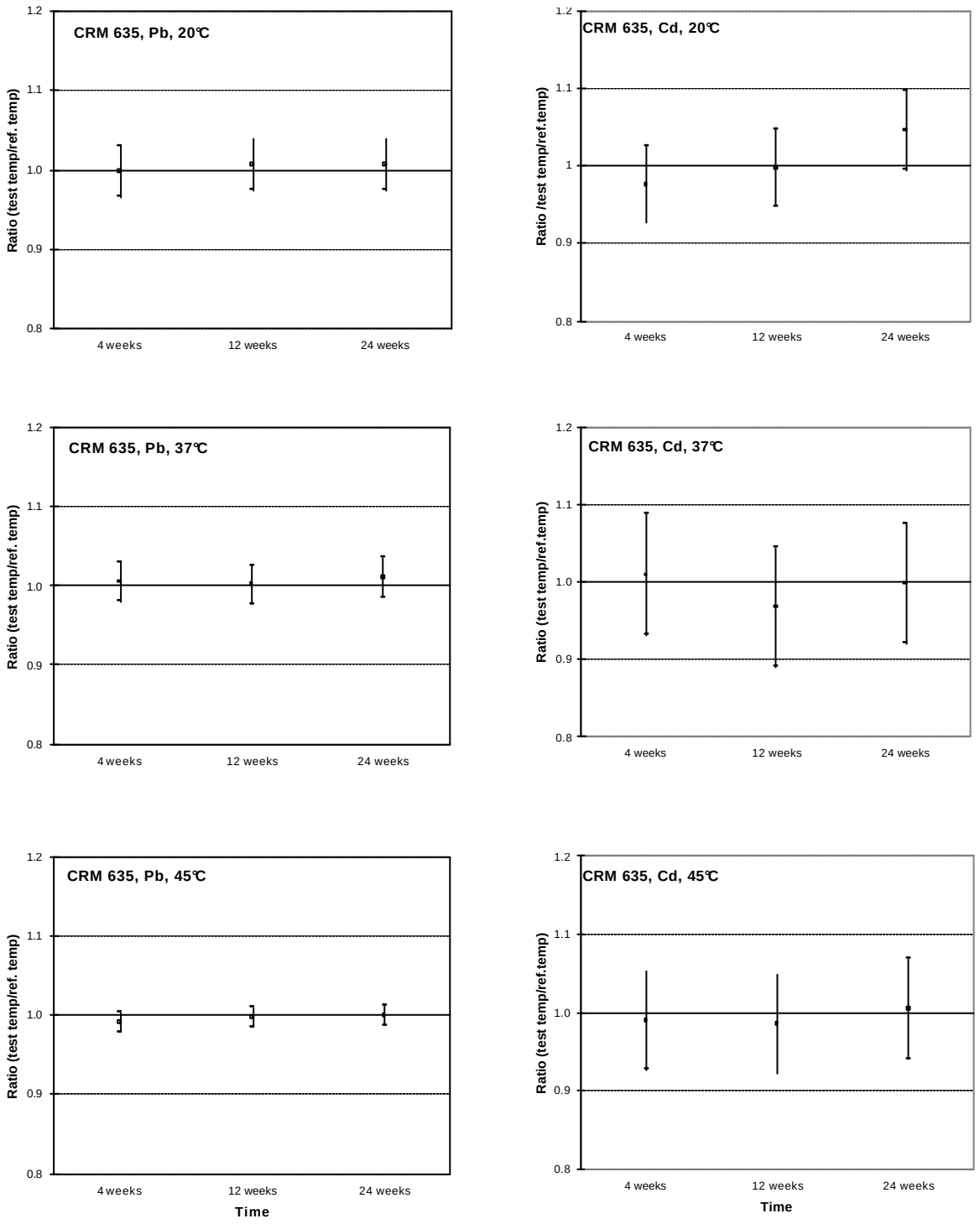

Figure 6.2 - Accelerated degradation study. Determinations of Pb and Cd in BCR- 635 at three different time intervals. Ratio (test temp./ref. temp.) is the ratio of the peak area obtained at the test temperature and the peak area obtained at the reference temperature.

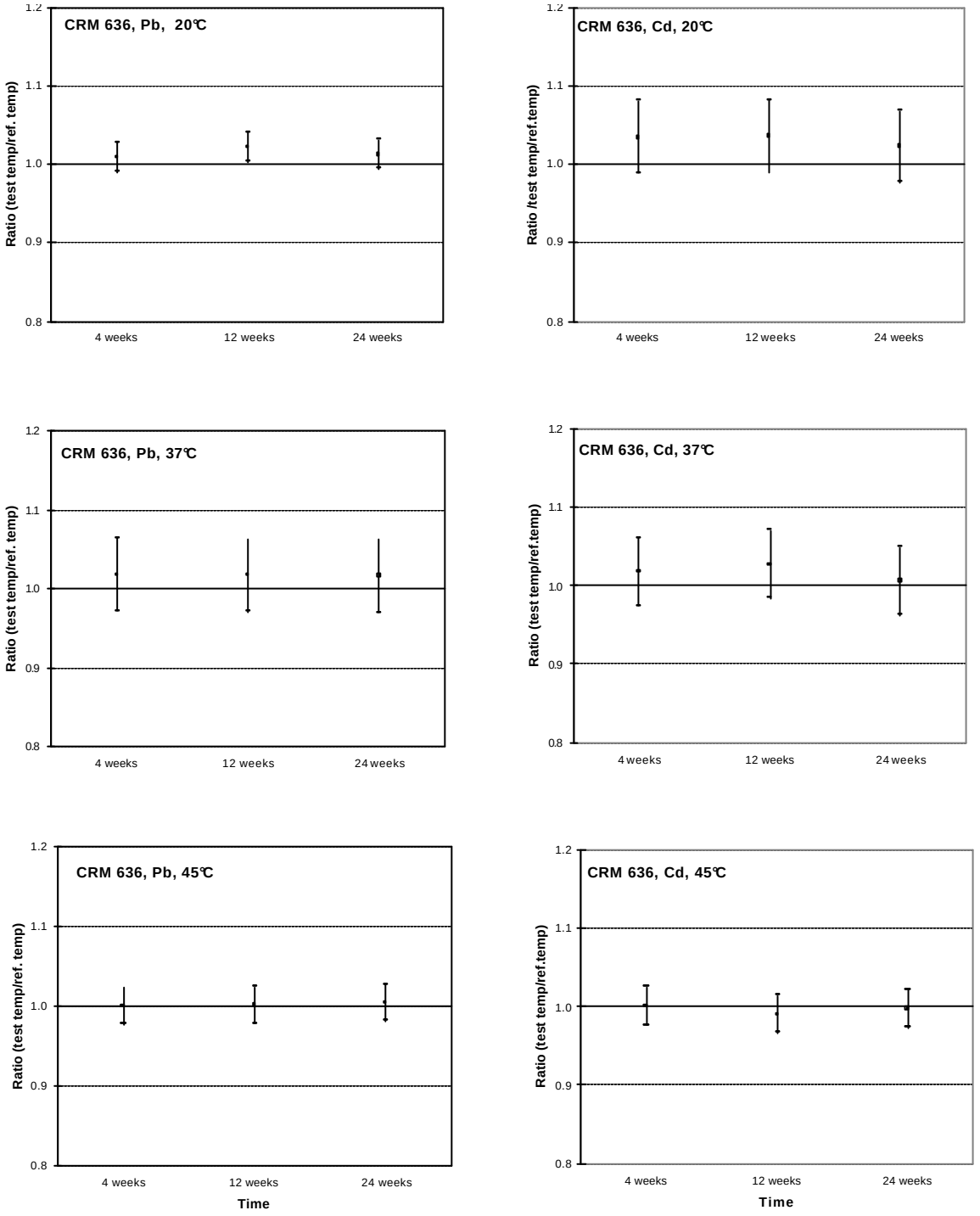

Figure 6.3 - Accelerated degradation study. Determinations of Pb and Cd in BCR-636 at three different time intervals. Ratio (test temp./ref. temp.) is the ratio of the peak area obtained at the test temperature and the peak area obtained at the reference temperature.

7. CERTIFICATION MEASUREMENTS

The participants in the certification campaign were invited as a consequence of a successful participation in the feasibility study. Each participant was requested to read and complete a detailed protocol for quality assurance giving detailed information about the reconstitution of the samples, accuracy of the volumetric equipment used for sampling and in the measurement procedure, the calibrants used and the applied analytical procedure. Furthermore, the participants had to document and formally guarantee that the measurements were performed with the analytical procedure in statistical control, i.e.

- no bias was detectable;
- the reproducibility was consistent with what the laboratory was able to achieve under the best circumstances.

Special emphasis was on the traceability of the measurements, i.e. the identity, purity and stoichiometry of the substances used for preparation of the calibrants. Acceptable calibrants were:

- A pure substance, weighed and dissolved by the participant. Stoichiometry was verified by checking the working standards against another fresh gravimetrically prepared standard.
- A certified material of the substance in simple solution (i.e. not a matrix certified material), provided that the uncertainty of the certified value is consistent with its use as a calibrant. The certified value was checked against a fresh gravimetrically prepared standard.
- Other substances, either pure or in simple solution, traceable to the types of materials mentioned above. The traceability chain was documented. Matrix reference materials were not allowed as calibrants.

