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COOPERATIVE STUDY ON MEASUREMENT OF CONCENTRATIONS OF SELENIUM IN FREEZE-DRIED (HUMAN WHOLE) BLOOD

(Technical Report)

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Cooperative study on measurement of concentrations of selenium in freeze-dried (human whole) blood (Technical Report)

SUMMARY

A cooperative study was conducted under the auspices of the IUPAC Commission on Toxicology to measure total concentrations of selenium in three lots of commercially available freeze-dried human whole blood (Seronorm, NycoMed A/S, Norway). A serum material already examined in two previous studies was simultaneously distributed to assess accuracy of the analytical procedures better. A total of 39 laboratories from 15 countries participated in the trial using seven inherently different methods: acid decomposition-fluorimetry (ADF); electrothermal atomic absorption spectrometry (EAAS); acid digestion-hydride generation/atomic absorption spectrometry (ADHG-AAS); protoninduced X-ray emission (PIXE); instrumental neutron activation analysis (INAA) or instrumental activation analysis with radiochemical separation (RNAA); energy dispersive-X-ray-fluorescence spectrometry (EDXRF) and acid digestion-isotope-dilution mass spectrometry (ADIDMS). The performance of the different analytic methods was critically assessed. For serum, all laboratories using PIXE, RNAA and EDXRF had to be excluded in the final estimation as well as two of the three laboratories using INAA. For whole blood too, laboratories applying PIXE and RNAA had to be excluded, but performances for INAA were acceptable. Better agreement and satisfactory variance components were found between other methods and the exclusion rate was considerably lower. The following mean concentrations [\pm 68 % confidence intervals for one future observation] were established for whole blood: Batch 904, 96.4 \pm 4.4 µg/L; Batch 905, 97.5 \pm 5.1 µg/L; Batch 906, 96.0 \pm 4.4 µg/L. The concentrations in these materials were similar to those found in population studies in most European countries. The material is suitable as quality assurance material for the measurement of typical concentrations of selenium in blood.

INTRODUCTION

The recognition of selenium as an essential element in human metabolism has stimulated the measurement of selenium in biological materials. Many selenium measurements are performed in clinical laboratories in order to establish intake of selenium or guidelines for supplementation, and to monitor environmental and occupational exposures.

In recognition of the importance of analytical accuracy and precision in assessing the biomedical and environmental effects of selenium, the Commission on Toxicology of the IUPAC Clinical Chemistry Division has encouraged the use of freeze-dried (human) body fluids as quality control materials. Thus cooperative studies were conducted to measure total mass concentration of selenium in lots of commercially available freeze-dried human serum and urine to arrive at consensus values (1). A secondary goal was to assess the performance of the broad range of analytical methods capable of measuring selenium in clinical materials (2,3,4).

In extension of this work, three batches of freeze-dried human whole blood were characterized for total selenium, since this biological fluid was considered to provide a long-term indication of general selenium status. Plasma selenium, which is a commonly used parameter, better reflects short-term changes in selenium status while whole blood selenium is considered to provide a long-term indication of general selenium status.

EXPERIMENTAL

Participant laboratories, analytical methods, and general design

Invitations to participate in the study were sent to 91 laboratories in 22 countries selected on the basis of their experience or interest in selenium measurements in biological materials. They were advised of the difficulties in analysing the whole blood matrix and

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Method	Code	Number of laboratories	Method	Code	Number of laboratories
Acid-decomposition fluorimetry	ADF	10	Neutron-activation analysis with	RNAA	2 *
Electrothermal atomic absorption spectrometry	EAAS	6	Energy-dispersive X-ray		-
Acid-decomposition hydride-generation atomic absorption spectrometry	ADH-AAS	9	fluorescence Acid-decomposition/isotope-	EDXRF	1 **
Proton-induced X-ray emission	PIXE	2	-dilution mass spectrometry	ADIDMS	1
Instrumental neutron-activation analysis	INAA	4 *			
· · · · · · · · · · · · · · · · · · ·			**		•

 Table 1:
 Analytical methods used for determination of selenium in human serum and whole blood in the study (35 laboratories).

* one of these laboratories did not report results for serum

** this laboratory did not report results for whole blood

were requested to analyse vials from three batches, each with the natural concentration of selenium. They were also informed that a freeze-dried serum would be included to test performance of the methods for this more common matrix. No constraint was imposed on the analytical method to be used. A total of 39 laboratories agreed to take part in the proposed study. Each were asked to report a minimum of three independent assay results for each batch and had approximately 12 weeks in which to perform the analytical work; 35 laboratories (Appendix A) finally reported results (8 in US, 6 in Belgium, 4 in DE, 3 in Finland, 2 in France, 2 in Great Britain, 2 in Italy, 2 in New Zealand, and one each in Norway, Canada, Netherlands, Denmark, Australia, China and Poland). The numbers of laboratories using the various analytical methods are listed in Table 1.

