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The certification of the mass concentrations of aluminium, selenium and zinc in human serum

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BCR information
REFERENCE MATERIALS

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ABSTRACT

This report documents the preparation, homogeneity, stability and certification of three liquid human serum materials, i.e. BCR-637, BCR-638 and BCR-639 with certified concentrations of aluminium, selenium and zinc. The homogeneity studies demonstrated that the materials are homogeneous with respect to the content of aluminium, zinc and selenium. The materials were subjected to a certification campaign for which the analytical work is described.

Uncertainty contributions for stability, homogeneity and characterisation were included in the combined and expanded uncertainties assigned to the certified values.

The certified concentrations and uncertainties (expanded uncertainty with a coverage factor of k=2) of aluminium, selenium and zinc are

	Aluminium [µg/L]	Selenium [µg/L]	Zinc [µg/L]
BCR-637	12.5 ± 3.0	81 ± 7	$(1.11 \pm 0.22) \cdot 10^3$
BCR-638	55 ± 7	104 ± 7	$(1.43 \pm 0.21) \cdot 10^3$
BCR-639	194 ± 14	133 ± 12	$(2.36 \pm 0.14) \cdot 10^3$

ABBREVIATIONS AND SYMBOLS

In the report (including the annexes), the following abbreviations are used:

BCR	Community Bureau of Reference	RNAA	Radiochemical Neutron Activation Analysis
CRM	Certified Reference Material		
CI	Confidence interval	s	Standard deviation
CV	Coefficient of Variation	SF	Sector Field
ETAAS	Electrothermal Atomic Absorption Spectrometry	U	Uncertainty value
		u_{bb}	Uncertainty component from homogeneity
FAAS	Flame Atomic Absorption Spectrometry	u_{bb}^{*}	Upper limit of inhomogeneity that can be hidden by the method repeatability
FIAAS	Flow Injection Atomic Absorption Spectrometry		
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry	u_{char}	Uncertainty component from batch characterisation
ICP-MS	Inductively Coupled Plasma Mass Spectrometry	U_{CRM}	Expanded uncertainty of CRM
		u_{lts}	Uncertainty component from long-term stability
INAA	Instrumental Neutron Activation Analysis	u_{sts}	Uncertainty component from short-term stability
ISO	International Organization for Standardization		

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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 high concentrations of elements such as aluminium (Al), selenium (Se) and zinc (Zn) are considered as an 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 relating to exposure to chemical, biological and physical agents. Most recently a specific Directive proposal on chemical agents at work has been introduced [2].

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 [3]). However, biological monitoring is a very important tool to assess exposure to chemicals where air monitoring alone may not be a reliable indicator, and to assess overall exposure, occupational and non-occupational, by being a more accurate assessment of the actual body burden. When health risk has to be assessed and actions have to be taken to reduce exposure, reliable data from measurements for toxic elements in human tissues and biological fluids are essential. It is known from scientific literature that even low concentrations may have significant and specific biological effects on human tissue, such as blood vessel walls [4, 5].

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. In the EU, however, CRMs are only available for a limited number of trace elements in biological fluids and not for the elements Al, Se and Zn.

The Standards, Measurements and Testing Programme (SM&T, formerly BCR) of the European Commission, who has the task to assist in improving the quality of measurements, therefore launched a project dealing with the production of three sterile filtered human serum reference materials certified for the contents of Al, Se and Zn.

The concentrations of Al, Se and Zn in BCR-637 (low level), BCR-638 (medium level) and BCR-639 (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) [6] and German Biologische Arbeitsstoff-Toleranz-Werte (BAT values) [7]. Where no BEI or BAT values exists, the elevated concentrations were based on data from occupationally exposed workers.

The CRMs will harmonise methodological approaches and measurements of toxic trace elements between different member states to enable economic and political decisions to be made.

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 discussed carefully, 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 had all shown satisfactory analytical performance in a

preliminary intercomparison study. Each participant in the certification campaign was requested to analyse the candidate CRMs under reproducibility conditions, i.e. analyses 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 calibration standards etc.), the calibrant (reference material, spectroscopic standard etc.), the producer of the calibrant and the calibrant purity.

The different analytical techniques applied in the certification campaign were electrothermal atomic absorption spectrometry (Zeeman background correction), flame atomic absorption spectrometry, inductively coupled plasma mass spectrometry, high resolution inductively coupled plasma mass spectrometry and instrumental neutron activation analysis.

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
-	Trace Element Laboratory, Odense University Hospital, Odense	DK
-	State Serum Institute	DK

2.2 Analyses

-	Centre for Analytical Sciences, University of Southampton	UK
-	Friedrich-Alexander Universität, Erlangen-Nürnberg	DE
-	GSF – National Research Center for Environment and Health, Oberschleissheim	DE
-	Institut National de Recherche et de Sécurite, Vandeouvre	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
-	Laboratory of Analytical Chemistry, University of Gent	BE
-	National Institute of Occupational Health, Copenhagen	DK
-	Risø National Laboratory, Risø	DK
-	Robens Institute, University of Surrey	UK
-	Scientific Institute of Public Health, Brussels	BE
-	Trace Element Laboratory, Odense University Hospital, Odense	DK
-	Statens arbeidsmiljøinstitutt, Oslo	NO
-	University of Sheffield	UK

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 serum materials were produced in 1996 and a feasibility study was organised with the participation of 22 laboratories for determination of one or more of Al, Se and Zn. 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 the three concentration levels of the serum material, 300 samples were produced by State Serum Institute, Denmark under supervision from the co-ordinator. The Low concentration level was representative for an environmental exposure level and 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 [8]. 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 Aluminium

All results were based on ETAAS. The results of 2 laboratories using ICP-MS were excluded from the evaluation since this technique is not adequate for determination due to the low atomic mass of Al. Standard additions or matrix-matched standards were the preferred calibration technique. Aqueous solutions of aluminium for manufacturing the calibrants were obtained from 8 different companies.

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

Level	Target value (µg/L)	Spike (µg/L)	Mean value, (µg/L)	95 % CI of mean value, (µg/L)	Data sets Total	Accepted
Low	10	0	13.5	11.4 - 15.7	18	8
Medium	50	41	57	52 - 62	18	9
High	150	144	159	150 - 169	18	9

For aluminium in serum the target values were achieved within a reasonable margin. The low

level is satisfactory considering the fact that contamination is a serious problem with aluminium. A summary of the preliminary intercomparison study for aluminium is presented in table 3.a.

3.1.2 Selenium

Seven methods were based on ETAAS technique, one was based on digestion of the serum followed by FIAAS, and one method was INAA. The FIAAS method tended to give higher results, while the ETAAS methods and INAA agreed. The most used calibration techniques were standard additions followed by matrix matched calibrants. The baseline level (Low level) was lower than foreseen by the target value, and consequently the mean values at the Medium and High level were also lower than the target values. It is advantageous to have a Se baseline level as low as possible, because Se-deficiency is a more typical problem than selenium excess. The achieved baseline level reflects the current Danish reference level.

Aqueous solutions of selenium for manufacturing the calibrants were obtained from 6 different companies. For the INAA method solid SeO_2 was used as comparator. A summary of the preliminary intercomparison study for selenium is presented in table 3.b:

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

Level	Target value (µg/L)	Spike (µg/L)	Mean value, (µg/L)	95 % CI of mean value, (µg/L)	Data sets	
					Total	Accepted
Low	100	0	78	63 - 84	17	9
Medium	120	25	103	98 - 108	17	8
High	150	51	131	123 - 139	17	7

3.1.3 Zinc

Six methods were based on FAAS, while other methods included ETAAS, ICP-AES, high resolution-ICP-MS and INAA and RNAA at High level. For the calibration of FAAS methods, zinc in aqueous glycerol was most often used for calibration. Solutions of selenium for preparing the calibrants were obtained from 7 different companies. For the INAA method, solid ZnO_2 was used as comparator.

The baseline level (Low level) was higher than the target value. Since the achieved baseline level was at the upper end of the Danish reference level, Low level probably reflected a contamination during the production process (e.g. collection of serum). The homogeneity of the material was not questioned. Problems related to contamination were investigated before production of the candidate CRMs. A summary of the preliminary intercomparison study for zinc is presented in table 3.c.

Table 3.c - Summary of the preliminary intercomparison study for zinc. Target value: The value to be achieved according to the protocol. Spike: The concentration due to the spike. Mean value: The mean

of laboratory means of accepted data. Statistical outlier evaluation has not been performed. 95 % CI : The 95 % confidence interval of the mean value. Data sets: The total number of data sets received, and the number of data sets accepted for calculating the mean value and confidence interval.

Level	Target value (mg/L)	Spike (mg/L)	Mean value, (mg/L)	95 % CI of mean value, (mg/L)	Data sets	
					Total	Accepted
Low	0.65	0	1.36	1.32 - 1.40	19	10
Medium	1.0	0.36	1.73	1.68 - 1.79	19	10
High	2.0	1.35	2.70	2.61 - 2.79	21	12

3.2 Conclusion

The outcome of this feasibility study was promising enough to justify a certification campaign on materials produced with similar concentrations.

4. PREPARATION OF THE CANDIDATE CRMS

The materials were produced by Statens Serum Institut, Denmark under supervision of the co-ordinator. Spiking solutions were provided by the co-ordinator.

Twenty four liters of serum were prepared from fresh blood obtained from normal (healthy) Danish blood donors. The individual donations were tested negative for hepatitis B antigen, and hepatitis C and HIV 1+2 antibodies. The serum had been stored at -20 °C until processed. The production of the CRMs was performed from 24.09.1996 to 26.09.1996.

4.1 Filtration

The serum was thawed at room temperature overnight 23.09. – 24.09.1996. Pre-filtration and sterile filtration were performed at day 1 of the production.

4.1.1 Prefiltration

The serum was pooled in a sterile tank with a capacity of 30 L and pre-filtered through three 10" Millipore filters in series (CP 20→ CP 06 → CWSS) at low pressure into another sterile tank with a capacity of 30 L. Duration : 30 minutes.

4.1.2 Sterile filtration

Four B20 glass bottles (M, A, B and C) were equipped, connected and autoclaved. The pre-filtered serum was then sterile filtered through the 0.22 µm Millidisc filter 30#03 MCGL from the 30 L tank and into the sterile bottle M under elevated pressure. Duration: 45 minutes. The serum was stirred for 2 hours, the feed tube was closed and the filter disconnected.

The homogenised sterile filtered serum was then pumped through the feed tube into bottle A, B and C. Duration 50 minutes. The feed tubes of bottle A, B and C were closed and the bottles were disconnected and stored at +4 °C until spiking.

4.2 Spiking

Spiking of BCR-638 and BCR-639 was performed by injection of sterile trace element solutions into the bottles A and C, respectively. The solutions were injected using a sterile syringe with needle penetrating the rubber membrane of the bottle. After injection, the serum was stirred vigorously for 30 minutes. The materials were spiked according to the following scheme:

Table 4.a Target values and spiking amounts for Al, Se and Zn contents in BCR-637, BCR-638 and BCR-639. Values in brackets are expected endogenous concentrations.

Reference Material	Aluminium, Al		Selenium, Se		Zinc, Zn	
	Target value	Spike	Target value	Spike	Target value	Spike
	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)
BCR-637	(10)	0	(100)	0	(650)	0
BCR-638	50	40.6	120	20.3	1000	324.9
BCR-639	150	185.9	150	48.5	2000	1293.5

Spiking solutions were prepared by the co-ordinator using NIST SRM 3101a (10.00 ± 0.02 mg/mL Al), and NIST SRM 3168a (10.00 ± 0.03 mg/mL Zn) and Merck Titrisol Standard solution (1.000 ± 0.002 g/L Se). The solutions were autoclaved after preparation.