The participants had formally to guarantee and be able to provide evidence that the traceability in the measurements was ensured. The results from the certification campaign accepted on technical and statistical ground are presented in Annex II. Each set of results is identified by a Lab ID code and details on the analytical procedures and calibrants used are given in chapter 7.1.

7.1 Analytical methods

Three different analytical principles (AAS, ASV and ICP-MS) were applied in the certification campaign each of them being highly selective for the measurands. The method diversity therefore ensures the credibility of the certified values. In table 7.a-b, the analytical techniques from the certification measurements are listed. In table 7.c-d, the calibrants used by the participants are listed.

7.2 Technical evaluation of the results

To obtain the accuracy that is required for certification it is necessary to ensure that no substantial systematic error is left undetected. Therefore, each set of data was thoroughly scrutinised at a technical meeting with participation of the laboratories.

Table 7.a - Analytical techniques applied in the certification campaign for Pb in BCR-634, BCR-635 and BCR-636

Measurand	*LAB ID*	*Method*	*Sample pretreatment*	*Detection*
Lead	1	FAAS2	Dry, partly oxidised with H_2O_2 in Ni-cup: 10 µL sample are dried on on hot-plate, 20 µL 10 % H_2O_2 added dried for further 5 min.	Flame atomic absorption, microsampling cup Deuterium background corr.
Lead	2	AAS1	10 µL sample + 10 µL diluent/spike + 5 µL 1% $(NH_4)_2HPO_4$ Dilution 1+4 v/v or 1+5 v/v with 0.025 M $(NH_4)_2HPO_4$ in 1 % NH_4OH	ETAAS with Zeeman background correction pyrocoated furnace with platform
Lead	4	ASV1	Low + medium level:1.5 mL sample +3.0 mL HNO_3 + 0.4 mL $HClO_4$ High level: 0.5 mL sample + 1.5 mL HNO_3 + 0.25 mL $HClO_4$ Digested at 320 °C and evaporated until dryness. Reconstituted with 1.0 mL H_2O + 0.4 mL HCl (high level 0.2 mL HCl) + H_2O ad 10.0 mL.	Differential pulse anodic stripping voltammetry, DPASV
Lead	4	ICP2	Low + medium level:1.5 mL sample +3.0 mL HNO_3 + 0.4 mL $HClO_4$ High level: 0.5 mL sample + 1.5 mL HNO_3 + 0.25 mL $HClO_4$ Digested at 320 °C and evaporated until dryness. Reconstituted with 1.0 mL H_2O + 0.4 mL HCl (high level 0.2 mL HCl) + H_2O ad 10.0 mL.	High resolution ICP-MS ^{206}Pb, ^{208}Pb Resolution: 300 Internal standard:^{193}Ir
Lead	5	AAS1	100 µL sample diluted with 900 µL 0.2% Triton X-100	ETAAS with Zeeman background correction pyrocoated furnace with platform
Lead	7	AAS1	Dilution 1+10 with modifier 2 % $(NH_4)_2HPO_4$ + 0.2 % Triton X-100	ETAAS with Zeeman background correction, pyrocoated furnace with platform
Lead	13	AAS1	Samples diluted 1+ 4 with 0.2 % Triton X-100 modifier: 0.5 g/L $Mg(NO_3)_2$ + 5 g/L $NH_4H_2PO_4$	ETAAS with Zeeman background correction pyrocoated furnace with platform
Lead	19	AAS2	Samples diluted 1+12 with 1 % $NH_4H_2PO_4$ + 0.1 % Triton X-100	ETAAS with deuterium background correction pyrocoated furnace with platform
Lead	20	AAS2	30 µL sample diluted with 420 µL modifier : 0.05 % Triton X-100 + 7 g/L $(NH_4)_2HPO_4$	ETAAS with deuterium background correction pyrocoated furnace
Lead	26	AAS1	2.0 mL sample digested with 2.5 mL sub-distilled HNO_3. Solution made up to 10.0 mL with H_2O	ETAAS with Zeeman background correction Z-tek-tubes
Lead	31	AAS1	100 µL sample diluted with 100 µL standard and 300 µL modifier	ETAAS with Zeeman background correction pyrocoated furnace with platform
Lead	43	AAS2	Deproteinization with acid	ETAAS with deuterium background correction pyrocoated furnace
Lead	56	AAS1	Dilution 1:10	ETAAS with Zeeman background correction graphite furnace with platform

Table 7.b - Analytical techniques applied in the certification campaign for Cd in BCR-634, BCR-635 and BCR-636

Measurand	*LAB ID*	*Method*	*Sample pre-treatment*	*Detection*
Cadmium	2	AAS1	10 µL sample + 10 µL diluent/spike + 5 µL 1% $(NH_4)_2HPO_4$ Dilution 1+4 v/v or 1+5 v/v with 0.025 M $(NH_4)_2HPO_4$ in 1 % NH_4OH	ETAAS with Zeeman background correction pyrocoated furnace with platform
Cadmium	4	ASV1	Low + medium level:1.5 mL sample +3.0 mL HNO_3 + 0.4 mL $HClO_4$ High level: 0.5 mL sample + 1.5 mL HNO_3 + 0.25 mL $HClO_4$ Digested at 320 °C and evaporated until dryness. Reconstituted with 1.0 mL H_2O + 0.4 mL HCl (high level 0.2 mL HCl) + H_2O ad 10.0 mL.	Differential pulse anodic stripping voltammetry, DPASV
Cadmium	4	ICP2	Low + medium level:1.5 mL sample +3.0 mL HNO_3 + 0.4 mL $HClO_4$ High level: 0.5 mL sample + 1.5 mL HNO_3 + 0.25 mL $HClO_4$ Digested at 320 °C and evaporated until dryness. Reconstituted with 1.0 mL H_2O + 0.4 mL HCl (high level 0.2 mL HCl) + H_2O ad 10.0 mL.	High resolution ICP-MS ^{114}Cd Resolution: 300 Internal standard: ^{103}Rh
Cadmium	5	AAS1	100 µL sample is diluted with 400 µL $(NH_4)_2HPO_4$ solution with 2 % Triton X-100	ETAAS with Zeeman background correction pyrocoated furnace with platform
Cadmium	7	AAS1	Dilution 1+10 with modifier 2 % $(NH_4)_2HPO_4$ + 0.2 % Triton X-100	ETAAS with Zeeman background correction, pyrocoated furnace with platform
Cadmium	13	AAS1	Samples diluted 1+ 3 with 2 % Triton X-100 modifier: 0.5 g/L $Mg(NO_3)_2$ + 5 g/L $NH_4H_2PO_4$	ETAAS with Zeeman background correction pyrocoated furnace with platform
Cadmium	15	AAS1	Samples diluted fivefold with diluent/modifier: 5g/L Triton X-100 + 50 g/L $(NH_4)_2HPO_4$	ETAAS with Zeeman background correction pyrocoated furnace with platform
Cadmium	26	AAS1	2.0 mL sample digested with 2.5 mL sub-distilled HNO_3 . Solution made up to 10.0 mL with H_2O Modifier: 0.1 % Pd	ETAAS with Zeeman background correction Z-tek-tubes
Cadmium	56	AAS1	Dilution 1:10	ETAAS with Zeeman background correction graphite furnace with platform
Cadmium	63	AAS1	Dilution with Triton X-100 1:5 to 1:10 Modifier: $Pd(NO_3)_2$	ETAAS with Zeeman background correction graphite furnace

7.3 Statistical evaluation of the results

For each data set from the certification campaign (presented in Annex II) the mean value and the standard deviation were calculated. Each set in the evaluation reports has passed the technical scrutiny. After the technical scrutiny, the results were subjected to appropriate statistical techniques:

- Kolmogorov-Smirnov-Lilliefors test to assess the conformity of the distributions of the laboratory mean values to normal distribution;

- Cochran's test for detecting outlying laboratory variances;
- Grubb's test for detecting outlying laboratory mean values;
- Bartletts test to check the homogeneity of the laboratory variances;
- One way ANOVA (F-test) to compare and estimate the between and the within laboratory components of the overall variance of all individual results.

For the Cochran and Grubb tests, a value is called an outlier if it is rejected with a 1 % risk of error. If the risk lies between 1 % and 5 %, the values is called "a straggler" and it can be included in the calculation of the values for certification if it overlaps with the results from the same analytical principle.

The ANOVA showed that the between laboratory variation contributes considerably to the overall variability of the results and, consequently, the laboratory means were used for calculating the values for certification. A summary of the statistical evaluation of each CRM is presented in Tables 7.e-g. Details of the individual results from the certification campaign are presented in Annex II.