Materials

The pool materials for the study were made available by NycoMed A/S, Oslo, Norway -Batches 904, 905 and 906 of freeze-dried human whole blood and Batch 105 of freezedried human blood serum presented in vacuum-sealed vials (5 mL and 3 mL nominal values respectively). The maximum acceptable difference in weight in NycoMed's procedure is +0.3% with a coefficient of variation of 0.1%. Furthermore, vial to vial consistency within the same batch is assured by identical treatment in all production steps. The materials were prepared from thoroughly tested Norwegian donors (each individual was separately tested by official authorities and found negative for the presence of HBs antigen and HIV antibodies) and were expected to have typical concentrations of endogenous selenium. However the materials were spiked with other elements of interest in biochemistry and toxicology. Typical values for these elements were measured in another IUPAC interlaboratory study (4). Each participant received two vials of each batch, together with three 20-mL polypropylene tubes containing purified sterile water free from contamination with selenium ($<0.1 \mu g/L$) for reconstitution of the freeze-dried materials. On distributing the vials to participants, no indication was given about the manufacturer, nor was information supplied about Batch 105, which had previously been evaluated for its selenium concentration (2, 3).

Statistical analysis

Each set of results submitted by the participating laboratories was first investigated for outlier values by Dixon's test (5, 6). Mean concentrations and variances were calculated for each batch of material and each laboratory. Then, taking all the laboratories results, outlying mean values were excluded either on the basis of the Dixon's test (for serum) or by an extreme-rank sum test for outliers (7) for all the batches of whole blood and serum. Bartlett's test for homoscedasticity (8) was used to test for heterogeneity of variance between all the laboratories and within each group of laboratories that used the same analytical method: exclusion was performed by the chi-square test (8). Large variances were simultaneously identified by deviation from the upper 3-sigma control limit for the mean deviations of replicate measurements (9).



Figure 1 Two-pool sample chart for visual comparison of the analytical results.

Data for whole blood were further analysed for repeatability and reproducibility by plotting the results from a pair of pool materials, of similar concentration, analysed together as blind duplicates, in a X-Y graph (9). This allowed visual comparison of analytical results without elaborate statistical calculations. The dispersion along the 45° line and outside the 95 % confidence circle showed which laboratories had supplied outlying results or were not consistent with regard to repeatability. Figure 1 shows one of the two-sample plots used to exclude some of the outliers.

Exclusion for extreme mean values for each sample was considered when the difference between successive ranked means was higher than 3 %. In this step, analysis of variance (ANOVA), by one-way, two-way or three-way procedures, was performed to test the differences in mean concentrations among the laboratories by the same analytical method and among all the laboratories. These statistical procedures were repeated after removing several laboratories' results on the basis of criteria outlined in the next section. Withinlaboratory and between-laboratories variance components (9) were also estimated to assess the repeatability (\mathbf{r}) and reproducibility (\mathbf{R}) (6) of the analytical methods and to estimate the precision of the reported selenium concentrations for the serum and whole blood materials.

RESULTS AND DISCUSSION

Presentation of the experimental results

Of the 34 laboratories that submitted values for the three batches of whole blood, only 2 (using INAA or RNAA) did not simultaneously report data for serum. The number of replicates for the various assays performed on each batch ranged from 3 to 10. The few laboratories that only reported the mean values [\pm the standard deviation (s)] submitted individual results after written request, except for two which did not answer and were therefore excluded from the final calculations. One investigator using EDXRF admitted its inability to analyse the whole blood sample because of difficulties in sample preparations, but values for serum were submitted and were included in the statistical evaluation for serum.

The majority of investigators carried out their measurements on volumetric aliquots of the reconstituted solutions and expressed results as mass concentration of Se (μ g/L). Two laboratories, using INAA and ADIDMS, reported results as mass fraction of Se in wet

material ($\mu g/kg$), and 6 others, using INAA, RNAA or PIXE, as mass fraction of Se in dry material ($\mu g/g=mg/kg$). To achieve comparability of the results, these last values were converted to mass concentrations ($\mu g/L$) by multiplying values by experimentally measured volumic mass (or mass density, kg/L). Volumic mass was 1.003 (Batch 105), 1.037 (Batch 904), 1.035 (Batch 905) and 1.035 (Batch 906) g/mL, respectively. The reconstituted volumes by addition of the recommended volume of pure water were estimated to be 3.255, 5.722, 5.718 and 5.718 mL, respectively.