4.3 Filling

Prior to the filling process the filling, chambers and connected equipment were sterilised together with the vials and stoppers.

After spiking and homogenisation, dispensing of the serum for BCR-638 and BCR-639 was performed at day 2. Dispensing of BCR-637 was performed at day 3. The initial 200 mL of each CRM were discarded. Subsequently, 4.5 mL of serum was dispensed into each vial, the vials were stoppered in the filling chambers and encapsulated. The CRMs were stored at −80 °C.

5. HOMOGENEITY STUDY

The homogeneity study was performed after the filling process in order to determine the overall random variation originating from the variations during the filling and the production process. The homogeneity was examined by analysing 20 vials for Al and 10 vials for Se and Zn from each candidate CRM.

5.1 Method

Analysis of Al and Zn was performed by the Trace Element Laboratory, Odense University Hospital, Denmark. Analysis of Al content was performed by ETAAS (Perkin Elmer 4100 ZL). Analysis of Zn was performed by FAAS (Perkin Elmer 4100).

The samples were thawed at room temperature and the analyses were performed as duplicate concentration measurements (Al) or triplicate concentration measurements (Zn) under repeatability conditions in order to reduce any analytical variation.

Analysis of Se was performed by the co-ordinator. The samples were thawed at room temperature and analysis was performed by ETAAS (Perkin Elmer 5100-Z) as triplicate peak area measurements under repeatability conditions in order to reduce any analytical variation.

5.2 Results

The variation of Al, Se and Zn peak signals was evaluated by analysis of variance, ANOVA, and the outcome of the statistical evaluation is presented in tables 5.a-c. A graphical survey of the results is presented in Annex I. The between-sample variance 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 the 5 % significance level. The evaluation did not reveal any statistical significant between-sample variation at the 5 % significance level. The materials were consequently deemed homogeneous with respect to Al and Se contents and judged suitable for certification.

Table 5.a - Homogeneity test of Al, Se and Zn contents in BCR-637. The material is homogeneous with respect to Al and Se as the P values are ≥ 5 %, but not homogeneous with respect to Zn as the P value is < 5 %.

	Element	Source of variation	ANOVA estimated variance, s^2	F-test value	P-value
BCR-637	Al	Samples	0.496	0.82	66.3 %
		Analytical	0.603	-	-
	Se	Samples	0.26	1	58.0 %
		Analytical	0.30	-	-
	Zn	Samples	0.291	5.3	0 %
		Analytical	0.055	-	-

Table 5.b - Homogeneity test of Al, Se and Zn contents in BCR-638. The material is homogeneous with respect to Al and Se as the P values are ≥ 5 %, but not homogeneous with respect to Zn as the P value is < 5 %

	Element	Source of variation	ANOVA estimated variance, s^2	F-test value	P-value
BCR-638	Al	Samples	2.308	1.13	39.7 %
		Analytical	2.051	-	-
	Se	Samples	0.21	1	70.9 %
		Analytical	0.30	-	-
	Zn	Samples	1.201	6.8	0 %
		Analytical	0.177	-	-

Table 5.c Homogeneity test of Al, Se and Zn contents in BCR-638. The material is homogeneous with respect to Al, Se and Zn as the P values are ≥ 5 %.

	Element	Source of variation	ANOVA estimated variance, s^2	F-test value	P-value
BCR-639	Al	Samples	11.413	1.2	34.4 %
		Analytical	9.507	-	-
	Se	Samples	156.77	1	45.4 %
		Analytical	153.00	-	-
	Zn	Samples	0.482	1.6	17.3 %
		Analytical	0.293	-	-

For BCR-637 and BCR-638 homogeneity could not be verified with respect to the Zn content. This might be due to a very low analytical variance for the Zn analysis. However, it was decided to calculate the relative standard uncertainty for the inhomogeneity of all three materials and to include them into the combined uncertainty assigned to the certified values. For further information refer to Chapter 8.

5.3   Conclusion

For all three candidate CRMs, no statistical significant between-sample variation was revealed at the 5 % significance level for Al and Se. Homogeneity could not be demonstrated for Zn in BCR-637 and BCR-638 due to a very low analytical variation. However, the relative between-sample standard deviation (which includes the variation caused by inhomogeneity) was lower than the repeatability standard deviation from the preliminary intercomparison study. Therefore, it was decided that the materials were sufficiently homogeneous to be subjected to certification. Uncertainty allowances due to inhomogeneity were included in the respective uncertainty of the certified value.

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 – 80 °C at two occasions (August 1997 and January/February 1998).

6.1.1 Method

The samples were analysed for Al by ETAAS (Perkin Elmer 4100-ZL) using a calibration curve prepared in bovine serum by spiking with Merck Titrisol solution (1.000 ± 0.002 g/L Al) to appropriate concentrations. The samples were analysed for Se by ETAAS (Perkin Elmer 5000-ZL) using a calibration curve prepared in bovine serum by spiking with Merck standard solution (1000 mg/L Se) to appropriate concentrations.

Analysis of Zn was performed by FAAS (PE 2100) using a calibration curve prepared in bovine serum by spiking with Merck standard solution (1000 mg/L Zn) to appropriate concentrations. Statistical control of the analytical procedures was documented by the use of control charts.

6.1.2 Results

Any change of the 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 Al, Se and Zn at the two study occasions using ANOVA. The stability was tested at the 5 % significance level and the evaluation did not reveal any statistical significant long-term variation for Al and Se. Results from the ANOVAs are presented in the tables 6.a-c. The data were used to estimate uncertainty allowances due to stability issues. For further details refer to Chapter 8.

Table 6.a - Long-term stability test of Al, Se and Zn contents in BCR-637. The material is stable with respect to Al, Se and Zn as the P values are ≥ 5 %.

	Element	Source of variation	ANOVA estimated variance, s^2	F-test value	P-value
BCR-637	Al	Time	1.576	1.88	22.0 %
		Analytical	0.839	-	-
	Se	Time	9.976	0.87	38.8 %
		Analytical	11.498	-	-
	Zn	Time	1.323	6.0	5.0 %
		Analytical	0.47	-	-

Table 6.b - Long- term stability test of Al, Se and Zn contents in BCR-638. The material is stable with respect to Al and Se as the P values are ≥ 5 %, but not stable with respect to Zn as P is < 5 %.

	Element	Source of variation	ANOVA estimated variance, s^2	F-test value	P-value
BCR-638	Al	Time	8.670	4.98	6.7 %
		Analytical	1.747	-	-
	Se	Time	5.246	0.86	39.0 %
		Analytical	6.122	-	-
	Zn	Time	3.234	7.1	3.7 %
		Analytical	0.177	-	-

Table 6.c - Long- term stability test of Al, Se and Zn contents in BCR-639. The material is stable with respect to Al, Se and Zn as the P values are ≥ 5 %.

	Element	Source of variation	ANOVA estimated variance, s^2	F-test value	P-value
BCR-639	Al	Time	3.069	1.18	32.0 %
		Analytical	2.611	-	-
	Se	Time	9.988	0.28	61.3 %
		Analytical	35.183	-	-
	Zn	Time	1.027	1.0	35.4 %
		Analytical	1.019	-	-

6.1.3 Conclusion

The materials were deemed stable with respect to Al and Se contents and judged suitable for certification. For BCR-638 there was a significant decrease in the Zn content. However, this was not verified in the accelerated stability study (see below), where samples of BCR-638 were stored at 20 °C for 24 weeks. In this case, no instability was indicated.

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 for Al and Se by ETAAS (Perkin Elmer 4100–ZL and PE 5100-ZL, respectively). 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. For BCR-637, there was no indication of instability for the samples kept at 20 °C. The content of Al showed a significant decrease in the ratio (peak area at test temperature/ peak area at reference temperature) after 12 weeks. However, after 24 weeks the ratio has returned to the starting level with the CI including the value 1. For the samples stored at 37 °C, no thermal instability was indicated for Al and Se. However, the content of Zn showed a significant decrease after 24 weeks. At 45 °C, the contents of Al and Se significantly increased after 24 weeks of storage. For the same test temperature, the content of Zn significantly decreased after 12 weeks and remained at the decreased level for the rest of the test period.

Figure 6.1 - Accelerated degradation study. Determinations of Al, Se and Zn in BCR-637 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.

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For BCR-638, there was no indication of instability for the samples kept at 20 °C. The content of Al showed a significant increase in the ratio after 4 weeks. However, after 12 weeks the ratio has returned to the starting level with the CI including the value 1. No thermal instability was indicated for Al and Se for the samples stored at 37 °C.The content of Zn showed a significant decrease in the ratio after 12 and 24 weeks. At 45 °C, the contents of Al significantly increased after 4 weeks and after 24 weeks. The content of Se significantly increased after 24 weeks and the content of Zn showed a significant decrease in the ratio after 12 and 24 weeks.
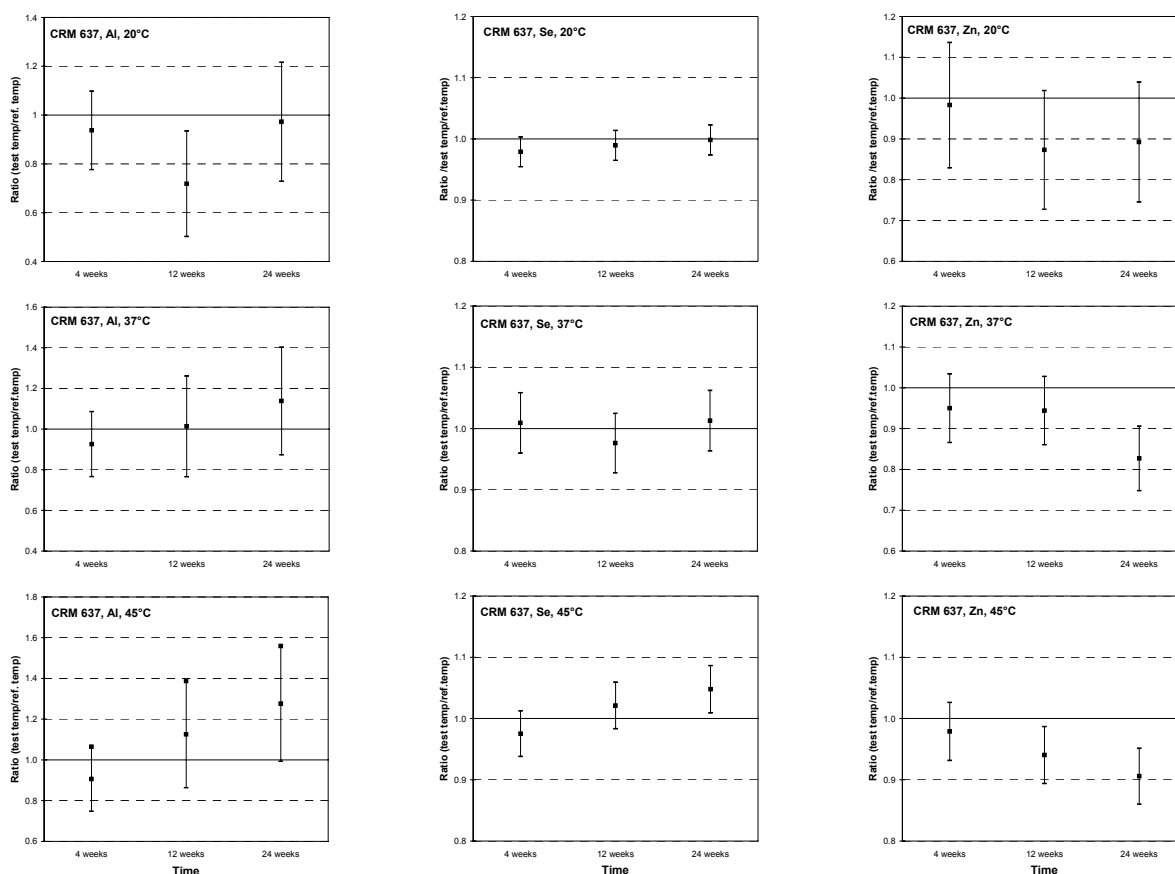

Figure 6.2 - Accelerated degradation study. Determinations of Al, Se and Zn in CRM 638 at three different time intervals. Ratio (test temp./ref. temp.) is the ratio of the peak area obtained at the test temperature and the peak area obtained at the reference temperature.