Table 7.c - Calibrants used in the certification campaign for Pb in BCR-634, BCR-635 and BCR-636

Measurand	*LAB ID*	*Method*	*Type of calibration*	*Calibrant*	*Producer*	*Purity*
Lead	1	FAA2	Matrix matching standards (bovine blood)	Lead standard solution no. 2291	Fisons	1000 µg /mL
Lead	2	AAS1	Standard addition, Instrument automated	PE Pure GFAAS Mixed standard Spectroscan AAS Standards 8017	Perkin Elmer Technolab A/S	100 ± 0.04 µg Pb/mL 1000 ± 3 mg Pb/L
Lead	4	ASV	Standard addition	Spex plasma standard, Pb PLPB2-24	Spex Ind.USA	1000 µg /mL ± 0.2 %
Lead	4	ICP2	Standard curve	Spex plasma standard, Pb PLPB2-24	Spex Ind.USA	1 g /L ± 0.2 %
Lead	5	AAS1	Standard addition Low level: standard curve, aqueous standards	Lead AA std. solution no. 88075	ALFA Johnson Matthey Company	1000 µg /mL
Lead	7	AAS1	Standard curve, matrix matching standards	SRM 3128	NIST	10.00 ± 0.03 mg/mL
Lead	13	AAS1	Standard curve, matrix-matching standards	Pb standard AA solution	Merck	1.000 ± 0.002 g/L
Lead	19	AAS1	Standard curve, aqueous standards	Pb single element plasma emission sol.,	Perkin Elmer	999 µg/mL ± 0.5 %
Lead	20	AAS1	Standard curve, matrix-matching standards	Spectrosol Pb sol. no. 140362D	Merck	1000 ± 2 mg/L
Lead	26	AAS1	Standard curve, matrix-matching standards	Pb, no. 8017-2	Teknolab A/S	1000 ± 3 µg/mL
Lead	31	AAS1	Standard addition	Pb single element plasma emission sol.,	Perkin Elmer	1000 µg/mL ± 0.5 %

Lead	43	AAS2	Standard curve	Pb solution, no. 1.19776	Merck	1002 ± 2 mg/L
Lead	56	AAS1	Standard addition	Lead standard solution	Analyticals CARLO ERBA	1 mg/mL

Table 7.d - Calibrants used in the certification campaign for Cd in BCR-634, BCR-635 and BCR-636

Measurand	*LAB ID*	*Method*	*Type of calibration*	*Calibrant*	*Producer*	*Purity*
Cadmium	2	AAS1	Standard addition, Instrument automated	PE Pure GFAAS Mixed standard	Perkin Elmer	5 ± 0.05 µg /mL
				Titrisol AAS-ICP	Merck	1000 ± 2 mg /L
Cadmium	4	DPASV	Standard addition	Spex plasma standard, Cd PLCD2-24	Spex Ind.USA	1000 µg /mL ± 0.2 %
Cadmium	4	ICP2	Standard curve	Spex plasma standard, Cd PLCD2-24	Spex Ind.USA	1000 µg /mL ± 0.2 %
Cadmium	5	AAS1	Standard addition	Cadmium AA std. solution no. 88056	ALFA Johnson Matthey Company	1000 µg /mL
Cadmium	7	AAS1	Standard curve, Matrix matching standards	SRM 3108	NIST	10.00 ± 0.03 mg /mL
Cadmium	13	AAS1	Standard curve, Matrix-matching standards	Cd standard AA solution	Merck	1.000 ± 0.002 g/L
Cadmium	15	AAS1	Standard addition, matrix matching standards	Cadmium standard solution, ref.1.19777.500	Merck-Clevenot lab.	1000 ± 2 mg/L
Cadmium	26	AAS1	Standard curve, Matrix-matching standards	Cd, no. 8014-1	Teknolab A/S	1000 ± 3 µg/mL
Cadmium	56	AAS1	Standard addition	Cadmium standard solution	Analyticals CARLO ERBA	1 mg/mL
Cadmium	63	AAS1	Standard curve, Aqueous standards	PLCD2-24	SPEX	1002 µg/mL ± 0.5 %

Table 7.e - Summary of statistical data for BCR-634

Certified property	*Pb*	*Cd*
Number of data sets	12	10
Number of individual data	60	50
Outlying data sets (Grubbs test)	No	No
Outlying variances (Cochran test)	No	No
Mean of data set means	46.21 µg/L	1.36 µg/L
Within-data set SD	2.64 µg/L	0.11 µg/L
Between-data set SD	1.67 µg/L	0.07 µg/L
Variances homogeneous (Bartlett test)	No	Yes
SD of data set means	2.05 µg/L	0.07 µg/L

Data set means normally distributed	Yes	Yes
(Kolmogorov-Smirnov-Lilliefors test)		
Half width of the 95 % CI of the mean of data set means	1.30 µg/L	0.06 µg/L

Table 7.f - Summary of statistical data for BCR-635

Certified property	*Pb*	*Cd*
Number of data sets	12	9
Number of individual data	60	45
Outlying data sets (Grubbs test)	No (one straggler)	No
Outlying variances (Cochran test)	No (one straggler)	Yes (1 outlier detected)
Mean of data set means	209.83 µg/L	6.55 µg/L
Within-data set SD	5.91 µg/L	0.17 µg/L
Between-data set SD	8.69 µg/L	0.15 µg/L
Variances homogeneous (Bartlett test)	Yes	Yes
SD of data set means	9.09 µg/L	0.17 µg/L
Data set means normally distributed (Kolmogorov-Smirnov-Lilliefors test)	Yes	Yes
Half width of the 95 % CI of the mean of data set means	5.77 µg/L	0.14 µg/L

Table 7.g - Summary of statistical data for BCR-636

Certified property	*Pb*	*Cd*
Number of data sets	11	9
Number of individual data	55	45
Outlying data sets (Grubbs test)	No	No
Outlying variances (Cochran test)	No	Yes (1 outlier detected)
Mean of data set means	515.33 µg/L	11.64 µg/L
Within-data set SD	12.11 µg/L	0.35 µg/L
Between-data set SD	15.08 µg/L	0.35 µg/L
Variances homogeneous (Bartlett test)	Yes	Yes
SD of data set means	16.02 µg/L	0.38 µg/L
Data set means normally distributed (Kolmogorov-Smirnov-Lilliefors test)	Yes	Yes
Half width of the 95 % CI of the mean of data set means	10.76 µg/L	0.29 µg/L

8. CERTIFIED VALUES AND UNCERTAINTY EVALUATION

8.1 Uncertainty evaluation

The evaluation of uncertainties was done in compliance with the requirements made by GUM [10]. According to these requirements, the various uncertainty sources such as stability, homogeneity and batch characterisation (certification campaign) need to be properly estimated.

The evaluation described hereafter is based on a concept described by Pauwels *et al.* [11 and literature cited] and uses available data discussed in the previous chapters.

8.1.1 Introduction and statistical concept

In order to be complete, the combined (and expanded) standard uncertainty on a reference material should consider that in addition to the characterisation of the batch, homogeneity, and long- and short-term stability play an important role. Therefore, the uncertainty can be expressed as:

- Uncertainty of the certified value as obtained for the batch (characterisation, u_{char});
- Transferred to a single package (homogeneity, u_{bb});
- As dispatch to the customer (short-term stability, u_{sts});
- At the time of sale (long-term stability, u_{lts}).

Following this and based on the data obtained in the stability and homogeneity studies as well as the results of the batch characterisation, estimates for u_{bb} (homogeneity), u_{lts} (long-term-stability) and u_{char} (batch characterisation) were obtained and combined according the following equation [11 and literature cited]:

$$U_{CRM} = 2 \cdot \sqrt{u_{bb}^2 + u_{lts}^2 + u_{char}^2}$$

Due to the transport conditions selected for dispatch, the uncertainty constituent for short-term stabililty (u_{sts}) is negligible and consequently not included in the overall uncertainty. The estimation of the other uncertainty sources is described below.

8.1.2 Uncertainty source "homogeneity"

The homogeneity study is exhaustively described in chapter 5 and results have been evaluated by means of a ANOVA. Using the variances described in Tables 5a-b, estimates of u_{bb} were derived as described by Linsinger *et al.* [12]. According to this approach, s_{bb} (being the standard deviation between units) or u^*_{bb} (being the upper limit of inhomogeneity that can be hidden by the method repeatability) are used as estimates of u_{bb}. To this end values for u^*_{bb} and s_{bb} were calculated accordingly:

$$u_{bb}^* = \sqrt{\frac{MS_{within}}{n}} \cdot \sqrt[4]{\frac{2}{\nu_{MSwithin}}} \quad \text{and} \quad MS_{within} = s_{Analysis}^2,$$

where n is the number of replicates per unit, $\nu_{MSwithin}$ the degrees of freedom of MS_{within} (ANOVA estimated variance for 3 replicates, Table 5a-c);

and

$$s_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}} = \sqrt{s^2{}_{samples} - \frac{s^2_{Analysis}}{n}}$$

where $MS_{between}$ is the ANOVA estimated variance on 10 samples (Table 5a-c).

As a principle, the respective higher value of s_{bb} or u^*_{bb} is adopted as u_{bb} and included in the expanded uncertainty. The results of these calculations are shown in Tables 8a-c.

8.1.3 Uncertainty source "stability"

The stability data discussed in chapter 6 are sufficient to deem the material to be stable. However, they do not allow the establishment of a shelf-life. In order to obtain a reasonable estimate of the related uncertainty component u_{lts}, the ANOVA data of Table 7e-g were used.

First, a rough estimate for u_{lts} was derived evaluating the ANOVA data as simple homogeneity study and using the respective s_{bb}-value (see equation above). The obtained values were then divided by the mean values of the certification experiments in order to obtain an expression in percentage. These data are compiled in Tables 8a-c.