For BCR-639, there was no indication of instability for the samples kept at 20 °C. The content of Zn showed a significant decrease in the ratio after 4 weeks. However, after 12 weeks the ratio has returned to the starting level with the CI including the value 1. At the test temperature 37 °C, a significant decrease in the Al content was observed after 4 and 12 weeks. After 24 weeks the CI of the ratio included the value 1. The content of Se showed a significantly increase after 24 weeks and the Zn content showed a significantly decrease after 24 weeks. At 45 °C, there was no indication of thermal instability of the samples with respect to the Al content. However, the content of Se in the exposed samples showed a significantly increase after 12 and 24 weeks and a significantly decrease in the Zn content in the same period.
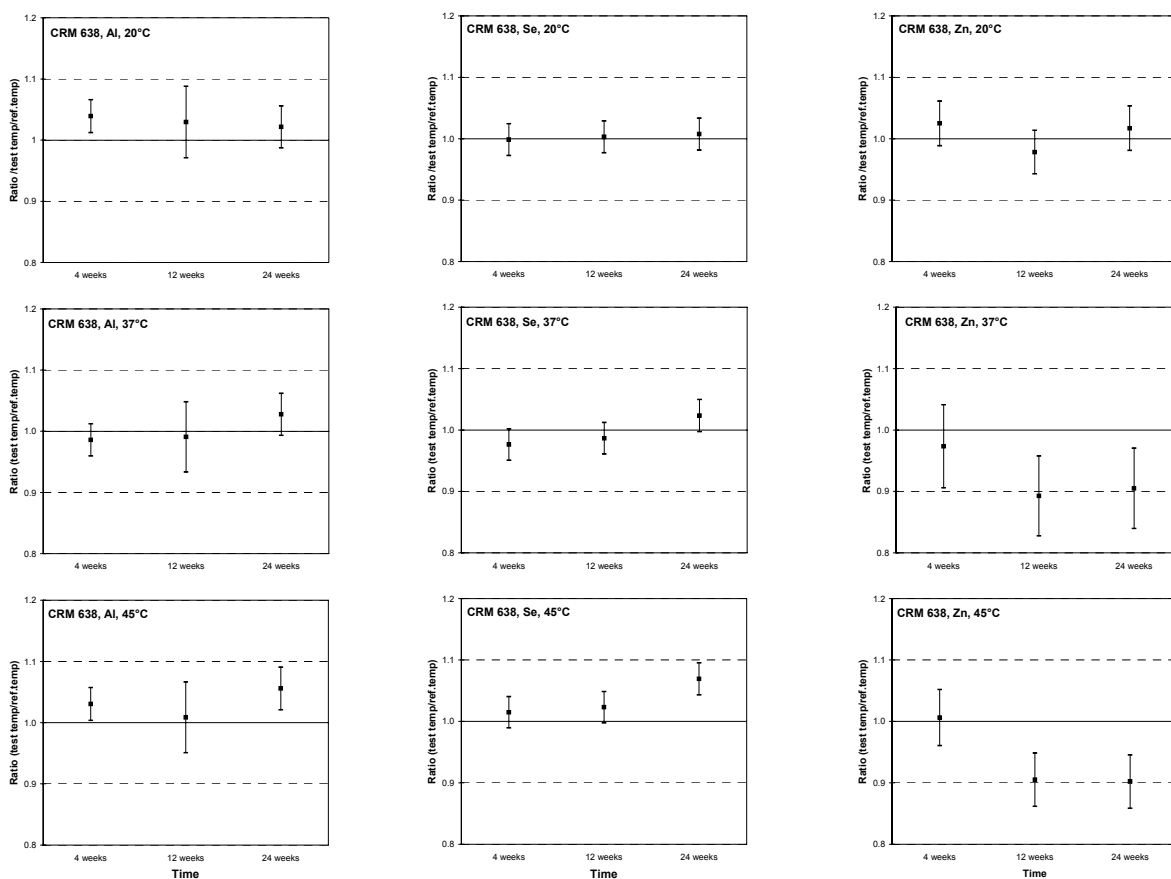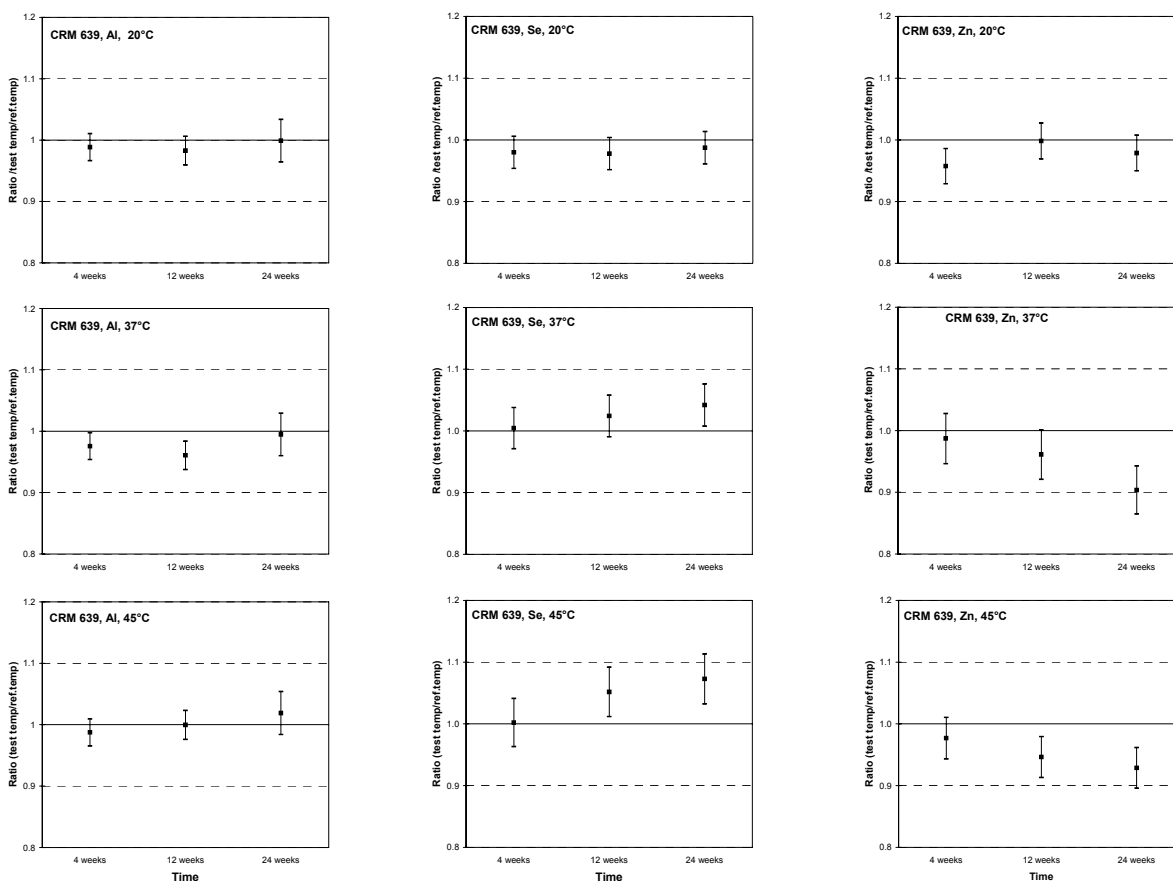
Figure 6.3 - Accelerated degradation study. Determinations of Al, Se and Zn in BCR-639 at three different time intervals. Ratio (test temp./ref. temp.) is the ratio of the peak area obtained at the test temperature and the peak area obtained at the reference temperature.

The materials demonstrate a sufficient stability with respect to the contents of Al, Se and Zn when stored at −80 °C. The apparent instability of BCR-638 with respect to the Zn content was not verified by the accelerated degradation study, where samples kept at 20 °C were stable for 24 weeks. The accelerated degradation study indicated that the CRMs are resistant to long term storage and transport at temperatures below 20 °C.

7. CERTIFICATIONS 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

At least two different analytical principles were applied in the certification campaign each of them being highly selective for the measurands. For Se and Zn, neutron activation analysis was applied. The method selectivity and diversity ensures the credibility of the certified values.

In table 7.a, the analytical techniques from the certification measurements are listed. In table 7.b, the type of calibration used by the participants is listed.

7.2 Technical evaluation of the results

In order to obtain the accuracy 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.

7.2.1 Aluminium

The participants discussed the need for working under clean room conditions when performing aluminium analyses. Aluminium analysis is very sensitive to contamination and, consequently, special efforts were considered in order to prevent contamination of the material during the analysis.

Table 7.a - Analytical techniques applied in the certification campaign for Al, Se and Zn in BCR-637, BCR-638 and BCR-639

Measurand	LAB ID	Method	Sample pretreatment	Detection
Aluminium	3	AAS1	100 µL sample were mixed with 100 µL 0.1 % Triton-X 100 in 0.5 % HNO_3 and 800 µL 0.5 % HNO_3	ETAAS with Zeeman background correction, pyrocoated furnace
Aluminium	4	ICP2	Dilution with ultrapure H_2O	SF ICP-MS, resolution: 300 Detection of ^{27}Al , internal standard: ^{45}Sc
Aluminium	7	AAS1	Dilution 1+3 with modifier (0.05 % Triton X-100), 1mM citrate)	ETAAS with Zeeman background correction, pyrocoated furnace
Aluminium	13	AAS1	50 µL sample diluted with 150 µL 0.2 % Triton X-100	ETAAS with Zeeman background correction, pyrocoated furnace with platform
Aluminium	15	AAS1	Samples diluted fivefold with 0.5 % Triton X-100 + 0.5 % EDTA	ETAAS with Zeeman background correction, pyrocoated furnace
Aluminium	20	AAS1	Samples diluted 1+2 with 2.5 % HNO_3	ETAAS with graphite furnace
Aluminium	31	AAS1	100 µL sample diluted with 100 µL standard and 200 µL modifier	ETAAS with Zeeman background correction, pyrocoated furnace with platform
Aluminium	56	AAS1	Dilution 1: 5	ETAAS with Zeeman background correction, graphite furnace with platform
Selenium	1	ICP1	Dilution 1+15 with 1 % v/v butan-1-ol, 0.66 % Triton X-100, 0.01 M NH_3, 0.0002 M $(NH_4)H_2EDTA$ and 0.002 M $NH_4H_2PO_4$, eliminates Ar-adduct ion interferences	ICP-MS Detection of ^{78}Se, Internal standard: ^{115}Ir
Selenium	4	AAS1	Modifier: Mg, Pd	ETAAS with Zeeman background correction, pyrocoated furnace
Selenium	5	AAS1	200 µL sample was diluted with 600 mL 0.2 % Triton X-100 Modifier: $(NH_4)I_2Cl_4$ in 5 % $Mg(NO_3)_2$	ETAAS with Zeeman background correction, pyrocoated furnace with platform
Selenium	6	INAA	Samples were lyophilized and the equivalent of 2 mL was irradiated	Cooling time: approx. 50 days Ge(Li) detector (FWHM 2.0 keV) coupled to multichannel analyser, ^{75}Se was measured
Selenium	7	AAS1	100 µL sample was diluted with 100 µL standard and 800 µL modifier, Modifier: $Ni(NO_3)_2 \cdot 6 H_2O$ in 0.05 %Triton X-100	ETAAS with Zeeman background correction, pyrocoated furnace
Selenium	13	AAS1	100 µL sample diluted with 150 µL 0.2 % Triton X-100 Modifier: 1 g/L $AgNO_3$ + 2 g/L $Cu(NO_3)_2 \cdot 3 H_2O$ + 2 g/L $Mg(NO_3)_2 \cdot 6 H_2O$+ 0.4 % HNO_3 65 %	ETAAS with Zeeman background correction, pyrocoated furnace with platform
Selenium	14	INAA	2.5 mL sample was lyophilized and irradiated	Cooling time: 3 weeks Ge detector coupled to multichannel analyser, ^{75}Se was measured