8.1.4 Uncertainty source "batch characterisation"

An estimate for u_{char} was derived from the standard error obtained on the mean of laboratories means.

8.1.5 Uncertainty budget

Based on the uncertainty contributions mentioned in sections above the following uncertainty budgets are established:

Table 8.a – Uncertainty budget and certified values for BCR-634

	Pb	*Cd*
u_{bb} (s_{bb}) *[in rel. %]*	3.4	9.6
u^*_{bb} *[in rel. %]*	2.1[a]	3.7[a]
u_{lts} *[in rel. %]*	3.7	6.6
u_{char} *[in rel. %]*	1.3	1.6
coverage factor k	2	2
U_{CRM} *[in rel. %]*	10.4	23.5
Mean [in µg/L]	***46.21***	***1.36***
Uncertainty [in µg/L]	***4.81***	***0.32***
Certified values expressed in µg/L	**46 ± 5**	**1.4 ± 0.4**

[a] not used for combined uncertainty

Table 8.b – Uncertainty budget and certified values for BCR-635

	Pb	*Cd*
u_{bb} (s_{bb}) *[in rel. %]*	0.8[a]	2.1
u^*_{bb} *[in rel. %]*[a]	1.0	1.2[a]
u_{lts} *[in rel. %]*	5.4	3.2
u_{char} *[in rel. %]*	1.3	0.9
coverage factor k	2	2
U_{CRM} *[in rel. %]*	11.3	7.9
Mean [in µg/L]	***209.83***	***6.55***
Uncertainty [in µg/L]	***23.71***	***0.52***
Certified values expressed in µg/L	**210 ± 24**	**6.6 ± 0.6**

[a] not used for combined uncertainty

Table 8.c – Uncertainty budget and certified values for BCR-636

	Pb	*Cd*
u_{bb} (s_{bb}) *[in rel. %]*	1.0	[b]
u^*_{bb} *[in rel. %]*[a]	0.7[a]	1.1
u_{lts} *[in rel. %]*	3.7	2.0
u_{char} *[in rel. %]*	0.9	1.1
Coverage factor k	2	2
U_{CRM} *[in rel. %]*	7.9	5.1
Mean [in µg/L]	***515.33***	***11.64***
Uncertainty [in µg/L]	***40.71***	***0.59***
Certified values and expressed in µg/L	**(0.52 ± 0.05)·10^3**	**11.6 ± 0.6**

[a] not used for combined uncertainty

[b] not calculable

8.2 Certified values

The certified values (unweighed mean of the accepted sets of results) and their uncertainties (expanded uncertainty with a coverage factor of k=2)) are summarised in tables 8.a-c.

Table 8.d - Certified values for Pb and Cd in BCR-634, expressed in reconstituted sample

BCR-634 *Component*	*Certified content, µg/L*	*Uncertainty, µg/L*	*Number of accepted sets of results*
Lead, Pb	46	5	12
Cadmium, Cd	1.4	0.4	10

Table 8.e - Certified values for Pb and Cd in BCR-635, expressed in reconstituted sample

BCR-635 *Component*	*Certified content, µg/L*	*Uncertainty, µg/L*	*Number of accepted sets of results*
Lead, Pb	210	24	12
Cadmium, Cd	6.6	0.6	9

Table 8.f - Certified values for Pb and Cd in BCR-636, expressed in reconstituted sample

BCR- 636 *Component*	*Certified content, µg/L*	*Uncertainty, µg/L*	*Number of accepted sets of results*
Lead, Pb	$0.52 \cdot 10^3$	$0.05 \cdot 10^3$	11
Cadmium, Cd	11.6	0.6	9

9. INSTRUCTIONS FOR USE

9.1 Description

Samples of BCR-634, BCR-635 and BCR-636 consist of lyophilised human whole blood. Each unit contains approximately 0.6 g dry matter with a residual moisture content of less than 2 %. Sodium-EDTA was used as anti-coagulant. No further preservatives are added.

9.2 Storage conditions

Unopened vials should be stored at -20°C or lower for long-term storage.

9.3 Reconstitution

Before use the following instructions for reconstitution are to be executed:

1. Allow the vial to reach ambient temperature before opening.
2. Tap the bottom of the vial to loosen any blood material adhering to the stopper.
3. Carefully remove the rubber stopper.
4. Add 3.00 mL water (room temperature). Acceptable CV = 0.5 %
5. Replace rubber stopper and homogenise by continuous agitation on a mixing apparatus for at least 1 h.

9.4 Safety

The materials were produced from blood from healthy Danish blood donors. Each portion of blood was tested negative for Anti-HIV-1&2, Anti-HCV and Anti-HTLV-I&II. However, as all biological material of human origin the blood should be treated as contagious material. The materials are **for in vitro use only.**

9.5 Use of the certified value and uncertainty

If the reference material is used for checking an analytical procedure or for evaluation of the performance of the procedure, the user can refer to the results from the certification campaign after having ascertained that the repeatability of the method is satisfactory.

The user may assess the bias of the method from the difference between the mean value of replicate measurements (x) and the certified value (μ). The following general criterion for acceptance is given in ISO Guide 33 [14]:

$$-a_2 - 2\,\sigma_L < x - \mu < a_1 + 2\,\sigma_L$$

in which a_1 and a_2 are adjustment values chosen by the user according to economic or technical limitations or stipulation, and σ_L is the long-term-within laboratory standard deviation (reproducibility SD) of the method (e.g. as determined during a method evaluation and used for setting acceptance limits in a control chart).

Matrix reference materials like BCR- 634, BCR-635 and BCR-636 should not be used for

calibration due to possible differences in matrix between calibrant and sample.

10. REFERENCES

1) EC Directive 80/1107/EEC on the protection of workers from the risks related to exposure to chemical, biological and physical agents at work.

2) EC Directive 77/312/EEC on biological screening of the general population for lead.

3) EC Directive 82/605/EEC on the protection of workers from the risks related to exposure to metallic lead and its ionic compounds at work.

4) EC Directive 90/394/EEC on the protection of workers from the risks related to exposure to carcinogens at work.

5) EC Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work.

6) EC Directive 91/322/EEC on establishment of recommended threshold limit values for implementation of Council Directive 80/1107/EEC on the protection of workers from risks related to exposure to chemical, physical and biological agents at work.

7) American Conference of Governmental Industrial Hygienists, *Threshold limit values and biological exposure indices for 1994-1995*, Cincinnati USA, 55-70

8) Deutsche Forschungsgemeinschaft. *List of MAK and BAT values*, VCH, Weinheim, 1994

9) European Commission, *Guidelines for the production and certification of BCR reference materials* Doc. BCR/48/93, Brussels 1994

10) ISO-IEC-BIPM-IFCC-IUPAC-IUPAP-OIML. *Guide to the Expression of Uncertainties in Measurement (GUM)*, Geneva, Switzerland (ISBN 92-67-10188-9).

11) A.M.H. van der Veen, T.P.J. Linsinger, H. Schimmel, A. Lamberty, J. Pauwels. *Accreditation and Quality Assurance* **6** (2001), 290-294.

12) T.P.J. Linsinger, J. Pauwels, A.M.H. van der Veen, H. Schimmel, A. Lamberty. *Accreditation and Quality Assurance* **6** (2001), 20-25.

13) ISO. *ISO-Standard 31 - Quantities and units – Part 0: General principles*. Geneva, Switzerland (1992).

14) ISO Guide 33. *Uses of Certified Reference Materials*, International Organization for Standardization, Geneva 1989

11. ANNEX I – GRAPHICAL PRESENTATION OF THE HOMOGENEITY STUDY

11.1 Graphical survey of the results from the homogeneity study on BCR-634

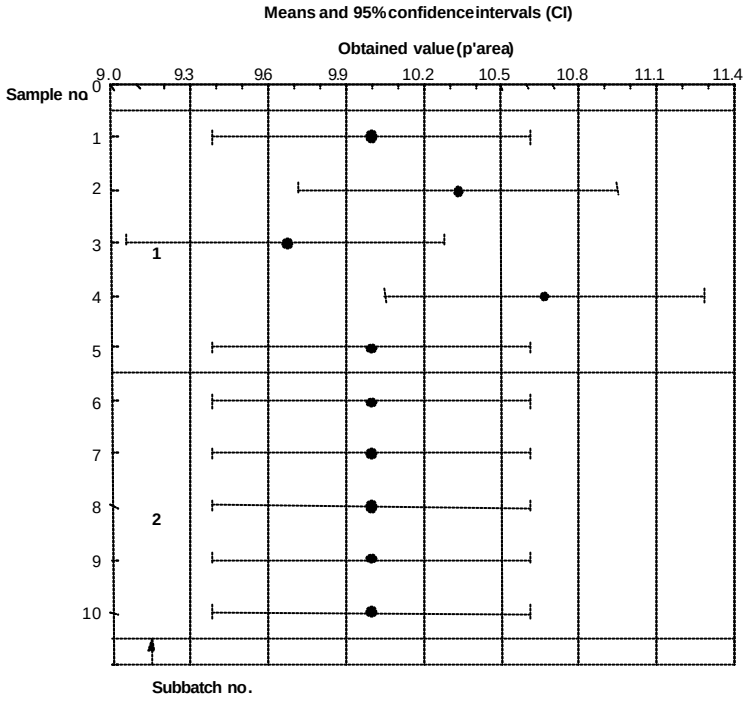

Figure 11.1 - Means and confidence intervals (CI) for the results of Pb determination in individual samples of BCR-634