Measurand	LAB ID	Method	Sample pretreatment	Detection
Selenium	15	AAS1	Samples diluted fivefold with diluent/modifier (1:1) Diluent: 3 g/L Triton X-100 + 4 mL/L HNO$_3$ 65 % Modifier: 3.80 g /L Cu(NO$_3$)$_3$, 3 H$_2$O + 21.10 g/L Mg(NO$_3$)$_2$, 6 H$_2$O	ETAAS with Zeeman background correction pyrocoated furnace with platform
Selenium	20	AAS2	Samples prediluted with standards made up in modifier Modifier: 1 % Ni(NO$_3$)$_2$ in 0.144 M HNO$_3$	ETAAS with D$_2$ background correction, graphite furnace
Selenium	24	ICP1	0.5 mL sample diluted to 5 mL with 0.1 % ammonium EDTA, 0.1 % Triton X-100 and 0.5 % NH$_3$	ICP-MS [82]Se was measured, Internal standard: Y, Rh, Tl
Selenium	26	AAS1	2.0 mL sample digested with 2.5 mL sub-distilled HNO$_3$. Solution made up to 10.0 mL with H$_2$O, modifier: 0.5 % Pd	ETAAS with Zeeman background correction, Z-tek-tubes
Selenium	31	AAS1	50 µL sample diluted with 50 µL standard and 350 µL modifier	ETAAS with Zeeman background correction, pyrocoated furnace with platform
Selenium	56	AAS1	Dilution 1:10 Modifier: Pd	ETAAS with Zeeman background correction , graphite furnace
Zinc	1	FAAS	1+ 9 dilution in butan-1-ol	Flame AAS
Zinc	4	ICPA	1:20 dilution with 3 % HCl	ICP-AES
Zinc	5	FAAS	600 µL sample was diluted with 1800 mL H$_2$O	Flame AAS, no background correction
Zinc	6	INAA	Samples were lyophilized and the equivalent of 2 mL was irradiated	Cooling time: approx. 50 days Ge(Li) detector (FWHM 2.0 keV) coupled to multichannel analyser [65]Zn was measured
Zinc	13	FAAS	200 µL sample diluted with 1200 µL 0.04 % Triton X-100	Flame AAS
Zinc	14	INAA	2.5 mL sample was lyophilized and irradiated	Cooling time: 3 weeks Ge detector coupled to multichannel analyser [65]Zn was measured
Zinc	19	FAAS	Samples diluted 1+4 with H$_2$O	Flame AAS
Zinc	20	FAAS	Samples prediluted 1+4 with DI/RO H$_2$O	Flame AAS
Zinc	26	ICPA	2.0 mL sample digested with 2.5 mL sub-distilled HNO$_3$. Solution made up to 10.0 mL with H$_2$O	ICP-AES Internal standard: Cd
Zinc	31	FAAS	200 µL sample diluted with 200 µL standard and 500 µL H$_2$O	Flame AAS

Table 7.b - Calibrants used in the certification campaign for Al, Se and Zn in BCR-637, BCR-638 and BCR-639

Measurand	LAB ID	Method	Type of calibration	Calibrant	Producer	Purity
Aluminium	3	AAS1	Standard curve	Aluminium A.A. Standard no. 6917	Mallinckrodt Baker	1000 µg /mL ± 0.2 %
Aluminium	4	ICP2	Standard curve	Aluminium standard solution	Spex Ind. USA	1 g /L ± 0.2 %
Aluminium	7	AAS1	Standard curve	SRM 3101a	NIST	10.00 ± 0.02 mg/mL
Aluminium	13	AAS1	Standard curve, matrix-matching standards	Al standard AA solution	Merck	1.000 ± 0.002 g/L
Aluminium	15	AAS1	Standard addition, matrix matching standards	Aluminium standard solution, ref.1.19770.500	Merck-Clevenot lab.	1001 ± 2 mg/L
Aluminium	20	AAS1	Standard curve, matrix-matching standards	Spectrosol Al sol. no. 140312Q	Merck Ltd Leicester	1000 ± 5 mg/L
Aluminium	31	AAS1	Standard addition	Al single element plasma emission sol., N930-00100	Perkin Elmer	1004 µg/mL ± 0.5 %
Aluminium	56	AAS1	Standard addition	Standard solution Aluminium	Analyticals CARLO ERBA	1 mg/mL
Selenium	1	ICP1	Standard addition	Selenium Atomic Abs. std. 50F S-9760	SIGMA	1020 µg/mL
Selenium	4	GFAAS	Standard addition	Se standard solution	Spex Ind.USA	1 g /L ± 0.2 %
Selenium	5	AAS1	Standard addition	Selenium AA std. solution no. 88094	ALFA Johnson Matthey Company	1000 µg /mL
Selenium	6	INAA	Ratio/ counts	Se (pure substance)	Metallurgic Hoboken, BE	99.999 %
Selenium	7	AAS1	Standard addition	PE N930-0182	Perkin Elmer	996 µg/mL ± 0.5 %
Selenium	13	AAS1	Standard curve, matrix-matching standards	Se standard AA solution	Merck	1.000 ± 0.002 g/L
Selenium	14	INAA	Ratio/ counts	SeO_2 Aldrich no. 20.431-5 verified against SRM 3149	Aldrich Chemical Co.	99.999 %
Selenium	15	AAS1	Standard addition, matrix matching standards	Selenium standard solution, ref.1.19796.000	Merck-Clevenot lab.	1001 ± 2 mg/L
Selenium	20	AAS1	Standard addition	Spectrosol Se sol. no. 141624K	Merck Ltd Leicester	1002 ± 2 mg/L
Selenium	24	ICP1	Standard curve, aqueous standards	Se standard solution, no. 45598 2W	BDH	1000 µg/mL
Selenium	26	AAS1	Standard curve, matrix-matching standards	Se standard solution, no. 8054-1	Teknolab A/S	1000 ± 3 µg/mL
Selenium	31	AAS1	Standard addition	Se single element plasma emission sol., N930-00149	Perkin Elmer	996 µg/mL ± 0.5 %
Selenium	56	AAS1	Standard addition	Standard solution Selenium	SIGMA Chemical Comp.	1020 µg/mL
Zinc	1	FAAS	Aquous standards	Spectrosol 14166	BDH	5 mmol/L
Zinc	4	ICP-AES	Standard curve	XSPEXF-95, XSPEXF-96	Spex Ind. USA	2.5 µg/mL ± 0.5 %
Zinc	5	FAAS	Standard curve, aqueous standards	Zinc AA std. solution no. 88118	ALFA Johnson Matthey Company	1000 µg /mL
Zinc	6	INAA	Ratio/ counts	Zn (pure substance)	Goodfellow metals	99.99 %

Measurand	LAB ID	Method	Type of calibration	Calibrant	Producer	Purity
Zinc	13	FAAS	Standard curve, aqueous standards	Zn standard AA solution	Merck	1.000 ± 0.002 g/L
Zinc	14	INAA	Ratio/ counts	Zn, SRM 728	NIST	99.999 %
Zinc	19	FAAS	Standard curve, aqueous standards	Zn single element plasma emission sol., N930-00168	Perkin Elmer	1000 µg/mL ± 0.5 %
Zinc	20	FAAS	Standard curve, aqueous standards	Spectrosol Zn sol. no. 141503C	Merck Ltd Leicester	1000 ± 5 mg/L
Zinc	26	ICP3	Standard curve, aqueous standards	Zn standard solution, no. 8054-1	Teknolab A/S	1000 ± 3 µg/mL
Zinc	31	FAAS	Standard addition	Zn single element plasma emission sol., N930-00168	Perkin Elmer	1000 µg/mL ± 0.5 %

7.2.2 Selenium

The certification campaign included 2 laboratories using INAA. However, for BCR-638 and BCR-639 the confidence intervals for the results from the INAA methods did not overlap. A profound discussion of the INAA results did not reveal any explanation of the deviation and it was decided to keep both sets of results for the certification. The very low CV of the INAA analysis was explained by a long counting time.

7.2.3 Zinc

The basic material for the CRMs was contaminated during an early step in the production (sampling or filtration), as the concentration in BCR-637, which is unspiked, is higher than the normal level.

The certification campaign included 2 laboratories using INAA. However, for BCR-637 the confidence intervals of the results from the INAA methods did not overlap due to a very low CV in both laboratories. A profound discussion of the INAA results did not reveal any explanation of the deviation and it was decided to keep both sets of results for the certification. The very low CV of the INAA analysis was explained by a long counting time.

7.3   Statistical evaluation of the results

For each data set from the certification campaign 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.c-e. Details of the individual results from the certification campaign are presented in Annex II.

Table 7.c - Summary of statistical data for BCR-637

Certified property	Al	Se	Zn
Number of data sets	7	13	9
Number of individual data	35	65	45
Outlying data sets (Grubbs test)	No	No	No
Outlying variances (Cochran test)	Yes (1 outlier detected)	No	Yes (1 detected)
Mean of data set means	12.47 µg/mL	81.12 µg/mL	1.1138 mg/mL
Within-data set SD	1.19 µg/mL	3.20 µg/mL	0.0219 mg/mL
Between-data set SD	2.14 µg/mL	4.73 µg/mL	0.0630 mg/mL
Variances homogeneous (Bartlett test)	Yes	Yes	No
SD of data set means	2.20 µg/mL	4.95 µg/mL	0.0637 mg/mL
Data set means normally distributed (Kolmogorov-Smirnov-Lilliefors test)	Yes	Yes	Yes
Half width of the 95 % CI of the mean of data set means	2.04 µg/mL	2.99 µg/mL	0.0490 mg/mL

Table 7.d - Summary of statistical data for BCR-638

Certified property	Al	Se	Zn
Number of data sets	8	13	10
Number of individual data	40	65	50
Outlying data sets (Grubbs test)	No	No	No
Outlying variances (Cochran test)	No	No	No
	(1 straggler detected)	(1 straggler detected)	
Mean of data set means	54.93 µg/mL	104.02 µg/mL	1.4264 mg/mL
Within-data set SD	2.13 µg/mL	3.41 µg/mL	0.0367 mg/mL
Between-data set SD	4.11 µg/mL	6.14 µg/mL	0.0589 mg/mL
Variances homogeneous (Bartlett test)	Yes	No	Yes
SD of data set means	4.22 µg/mL	6.32 µg/mL	0.0612 mg/mL
Data set means normally distributed (Kolmogorov-Smirnov-Lilliefors test)	Yes	Yes	Yes
Half width of the 95 % CI of the mean of data set means	3.52 µg/mL	3.83 µg/mL	0.0437 mg/mL

Table 7.e - Summary of statistical data for BCR-639

Certified property	Al	Se	Zn
Number of data sets	7	13	10
Number of individual data	35	65	50
Outlying data sets (Grubbs test)	No	No	No
Outlying variances (Cochran test)	No	No	No
Mean of data set means	194.24 µg/mL	132.53 µg/mL	2.3621 mg/mL
Within-data set SD	7.43 µg/mL	5.56 µg/mL	0.0549 mg/mL
Between-data set SD	15.31 µg/mL	8.30 µg/mL	0.0794 mg/mL
Variances homogeneous (Bartlett test)	Yes	No	Yes
SD of data set means	15.67 µg/mL	8.67 µg/mL	0.0731 mg/mL
Data set means normally distributed (Kolmogorov-Smirnov-Lilliefors test)	Yes	Yes	Yes
Half width of the 95 % CI of the mean of data set means	14.49 µg/mL	5.24 µg/mL	0.0595 mg/mL

8. CERTIFIED VALUES AND UNCERTAINTY EVALUATION

8.1 Uncertainty evaluation

The evaluation of uncertainties in the context of certification exercises has evolved over the past decade. Nowadays, certified values should be accompanied by uncertainty statements in compliance with the requirements made by GUM [9]. While the design of new certification projects consider the needs for a proper estimation of the various uncertainty sources such as stability and homogeneity, older campaigns aimed only on qualitative statements (yes/no decisions) whether a material was stable and homogeneous.