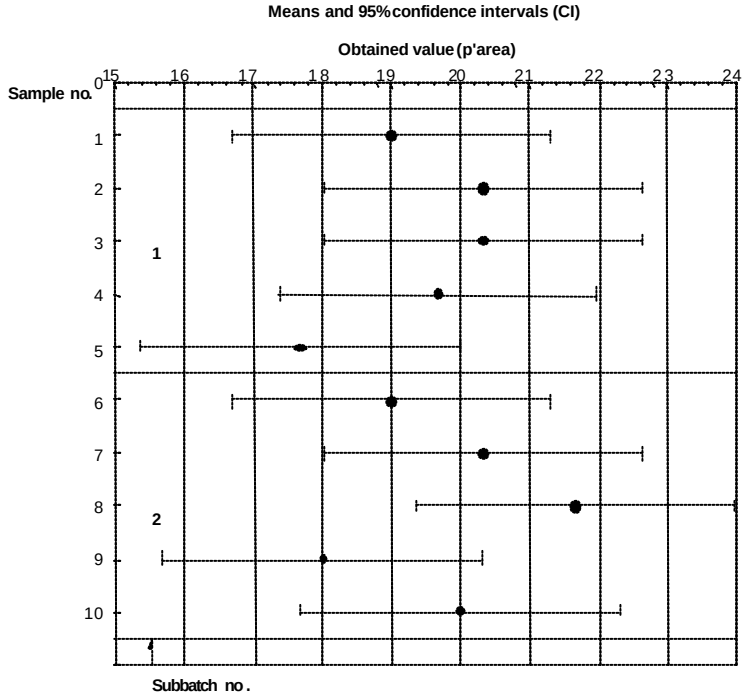

Figure 11.2 - Means and confidence intervals (CI) for the results of Cd determination in individual samples of BCR-634

11.2 Graphical survey of the results from the homogeneity study on BCR-635

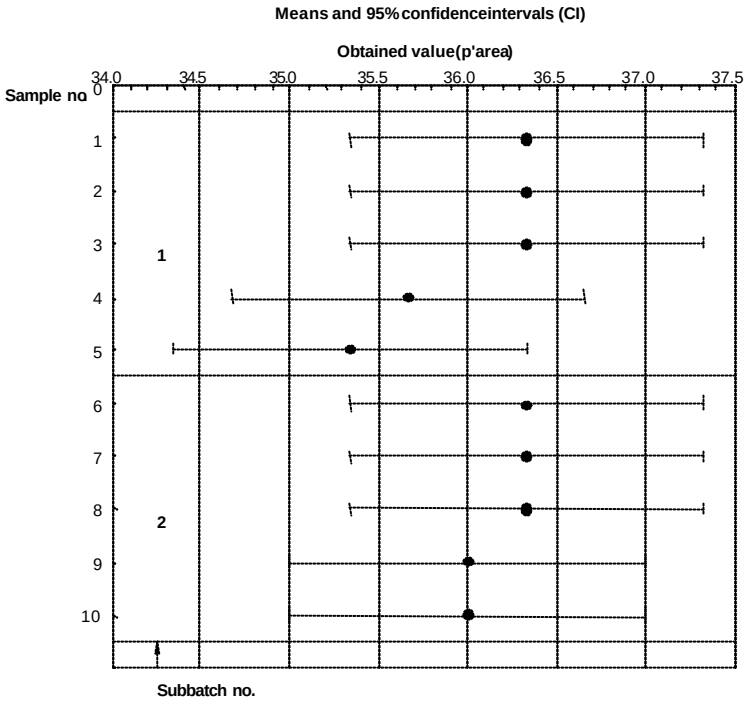

Figure 11.3 - Means and confidence intervals (CI) for the results of Pb determination in individual samples of BCR-635

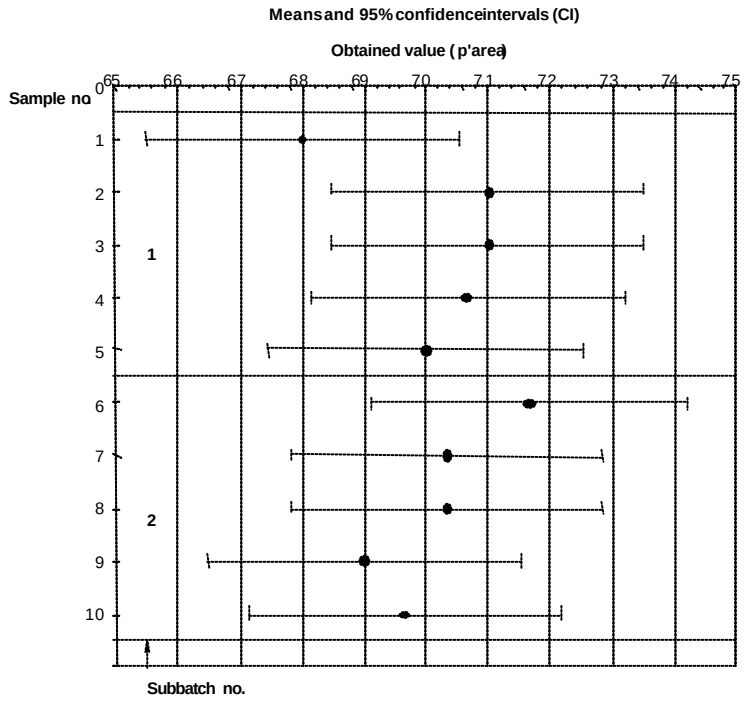

Figure 11.4 - Means and confidence intervals (CI) for the results of Cd determination in individual samples of BCR-635

11.3 Graphical survey of the results from the homogeneity study on BCR-636

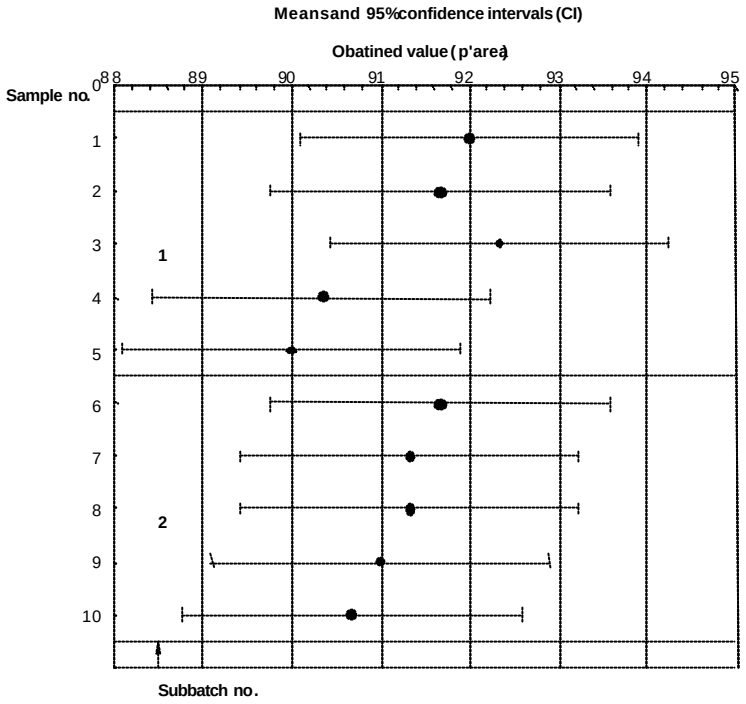

Figure 11.5 - Means and confidence intervals (CI) for the results of Pb determination in individual samples of BCR-636

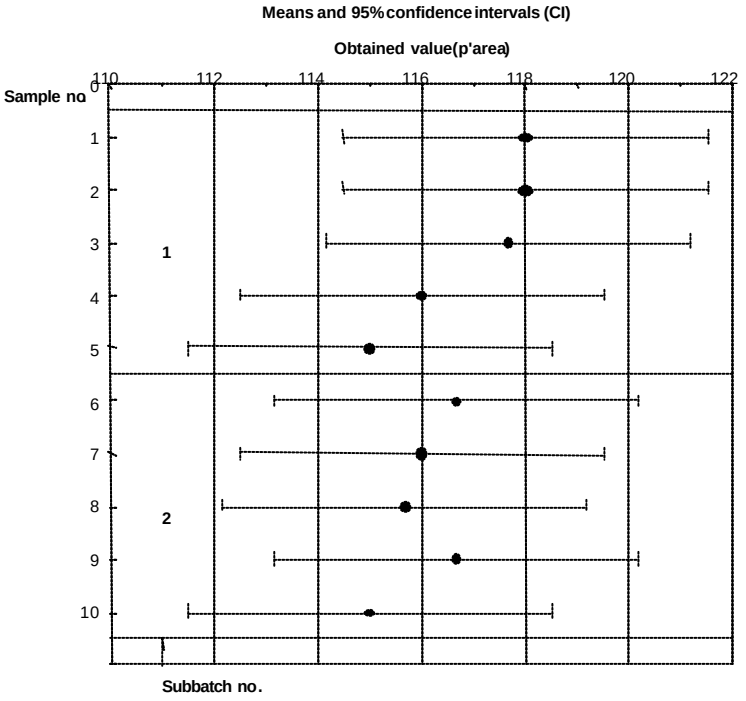

Figure 11.6 - Means and confidence intervals (CI) for the results of Cd determination in individual samples of BCR-636

12. ANNEX II – CERTIFICATION CAMPAIGN: TABLES OF INDIVIDUAL RESULTS AND GRAPHICAL PRESENTATION

Table 12.a - Table of individual results for BCR- 634, Pb (µg/L)

Lab ID	*Method*	*Individual measurement results*					*Mean*	*Std. Dev.*	*CV %*
L1	FAAS2	48.00	50.00	49.00	41.00	46.00	46.800	3.564	7.6
L2	AAS1	42.40	41.70	43.30	42.90	40.80	42.220	0.993	2.4
L4	ASV1	46.10	47.00	47.50	48.60	44.50	46.740	1.544	3.3
L4	ICP2	46.80	47.10	45.30	45.50	45.80	46.100	0.803	1.7
L5	AAS1	47.15	45.20	46.55	43.90	45.20	45.600	1.276	2.8
L7	AAS1	47.10	47.30	50.00	45.00	40.20	45.920	3.657	8.0
L13	AAS1	47.00	49.00	47.00	53.00	57.00	50.600	4.336	8.6
L20	AAS2	41.40	47.70	47.70	43.50	41.40	44.340	3.185	7.2
L26	AAS1	48.00	45.80	46.10	44.50	48.10	46.500	1.538	3.3
L31	AAS1	48.21	46.65	48.27	49.02	47.97	48.024	0.863	1.8
L43	AAS2	40.76	42.04	45.30	45.18	50.14	44.684	3.633	8.1
L56	AAS1	45.00	47.00	50.00	44.00	49.00	47.000	2.550	5.4

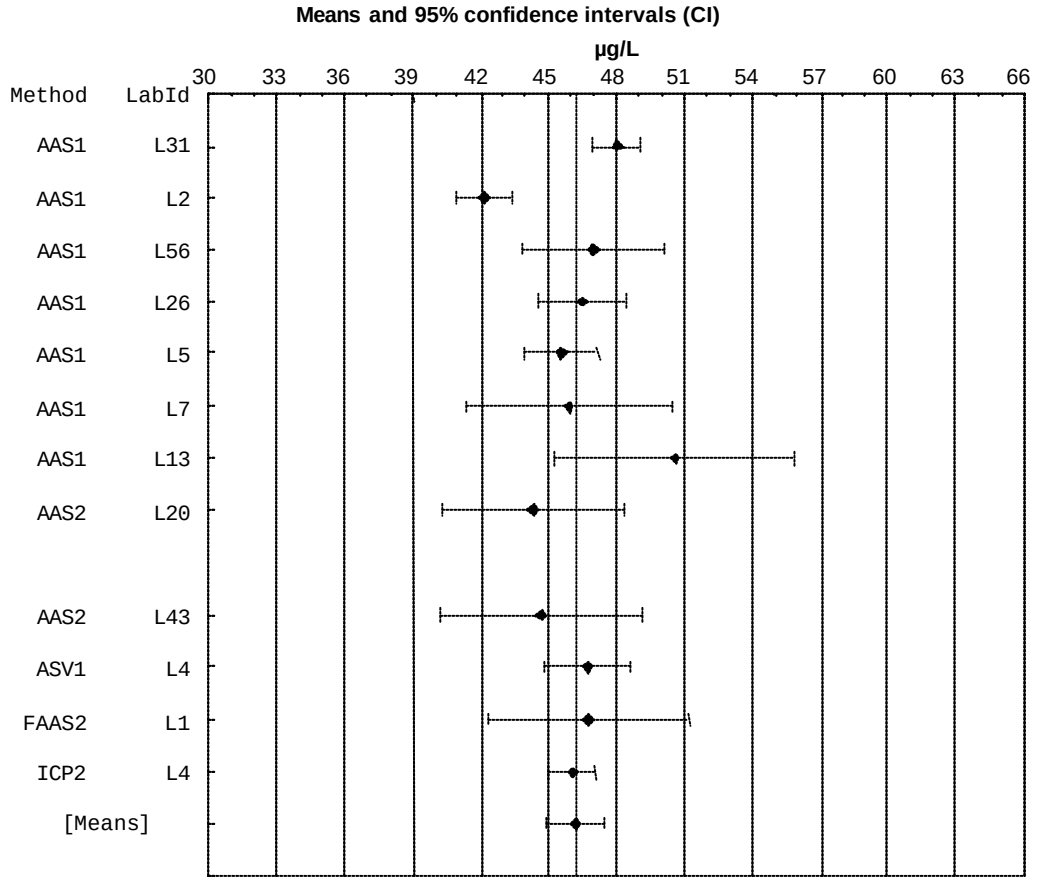

Figure 12.1 - Graphical presentation of individual results BCR- 634, Pb (µg/L)

Table 12.b - Table of individual results for BCR- 634, Cd (µg/L)

Lab ID	*Method*	*Individual measurement results*					*Mean*	*Std. Dev.*	*CV %*
L2	AAS1	1.16	1.38	1.24	1.30	1.40	1.296	0.099	7.7
L4	ASV1	1.30	1.30	1.50	1.20	1.30	1.320	0.110	8.3
L4	ICP2	1.30	1.50	1.60	1.40	1.50	1.460	0.114	7.8
L5	AAS1	1.40	1.30	1.50	1.30	1.30	1.360	0.089	6.6
L7	AAS1	1.53	1.46	1.58	1.38	1.45	1.480	0.077	5.2
L13	AAS1	1.29	1.30	1.58	1.21	1.15	1.306	0.165	12.6
L15	AAS1	1.19	1.34	1.26	1.28	1.26	1.266	0.054	4.2
L26	AAS1	1.24	1.19	1.27	1.24	1.35	1.258	0.059	4.7
L56	AAS1	1.40	1.50	1.30	1.50	1.60	1.460	0.114	7.8
L63	AAS1	1.20	1.50	1.50	1.40	1.40	1.400	0.122	8.7

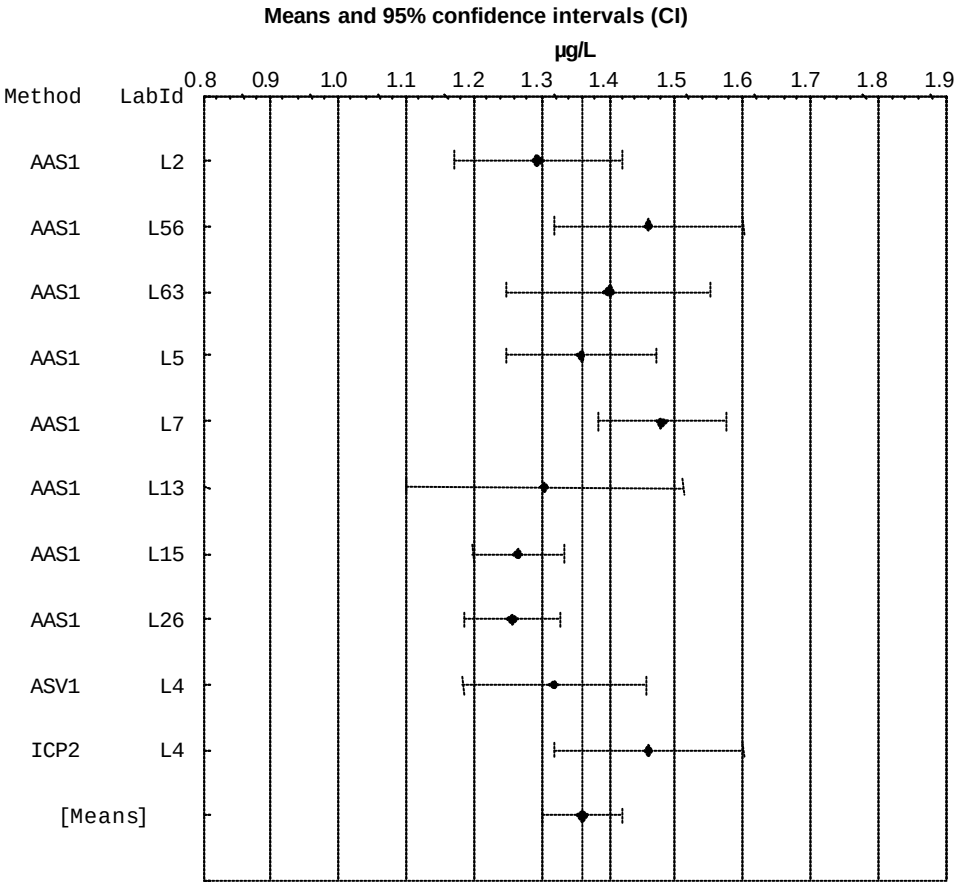

Figure 12.2 - Graphical presentation of individual results BCR- 634, Cd (µg/L)

Table 12.c - Table of individual results for BCR- 635, Pb (µg/L)