The evaluation described hereafter is based on a concept described by Pauwels *et al.* [10 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 [10 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* [11]. 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}. Values for u^*_{bb} and s_{bb} were calculated accordingly:

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

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 20 and 10 samples, respectively (Table 5a-c). As a principle higher values of s$_{bb}$ and u$^*_{bb}$ are adopted as u$_{bb}$. 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, although values for Zn are statistically insufficient. 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. As for Zn stability data were insufficient, the biggest uncertainty obtained in case of the other two elements was used as an estimate. In case of Se in BCR-639, u^*_{bb} was used, as s_{bb} could not be calculated. 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-637

	Al	*Se*	*Zn*
u$_{bb}$ (s$_{bb}$) [in rel. %]	4.0	2.2	3.2
u$^*_{bb}$ [in rel.%]	2.8 [a]	1.2 [a]	0.6 [a]
u$_{lts}$ [in rel.%]	9.1	3.1	9.1
u$_{char}$ [in rel.%]	6.5	1.7	1.9
coverage factor k	2	2	2
U$_{CRM}$ [in rel.%]	23.8	8.33	19.66
Mean [in µg/L]	*12.47*	*81.12*	*1113.8*
Uncertainty [in µg/L]	*2.97*	*6.76*	*219.0*
Certified values expressed in µg/L	*12.5 ± 3.0*	*81 ± 7*	*1110 ± 220*

[a] not used for combined uncertainty

Table 8.b – Uncertainty budget and certified values for BCR-638

	Al	Se	Zn
u_{bb} (s_{bb}) [in rel.%]	2.1	1.6	5.0
u^{*}_{bb} [in rel.%]	1.1 [a]	1.0 [a]	0.8 [a]
u_{lts} [in rel.%]	5.2	1.7	5.2
u_{char} [in rel.%]	2.7	1.7	1.4
coverage factor k	2	2	2
U_{CRM} [in rel.%]	12.4	5.8	14.7
Mean [in µg/L]	*54.93*	*104.02*	*1426.4*
Uncertainty [in µg/L]	*6.81*	*6.03*	*209.68*
Certified values expressed in µg/L	**55 ± 7**	**104 ± 7**	**1430 ± 210**

[a] not used for combined uncertainty

Table 8.c – Uncertainty budget for BCR-639

	Al	Se	Zn
u_{bb} (s_{bb}) [in rel.%]	1.4	3.4	1.7
u^{*}_{bb} [in rel.%]	0.7 [a]	1.6 [a]	0.6 [a]
u_{lts} [in rel.%]	0.8	2.1	2.1
u_{char} [in rel.%]	3.0	1.8	1.0
Coverage factor k	2	2	2
U_{CRM} [in rel.%]	6.8	8.8	5.8
Mean [in µg/L]	*194.24*	*132.53*	*2362.1*
Uncertainty [in µg/L]	*13.21*	*11.66*	*137.00*
Certified values expressed in µg/L	**194 ± 14**	**133 ± 12**	**2360 ± 140**

[a] not used for combined uncertainty

8.2 Certified values

The certified values (unweighed mean of the accepted sets of results) and their uncertainties (combined uncertainty with a coverage factor of k=2)) are given in tables 8.a-c. Results are rounded according to ISO Standard 31-0 [12].

Table 8.d - Certified values for Al, Se and Zn in BCR-637

BCR-637 Component	Certified content, µg/mL	Uncertainty, µg/mL	Number of accepted sets of results
Aluminium, Al	12.5	3.0	7
Selenium, Se	81	7	13
Zinc, Zn	$1.11 \cdot 10^{3}$	$0.22 \cdot 10^{3}$	9

Table 8.e - Certified values for Al, Se and Zn in BCR-638

BCR-638 Component	Certified content, μg/L	Uncertainty, μg/L	Number of accepted sets of results
Aluminium, Al	55	7	8
Selenium, Se	104	7	13
Zinc, Zn	$1.43 \cdot 10^3$	$0.21 \cdot 10^3$	10

Table 8.f - Certified values for Al, Se and Zn in BCR-639

BCR-639 Component	Certified content, μg/L	Uncertainty, μg/L	Number of accepted sets of results
Aluminium, Al	194	14	7
Selenium, Se	133	12	13
Zinc, Zn	$2.36 \cdot 10^3$	$0.14 \cdot 10^3$	10

9. INSTRUCTIONS FOR USE

9.1 Description

The materials consist of sterile filtered human serum. Each vial contains approximately 4.5 mL. No preservatives were added.

9.2 Storage conditions

Unopened vials should be stored at -70°C or lower for long-term storage.

9.3 Thawing

Thawing should be done at room temperature or at 37 °C. Note that a white "fluffy" precipitate may appear if thawed in the cold (e.g. 5 °C). The precipitate dissolves in few minutes at 37 °C or after some hours standing at room temperature.

9.4 Analysis of Aluminium

If the material is used for quality control of aluminium analyses, special efforts should be considered in order to prevent contamination of the material during the analysis, e.g. working under clean room conditions.

9.5 Safety

These serum 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 serum should be treated as contagious material. The materials are ***for in vitro use only***.

9.6 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 [12]:

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

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-637, BCR-638 and BCR-639 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

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[3]   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.

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[7]   Deutsche Forschungsgemeinschaft. *List of MAK and BAT values* , VCH, Weinheim (1994)

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

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

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

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

[12]   ISO Guide 33. *Uses of Certified Reference Materials*, International Organization for Standardization, Geneva 1989.

11. ANNEX I – GRAPHICAL PRESENTATION OF THE HOMOGENEITY STUDY

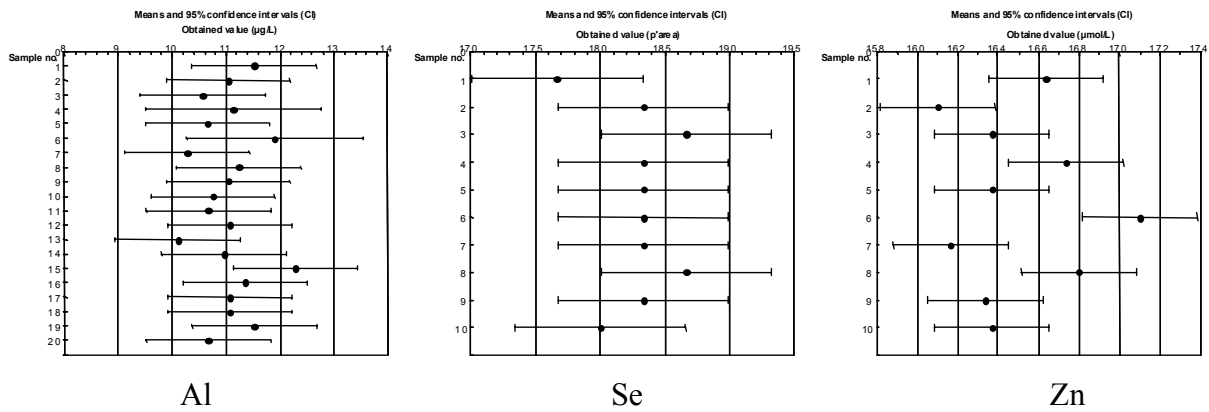## 11.1 Graphical survey of the results from the homogeneity study on BCR-637

Figure 11.1 - Means and confidence intervals (CI) of Al, Se and Zn determination in individual samples of BCR-637

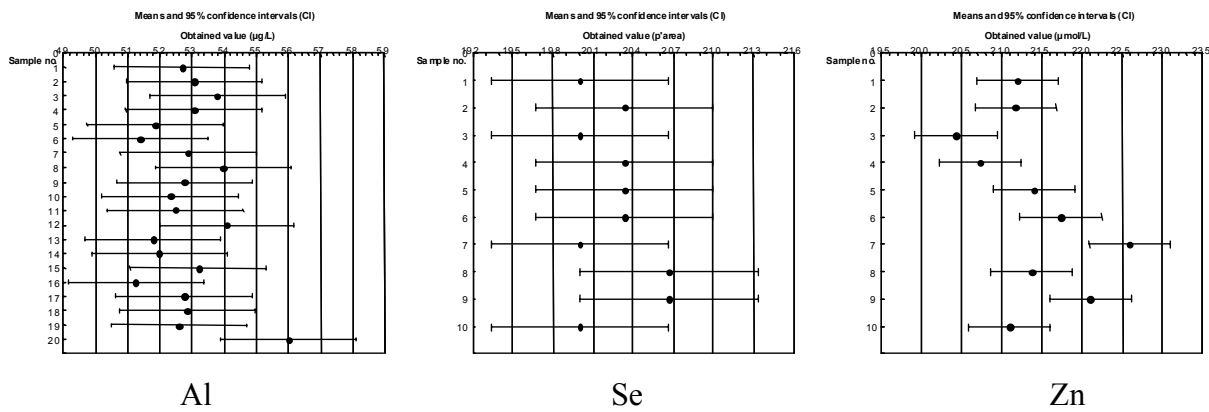## 11.2 Graphical survey of the results from the homogeneity study on BCR-638

Figure 11.2 - Means and confidence intervals (CI) of Al, Se and Zn determination in individual samples of BCR-638

11.3 Graphical survey of the results from the homogeneity study on BCR-639
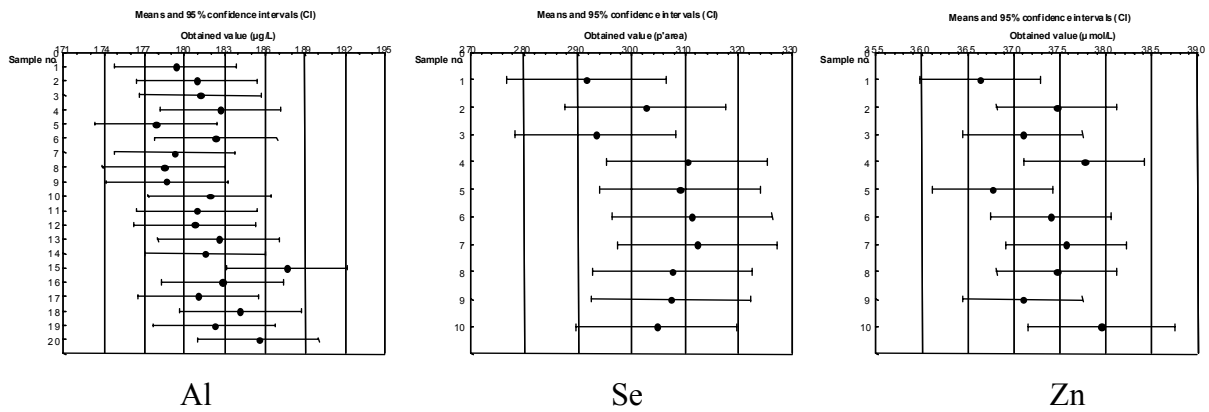

Figure 11.3 - Means and confidence intervals (CI) of Al, Se and Zn determination in individual samples of BCR-639

12. ANNEX II – INDIVIDUAL RESULT AND GRAPHICAL PRESENTATIONS OF THE CERTIFICATION CAMPAIGN

Numbers in square parentheses, "[ ]", are statistically found outliers (significance level 1 %). They take part in the Lillifors-test of normality and are shown in the graphs, but are ignored in all other calculations.