Lab ID	*Method*	*Individual measurement results*					*Mean*	*Std. Dev.*	*CV %*
L1	FAAS2	219.00	209.00	211.00	215.00	209.00	212.600	4.336	2.0
L2	AAS1	191.10	194.00	192.90	186.90	174.20	187.820	8.079	4.3
L4	ASV1	226.00	216.00	206.00	226.00	214.00	217.600	8.532	3.9
L4	ICP2	221.00	213.00	205.00	218.00	211.00	213.600	6.229	2.9
L5	AAS1	215.00	222.00	221.00	215.00	217.50	218.100	3.286	1.5
L7	AAS1	213.60	204.40	199.80	199.10	197.30	202.840	6.560	3.2
L13	AAS1	217.00	220.00	207.00	215.00	217.00	215.200	4.919	2.3
L19	AAS2	200.00	200.00	205.00	199.00	206.00	202.00	3.240	1.6
L20	AAS2	215.50	217.60	217.60	213.40	213.40	215.500	2.100	1.0
L26	AAS1	215.00	192.00	208.00	218.00	199.00	206.400	10.877	5.3
L31	AAS1	207.05	202.38	207.48	208.81	221.00	207.344	3.173	1.5
L56	AAS1	218.00	217.00	220.00	219.00	221.00	219.000	1.581	0.7

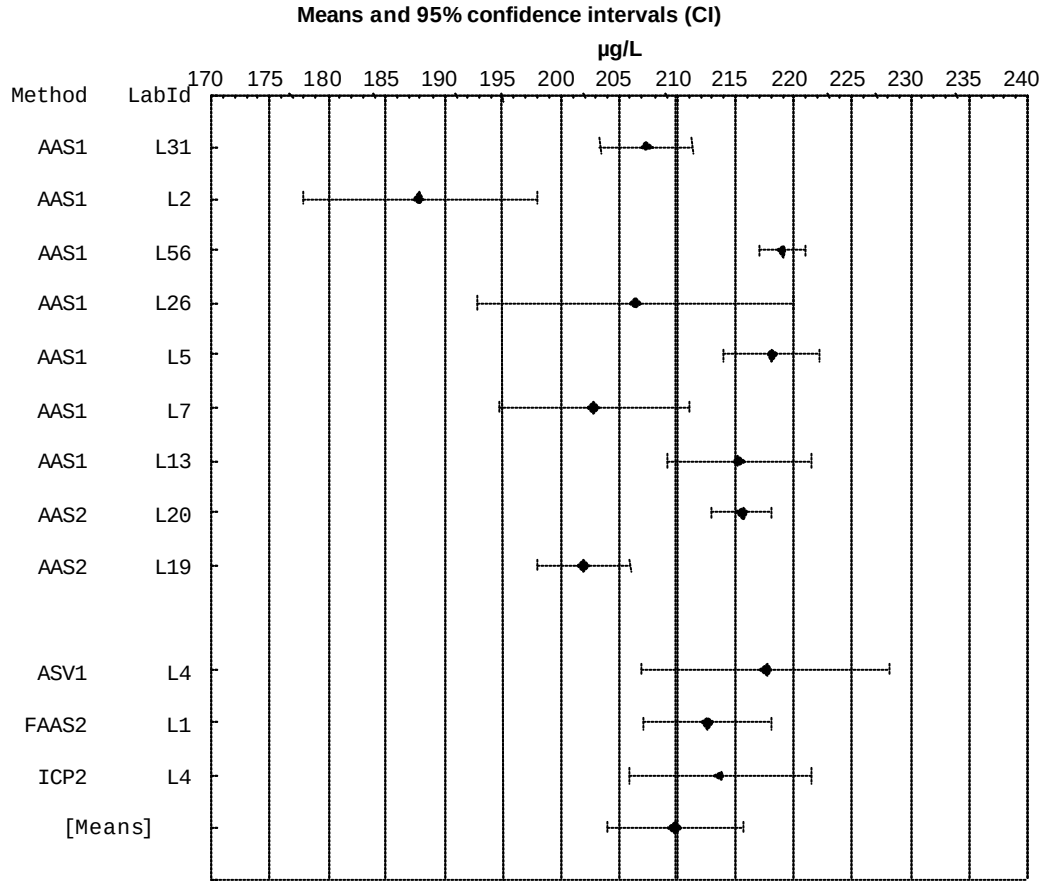

Figure 12.3 - Graphical presentation of individual results BCR- 635, Pb (µg/L)

Table 12.d - Table of individual results for BCR- 635, Cd (µg/L). Numbers in square parantheses,"[]", are statistically found outliers (significance level 1 %). They take part in the Lillifors-test for normality and are shown in the graphs, but are ignored in all other calculations.

Lab ID	*Method*	*Individual measurement results*					*Mean*	*Std. Dev.*	*CV %*
L2	AAS1	6.40	6.90	6.23	6.60	6.72	6.570	0.263	4.0
L4	ASV1	6.50	6.30	6.50	6.40	6.20	6.380	0.130	2.0
L4	ICP2	6.20	6.40	6.40	6.50	6.50	6.400	0.122	1.9
L5	AAS1	6.50	6.40	6.30	6.60	6.60	6.480	0.130	2.0
L7	AAS1	6.87	6.85	6.87	6.78	6.68	6.810	0.082	1.2
L13	AAS1	6.56	6.55	7.00	6.56	6.60	6.654	0.194	2.9
L15	AAS1	6.63	6.62	6.42	6.95	6.57	6.638	0.194	2.9
L26	AAS1	[6.40]	[6.13]	[7.03]	[5.90]	[6.50]	[6.392]	[0.427]	[6.7]
L56	AAS1	6.50	6.80	6.70	6.90	6.80	6.740	0.152	2.3
L63	AAS1	6.20	6.40	6.10	6.50	6.40	6.320	0.164	2.6

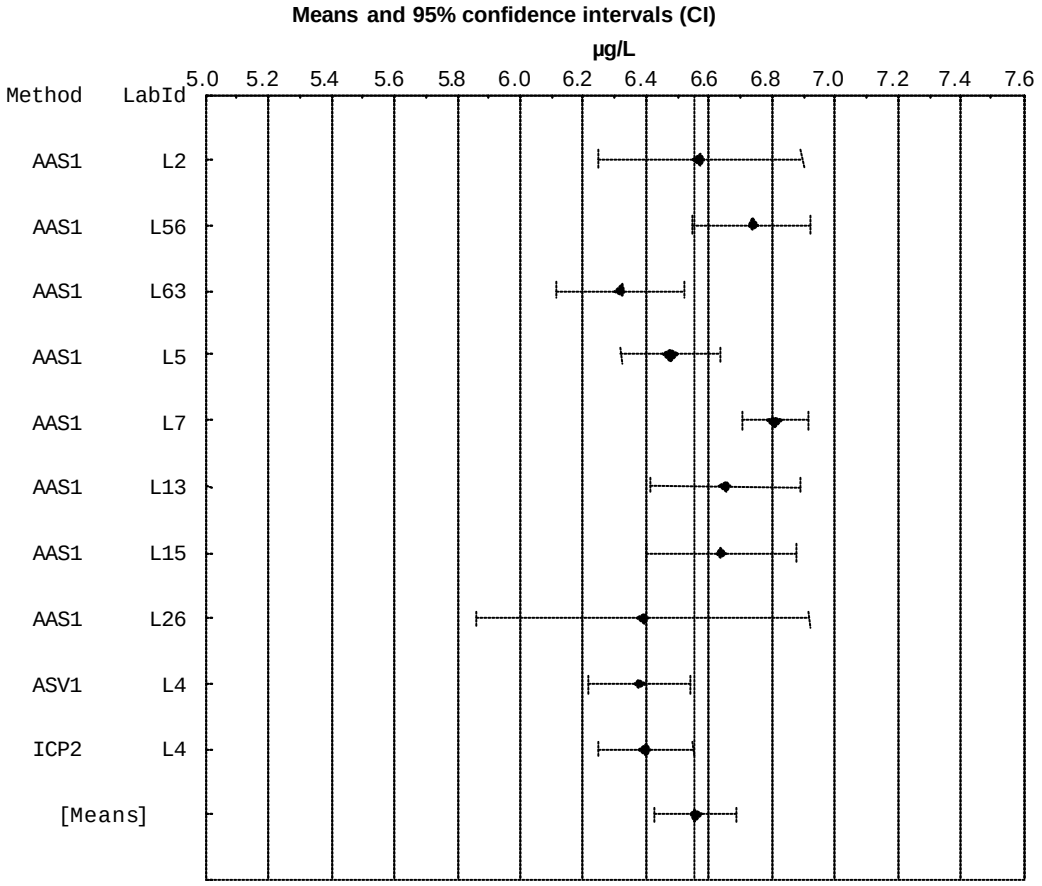

Figure 12.4 - Graphical presentation of individual results BCR-635, Cd (µg/L)

Table 12.e - Table of individual results for BCR-636, Pb (µg/L)

Lab ID	*Method*	*Individual measurement results*					*Mean*	*Std. Dev.*	*CV %*
L1	FAAS2	479.00	476.00	506.00	495.00	512.00	493.600	15.947	3.2
L4	ASV1	532.00	538.00	545.00	532.00	530.00	535.400	6.148	1.1
L4	ICP2	525.00	540.00	519.00	537.00	530.00	530.200	8.585	1.6
L5	AAS1	534.50	537.50	543.00	531.50	535.00	536.300	4.310	0.8
L7	AAS1	537.60	496.00	503.30	494.00	505.10	507.200	17.631	3.5
L13	AAS1	511.00	490.00	511.00	513.00	498.00	504.600	10.114	2.0
L19	AAS2	519.00	522.00	541.00	520.00	534.00	527.200	9.783	1.9
L20	AAS2	515.90	540.80	520.00	532.50	505.60	522.960	13.868	2.7
L26	AAS1	504.00	495.00	490.00	509.00	457.00	491.00	20.408	4.2
L31	AAS1	509.32	498.25	523.90	507.02	505.29	508.756	9.422	1.9
L56	AAS1	510.00	515.00	505.00	510.00	517.00	511.400	4.722	0.9

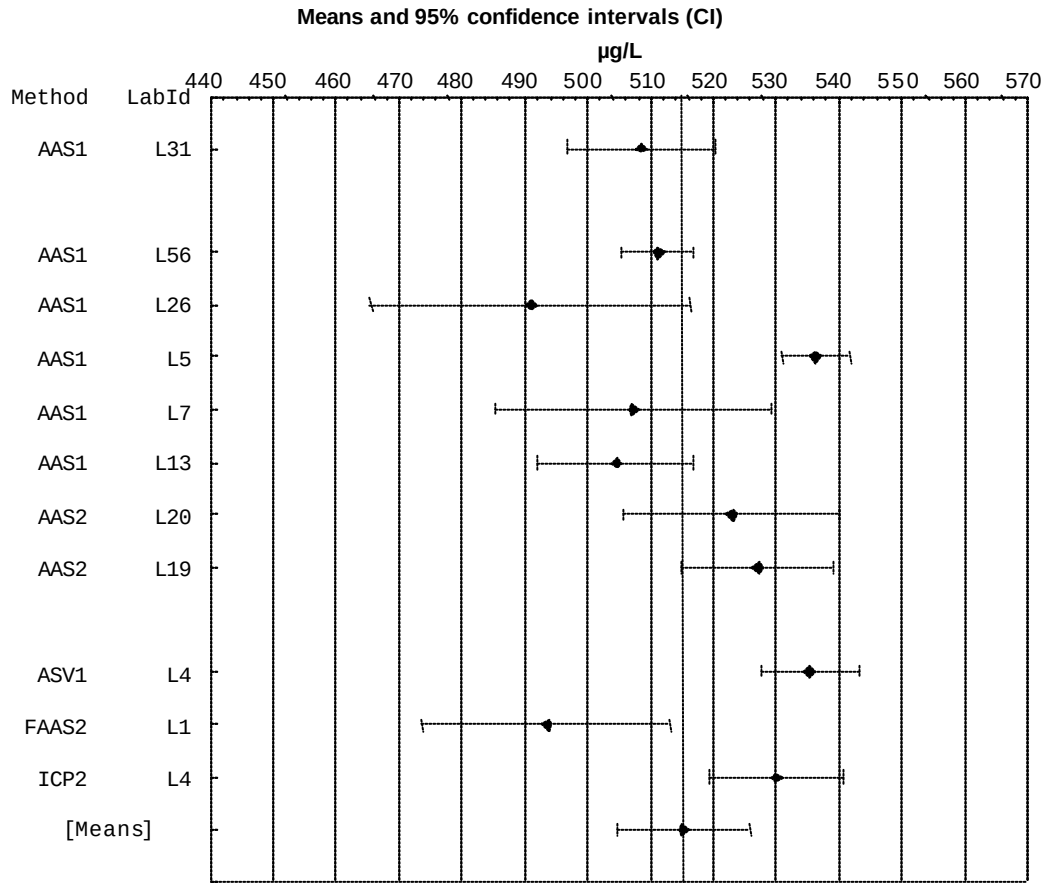

Figure 12.5 - Graphical presentation of individual results BCR- 636, Pb (µg/L)

Table 12.f - Table of individual results for BCR-636, Cd (µg/L). Numbers in square parantheses,"[ ]", are statistically found outliers (significance level 1 %). They take part in the Lillifors-test for normality and are shown in the graphs, but are ignored in all other calculations.

Lab ID	*Method*	*Individual measurement results*					*Mean*	*Std. Dev.*	*CV %*
L2	AAS1	12.20	12.51	11.24	12.15	12.50	12.120	0.519	4.3
L4	ASV1	10.80	10.30	11.20	11.30	11.40	11.000	0.453	4.1
L4	ICP2	11.00	11.50	11.10	11.30	11.20	11.220	0.192	1.7
L5	AAS1	11.20	11.40	11.70	11.50	11.70	11.500	0.212	1.8
L7	AAS1	11.98	12.00	12.03	12.00	11.80	11.962	0.092	0.8
L13	AAS1	11.56	11.96	12.03	11.79	11.56	11.780	0.219	1.9
L15	AAS1	11.30	11.37	11.52	11.93	11.31	11.486	0.263	2.3
L26	AAS1	[10.90]	[10.00]	[11.20]	[12.40]	[10.10]	[10.920]	[0.973]	[8.9]
L56	AAS1	11.50	12.00	11.50	12.50	12.80	12.060	0.586	4.9
L63	AAS1	11.60	11.90	11.30	11.70	11.70	11.640	0.219	1.9

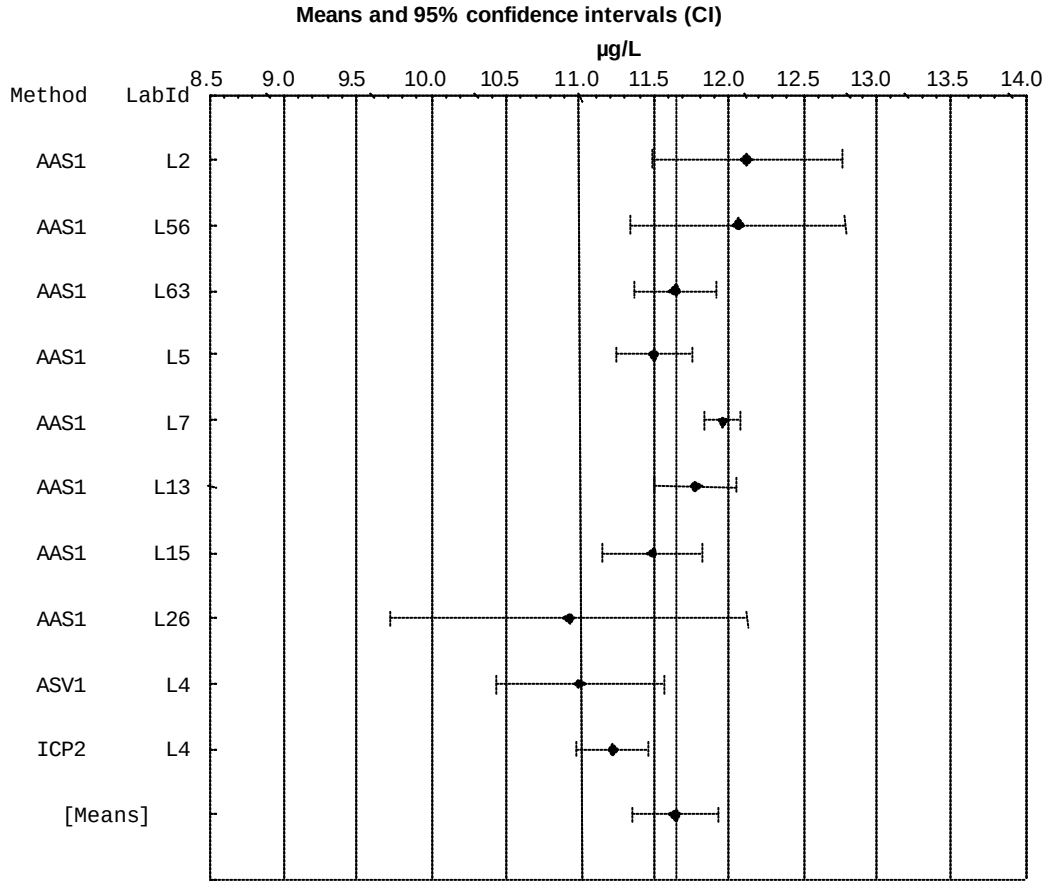

Figure 12.6 - Graphical presentation of individual results BCR 636, Cd (µg/L)

European Commission

EUR 21066 – DG Joint Research Centre, Institute for Reference Materials and Measurements –
The certification of the mass concentrations of lead and cadmium in reconstituted human blood, BCR-634, - 635, -636
Authors: K. Byrialsen, J. Kristiansen, J.M. Christensen, C. Dirscherl, B.M. Gawlik, C.L. Klein, A. Lamberty
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Abstract

This report documents the preparation, homogeneity and stability study as well as the certification campaign of three lyophilised human whole blood materials, i.e. BCR-634, BCR-635 and BCR- 636 with certified concentrations of lead and cadmium. The homogeneity and stability studies demonstrated that the materials are homogeneous and stable with respect to storage and transportation. The materials were subjected to a certification campaign for which the analytical work is described.

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