Table 12.a – Individual results for BCR-637, Al (µg/L)

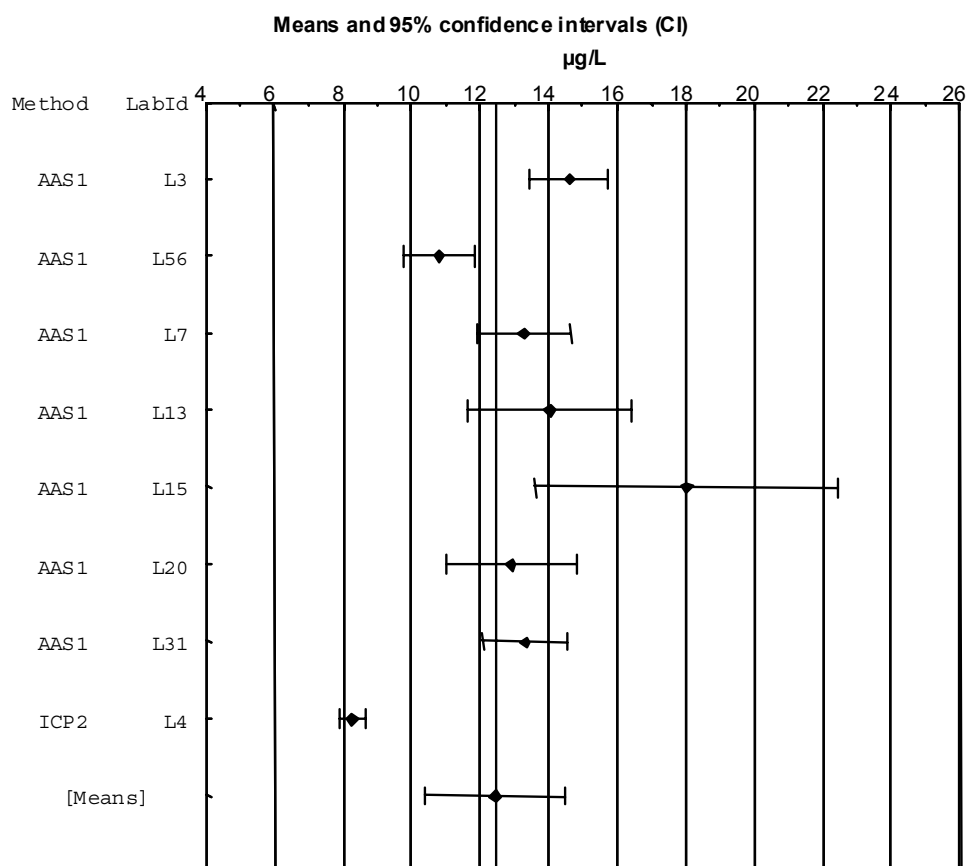| Lab ID	Method	Individual measurement results					Mean	Std. Dev.	CV %
L3	AAS1	14.70	13.40	15.80	15.10	14.10	14.620	0.920	6.3
L4	ICP2	8.60	8.50	8.40	7.90	8.00	8.280	0.311	3.8
L7	AAS1	14.70	12.70	13.60	13.70	11.80	13.300	1.098	8.3
L13	AAS1	13.80	13.60	16.10	11.20	15.50	14.040	1.917	13.7
L15	AAS1	[15.76]	[24.01]	[17.27]	[18.12]	[14.99]	[18.030]	[3.562]	[19.8]
L20	AAS1	15.30	11.10	12.30	12.90	13.00	12.920	1.530	11.8
L31	AAS1	14.34	13.66	14.11	12.29	12.25	13.330	0.998	7.5
L56	AAS1	11.00	12.00	10.00	11.00	10.00	10.800	0.837	7.7

Figure 12.1 - Graphical presentation of individual results BCR-637, Al (µg/L)

Table 12.b - Individual results for BCR-637, Se (µg/L)

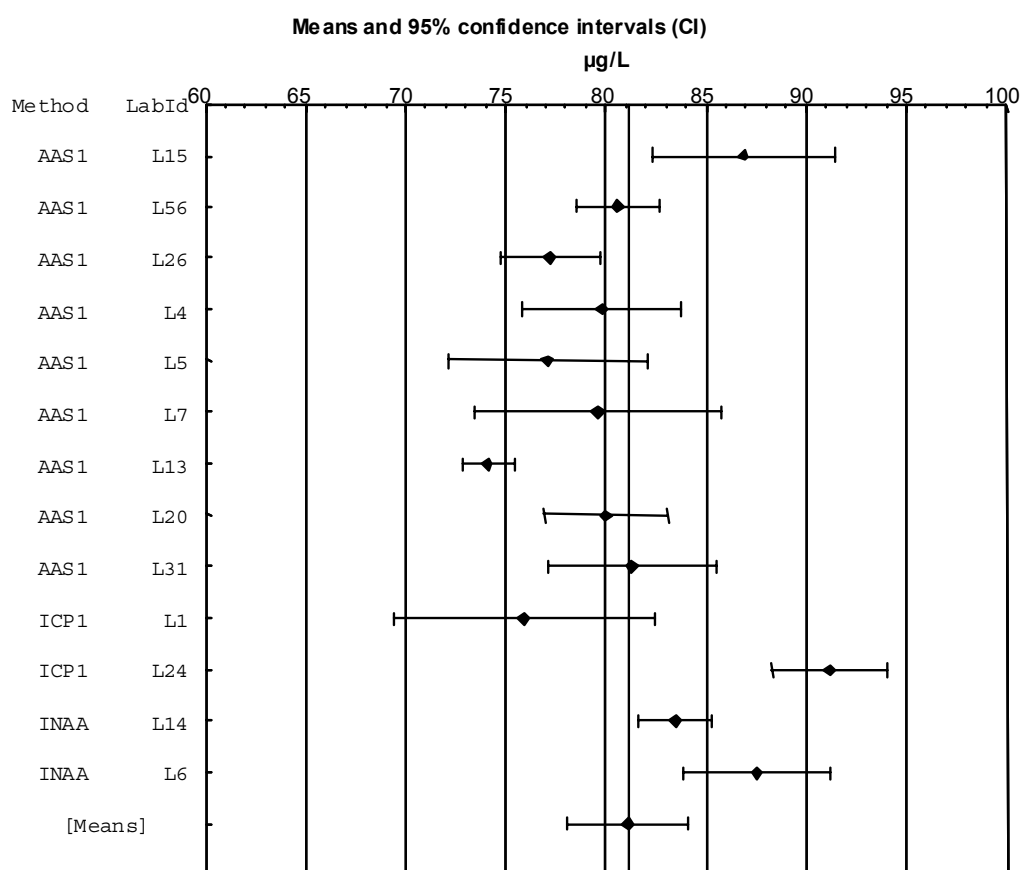| Lab ID	Method	Individual measurement results					Mean	Std. Dev.	CV %
L1	ICP1	76.70	83.50	76.30	69.00	74.00	75.900	5.239	6.9
L4	AAS1	79.00	84.00	76.00	78.00	82.00	79.800	3.194	4.0
L5	AAS1	79.20	74.10	71.60	80.40	80.20	77.100	4.005	5.2
L6	INAA	92.30	86.00	84.70	88.10	86.60	87.540	2.928	3.3
L7	AAS1	72.70	84.40	84.40	79.30	77.30	79.620	4.977	6.3
L13	AAS1	73.90	73.30	75.10	72.90	75.20	74.080	1.040	1.4
L14	INAA	82.60	85.30	83.10	81.60	84.60	83.440	1.501	1.8
L15	AAS1	88.00	89.80	87.00	80.50	88.90	86.840	3.694	4.3
L20	AAS1	80.00	82.00	80.00	82.00	76.00	80.000	2.449	3.1
L24	ICP1	89.20	93.40	93.80	89.50	89.80	91.140	2.260	2.5
L26	AAS1	75.20	74.80	78.20	78.90	78.90	77.200	2.033	2.6
L31	AAS1	78.58	81.42	77.29	85.20	83.96	81.290	3.383	4.2
L56	AAS1	78.00	81.00	82.00	80.00	82.00	80.600	1.673	2.1

Figure 12.2 - Graphical presentation of individual results BCR-637, Se (µg/L)

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Table 12.c – Individual results for BCR-637, Zn (mg/L)

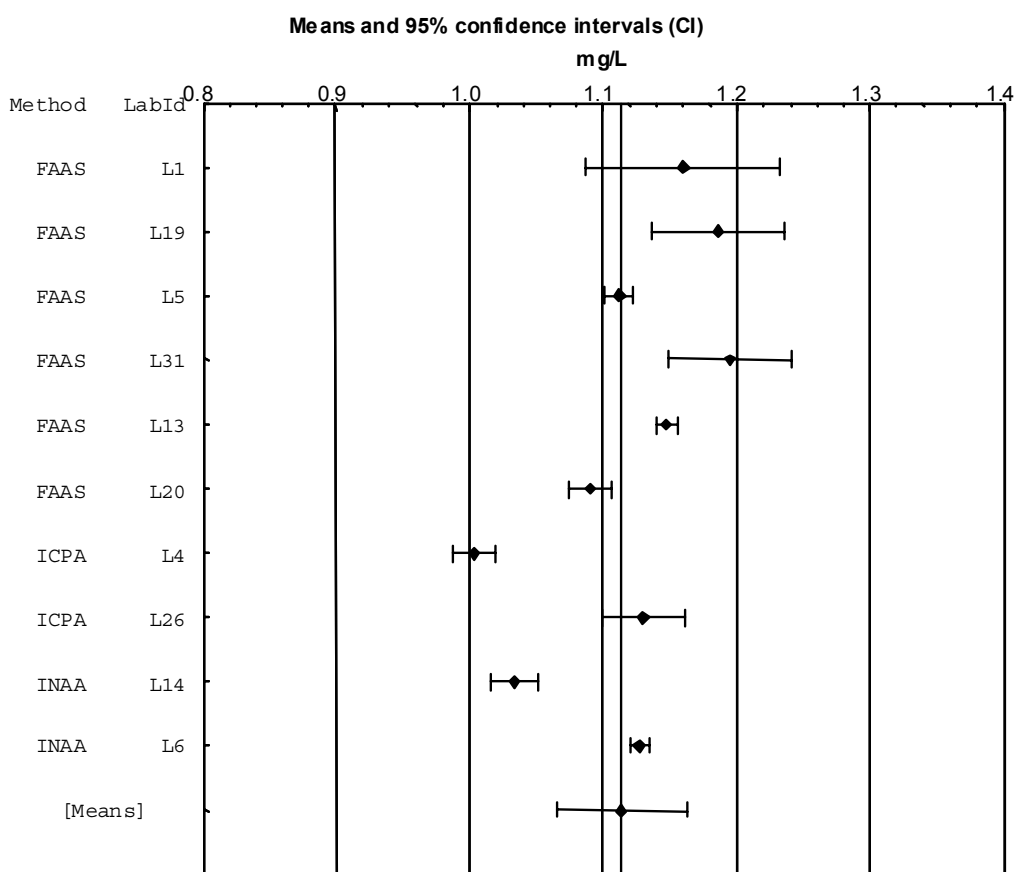| Lab ID	Method	Individual measurement results					Mean	Std. Dev.	CV %
L1	FAAS	[1.2420]	[1.1440]	[1.0790]	[1.1570]	[1.1770]	[1.15980]	[0.05882]	[5.1]
L4	ICPA	1.0100	0.9860	1.0200	1.0050	0.9960	1.00340	0.01303	1.3
L5	FAAS	1.1100	1.1200	1.1100	1.1000	1.1200	1.11200	0.00837	0.8
L6	INAA	1.1261	1.1192	1.1275	1.1290	1.1341	1.12718	0.00539	0.5
L13	FAAS	1.1480	1.1450	1.1410	1.1570	1.1450	1.14720	0.00602	0.5
L14	INAA	1.0490	1.0260	1.0480	1.0250	1.0200	1.03360	0.01379	1.3
L19	FAAS	1.1750	1.2200	1.2350	1.1600	1.1400	1.18600	0.04022	3.4
L20	FAAS	1.0920	1.0980	1.1050	1.0720	1.0850	1.09040	0.01266	1.2
L26	ICPA	1.1400	1.0900	1.1300	1.1300	1.1600	1.13000	0.02550	2.3
L31	FAAS	1.1600	1.2440	1.1730	1.1720	1.2250	1.19480	0.03721	3.1

Figure 12.3 - Graphical presentation of individual results BCR-637, Zn (mg/L)

Table 12.d - Table of individual results for BCR-638, Al (µg/L)

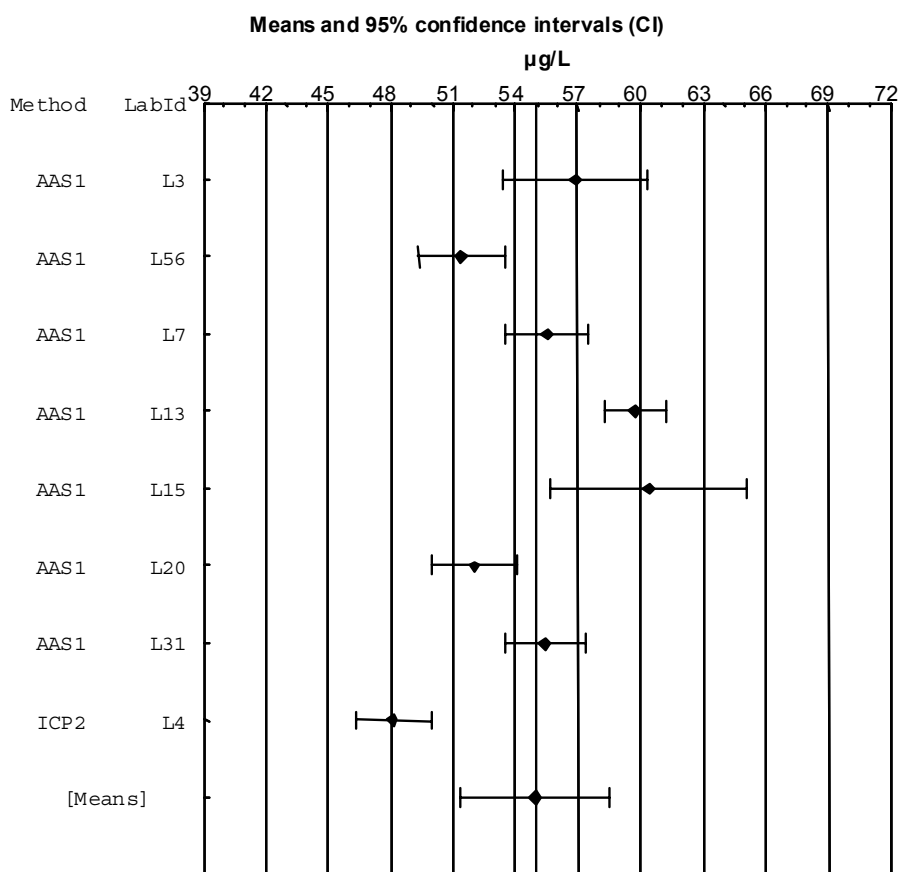| Lab ID	Method	Individual measurement results					Mean	Std. Dev.	CV %
L3	AAS1	60.30	57.00	58.10	56.10	52.80	56.860	2.759	4.9
L4	ICP2	48.30	48.60	45.60	49.50	48.50	48.100	1.471	3.1
L7	AAS1	56.80	57.20	55.00	55.30	53.20	55.500	1.594	2.9
L13	AAS1	60.00	58.30	61.40	59.10	59.80	59.720	1.152	1.9
L15	AAS1	59.63	55.40	65.92	61.60	59.45	60.400	3.821	6.3
L20	AAS1	49.80	52.80	50.70	53.10	53.70	52.020	1.678	3.2
L31	AAS1	56.66	56.63	55.51	52.85	55.37	55.404	1.550	2.8
L56	AAS1	50.00	51.00	50.00	54.00	52.00	51.400	1.673	3.3

Figure 12.4 - Graphical presentation of individual results BCR-638, Al (µg/L)

Table 12.e - Individual results for BCR-638, Se (µg/L)

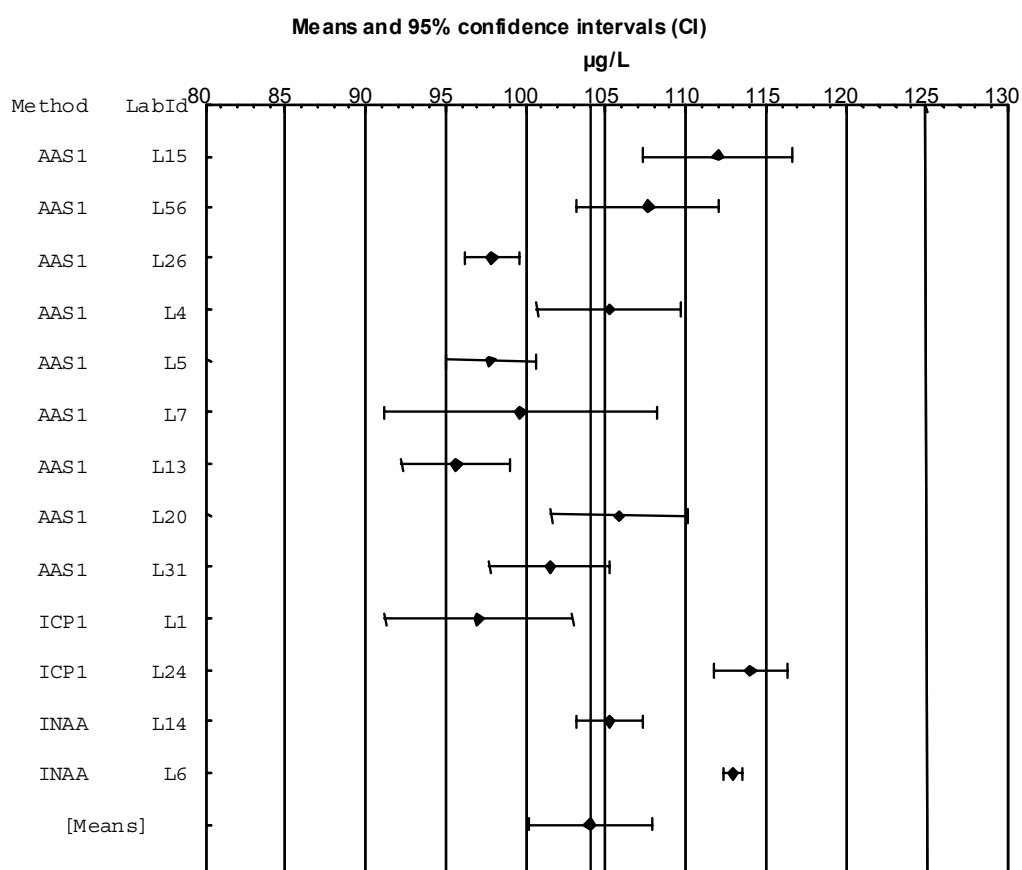| Lab ID	Method	Individual measurement results					Mean	Std. Dev.	CV %
L1	ICP1	96.70	104.60	95.20	91.70	97.10	97.060	4.722	4.9
L4	AAS1	102.00	102.00	110.00	104.00	108.00	105.200	3.633	3.5
L5	AAS1	93.80	98.90	99.30	98.90	98.10	97.800	2.278	2.3
L6	INAA	113.40	112.30	113.40	112.80	112.70	112.920	0.476	0.4
L7	AAS1	92.60	98.70	98.00	111.10	98.00	99.680	6.840	6.9
L13	AAS1	92.10	94.00	96.50	99.10	96.60	95.660	2.686	2.8
L14	INAA	104.30	103.20	106.60	104.70	107.20	105.200	1.660	1.6
L15	AAS1	110.70	115.60	108.20	109.00	116.30	111.960	3.761	3.4
L20	AAS1	107.00	104.00	105.00	111.00	102.00	105.800	3.421	3.2
L24	ICP1	112.00	116.00	113.00	116.00	113.00	114.00	1.871	1.6
L26	AAS1	99.20	96.70	98.80	96.20	98.60	97.900	1.353	1.4
L31	AAS1	100.94	97.76	100.29	102.97	105.76	101.544	3.003	3.0
L56	AAS1	103.00	110.00	112.00	108.00	105.00	107.600	3.647	3.4

Figure 12.5 - Graphical presentation of individual results BCR-638, Se (µg/L)

Table 12.f - Individual results for BCR-638, Zn (mg/L)

Lab ID	Method	Individual measurement results					Mean	Std. Dev.	CV %
L1	FAAS	1.4910	1.4770	1.4380	1.4120	1.4050	1.44460	0.03833	2.7
L4	ICPA	1.3350	1.3760	1.3440	1.3000	1.3800	1.34700	0.03276	2.4
L5	FAAS	1.4100	1.4400	1.4100	1.3900	1.3900	1.40800	0.02049	1.5
L6	INAA	1.4809	1.4518	1.3461	1.3989	1.4738	1.43030	0.05699	4.0
L13	FAAS	1.4200	1.4260	1.3960	1.4680	1.4650	1.43500	0.03089	2.2
L14	INAA	1.3840	1.3870	1.3400	1.3610	1.3380	1.36200	0.02329	1.7
L19	FAAS	1.5650	1.5250	1.4950	1.5000	1.4850	1.51400	0.03209	2.1
L20	FAAS	1.3920	1.3530	1.3470	1.3140	1.3660	1.35440	0.02845	2.1
L26	ICPA	1.4200	1.4100	1.4800	1.4500	1.4700	1.44600	0.03050	2.1
L31	FAAS	1.5560	1.5910	1.5300	1.4650	1.4700	1.52240	0.05462	3.6

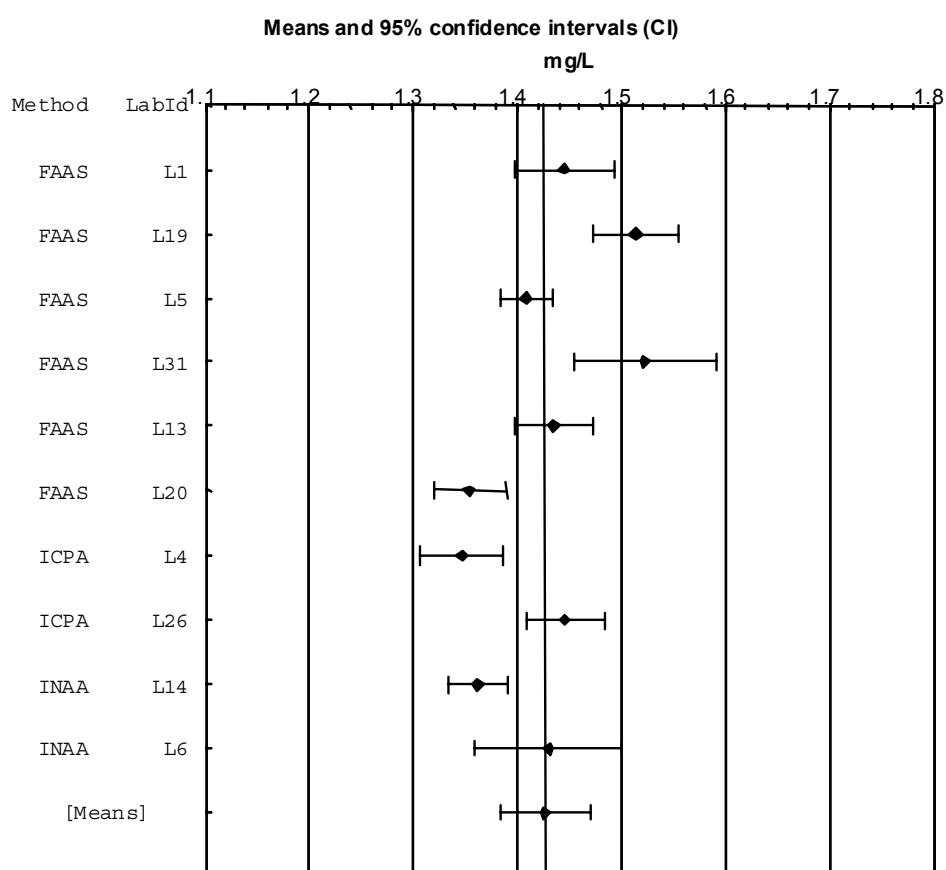*Figure 12.6 - Graphical presentation of individual results BCR-638, Zn (mg/L)*

Table 12.g - Individual results for BCR-639, Al (µg/L)

Lab ID	Method	Individual measurement results					Mean	Std. Dev.	CV %
L3	AAS1	169.50	171.40	157.50	167.00	172.30	16.540	5.969	3.6
L4	ICP2	178.00	207.00	197.00	210.00	202.00	198.800	12.637	6.4
L7	AAS1	198.90	194.40	183.10	190.10	184.00	190.100	6.748	3.5
L13	AAS1	204.30	199.50	212.50	199.60	200.40	203.260	5.527	2.7
L15	AAS1	214.31	191.72	203.16	194.89	203.34	201.484	8.797	4.4
L20	AAS1	216.00	217.80	224.10	208.80	211.50	215.640	5.922	2.7
L31	AAS1	184.10	182.39	184.36	180.45	182.87	182.834	1.566	0.9

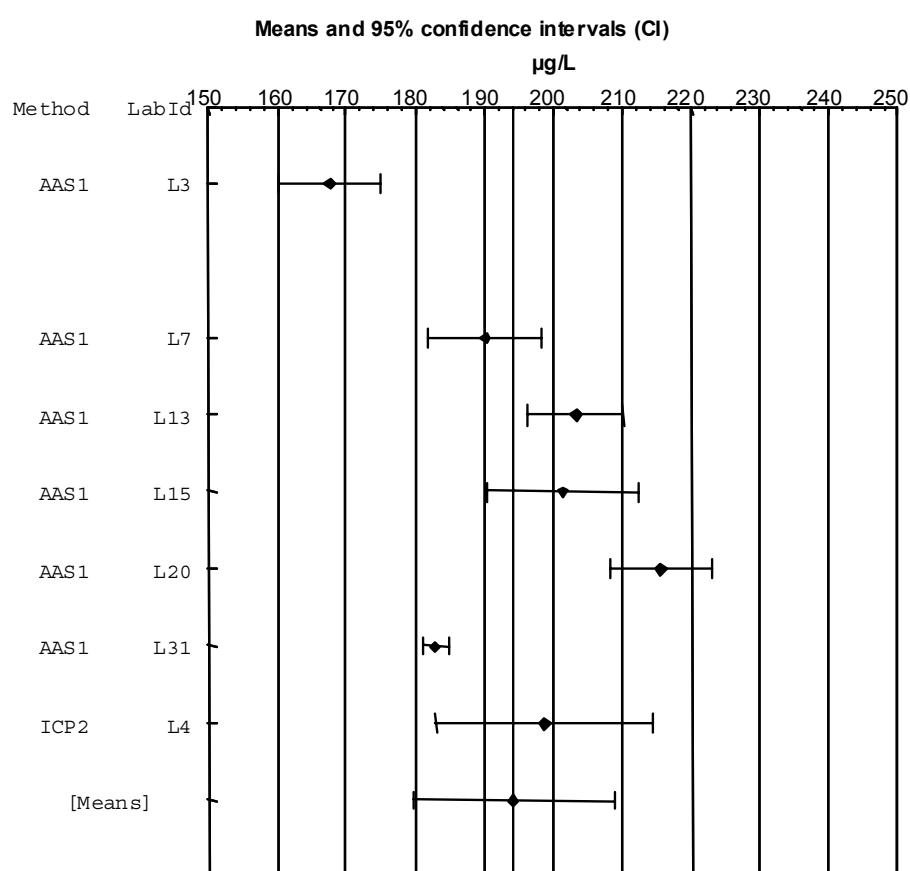*Table 12.h - Graphical presentation of individual results BCR-639, Al (µg/L)*

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Table 12.i - Individual results for BCR-639, Se (µg/L)

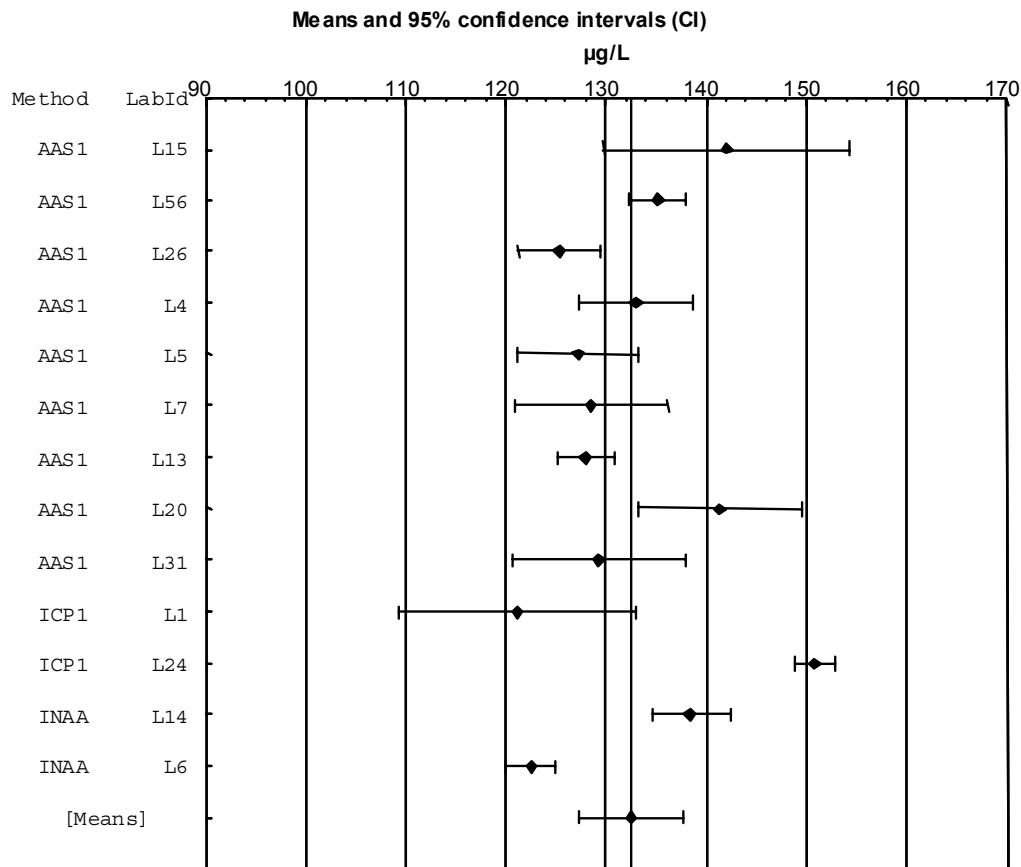| Lab ID	Method	Individual measurement results					Mean	Std. Dev.	CV %
L1	ICP1	126.50	132.00	111.60	110.50	125.00	121.120	9.563	7.9
L4	AAS1	130.00	134.00	128.00	133.00	140.00	133.000	4.583	3.4
L5	AAS1	135.00	126.30	122.80	123.80	128.00	127.180	4.827	3.8
L6	INAA	120.60	125.40	123.40	122.70	120.70	122.560	2.006	1.6
L7	AAS1	119.80	128.40	126.60	131.80	136.10	128.540	6.082	4.7
L13	AAS1	125.50	130.70	129.80	126.00	127.90	127.980	2.280	1.8
L14	INAA	142.60	138.40	140.00	134.30	136.80	138.420	3.145	2.3
L15	AAS1	140.00	157.90	136.80	131.60	144.00	142.060	9.950	7.0
L20	AAS1	147.00	143.00	134.00	135.00	148.00	141.400	6.580	4.7
L24	ICP1	150.00	152.00	150.00	149.00	153.00	150.800	1.643	1.1
L26	AAS1	128.00	126.00	125.00	128.00	120.00	125.400	3.286	2.6
L31	AAS1	128.32	131.10	126.64	120.58	139.82	129.292	7.035	5.4
L56	AAS1	132.00	138.00	136.00	134.00	136.00	135.200	2.280	1.7

Figure 12.7 - Graphical presentation of individual results BCR-639, Se (µg/L)

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Table 12.j - Individual results for BCR-639, Zn (mg/L)

Lab ID	Method	Individual measurement results					Mean	Std. Dev.	CV %
L1	FAAS	2.5180	2.4200	2.3540	2.3930	2.4190	2.42080	0.06059	2.5
L4	ICPA	2.2600	2.2500	2.2200	2.1700	2.3800	2.25600	0.07765	3.4
L5	FAAS	2.2580	2.2540	2.2800	2.3200	2.2590	2.27420	0.02754	1.2
L6	INAA	2.2628	2.2825	2.3216	2.3194	2.3355	2.30436	0.03041	1.3
L13	FAAS	2.3660	2.3580	2.2640	2.3060	2.3870	2.33620	0.05017	2.1
L14	INAA	2.2460	2.2870	2.2720	2.3070	2.3050	2.28340	0.02532	1.1
L19	FAAS	2.4000	2.5650	2.5100	2.6000	2.4450	2.50400	0.08257	3.3
L20	FAAS	2.3790	2.4190	2.3860	2.3930	2.3790	2.39120	0.01659	0.7
L26	ICPA	2.3600	2.3700	2.4700	2.4100	2.4400	2.41000	0.04637	1.9
L31	FAAS	2.4530	2.3250	2.5440	2.4180	2.4660	2.44120	0.07963	3.3

Figure 12.8 - Graphical presentation of individual results BCR-639, Zn (mg/L)

Abstract
This report documents the preparation, homogeneity, stability and certification of three liquid human serum materials, i.e. BCR-637, BCR-638 and BCR-639 with certified concentrations of aluminium, selenium and zinc. The homogeneity studies demonstrated that the materials are homogeneous with respect to the content of aluminium, zinc and selenium. The materials were subjected to a certification campaign for which the analytical work is described.

Uncertainty contributions for stability, homogeneity and characterisation were included in the combined and expanded uncertainties assigned to the certified values.

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