Before statistical evaluation, the raw data from all the laboratories were tabulated and sent to all the participants for checking. Two small typing errors were identified and corrected. Tables 2 to 5 assemble individual values for the four materials with the calculated means and standard deviations, after removing the outlying values for the serum and blood materials by Dixon's test (indicated by one asterisk) as well as by the extreme-rank sum test for the whole blood materials. The number of significant figures shown is as reported by the investigators, except in a few cases where we limited it to three or four (one digit after the decimal point). Figures 2 to 5 summarize the raw data and highlight those discarded after statistical evaluation. Two laboratories in particular were unable to obtain satisfactory results because of problems in sample preparation (laboratory 7) or interferences during specific detection (laboratory 26).

Relative performance of methods and exclusion of outlying results

Before assigning selenium concentrations to the serum and whole blood materials, the data were assessed for laboratory performance and method reliability. For this last purpose, the mean concentrations, the within-laboratory and between-laboratory standard deviations and the 95 % confidence intervals were estimated for each method (Table 6). As already observed in similar studies with serum or urine materials (1, 2, 3), the results (Tables 2 to 6) showed considerable variability in the statistical parameters for the different methods. However there was good concordance between ADF and ADH-AAS; sometimes between INAA and ADIDMS, but PIXE, and to a lesser extent EAAS and RNAA were erratic or biased. There was also considerable variation in repeatability, but apparently not related to a specific method, except for PIXE. Between-laboratory variance was high for EAAS and PIXE, and low for ADF and INAA.

Outliers were excluded separately for the serum and batches of whole blood on the basis of the criteria already described and are further documented in the footnotes to Tables 7 and 8, i.e. heterogeneity of variance (Bartlett's test) and differences in mean concentrations between laboratories (reproducibility). For serum, all the laboratories using PIXE, RNAA or EDXRF had to be excluded, as well as two of the three laboratories using INAA. The set of data from laboratories using ADIDMS was consistent, as well as the majority of those from laboratories using ADF, EAAS and ADH-AAS. Analytical performances for each group of methods disclosed, after exclusion of outliers, rather better agreement between the means and satisfactory components of variance (Table 6). The mean concentration derived from ADIDMS results was slightly lower than from other methods, but it could not be excluded by the ANOVA test for the retained laboratories. Even though IDMS can be the basis of a definitive method, the combined aciddigestion/isotope-dilution mass-spectrometry technique used in this study had the same disadvantage as ADHAAS and ADF (3) in digestion and recovery of the organic bound selenium present in body fluids. The lower concentration by this method may in fact be due to difficulty in conversion of protein-bound selenium to inorganic (tetravalent) selenium.

The inability of laboratories to characterize the batches of whole blood were further compared by a ranking procedure (9). This served to exclude 4 laboratories, including the two which used PIXE and RNAA (except for Batch 906). The two-sample plots (9) provided information on laboratory precision and relative accuracy that were in good agreement with other criteria, but the performance of laboratories using INAA were judged by this approuch to be satisfactory. The overall pattern of excluded laboratories and methods is presented in Table 8.

Figures 2-5 are printed on pp. 772-773. Tables 2-9 are printed on pp. 774-780.

Estimation of selenium concentrations

Table 9 shows the best estimates of mean concentrations and uncertainties for the four batches of biological materials derived from all of the acceptable results. Concordance between the means for whole blood was less satisfactory than that for serum, but the components of variance were generally better. ADIDMS and RNAA were associated with the lowest mean concentrations. Components of variance were of the same order of magnitude for all the methods, except for some high values associated with ADH-AAS and RNAA.

The concentration in the serum had already been assessed (2, 3). In the first study (2), the mean concentration and the 68 % confidence interval for one future observation from <u>n</u> data points ($\underline{m} \pm \underline{s} [\underline{n}]$) was estimated to be 90.7 ± 6.0 [159]. In the second study (3), two pairs of values were derived (91.7 ± 6.3 [53] and 93.3 ± 6.2 [77] depending on the digestion procedure by the participating laboratories. The mean concentration for the serum reported here is slightly higher than the previously reported values. Very little is known about the long-term stability of selenium in freeze-dried human serum, but it is not likely that there would be a real increase in concentration. Whether this higher concentration is a less biased estimate of the "true value" is difficult to judge. One distinction between the studies is that the participants in the previous trials had specialized experience in analysis of serum for selenium; however it is unlikely that the differences between the matrix components of serum and whole blood would have influenced the performance of the methods.

The selenium concentrations in the three blood batches were very similar to each other, and to the serum, about 95 μ g/L. Since the different batches were processed from the same batch of blood, this similarity was expected. Finally, the concentrations in whole blood were typical of values usually found in most European countries (but lower than in North America); thus the materials are useful for the analytical control of measurements of selenium in blood.

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Figure 2 : Estimation of mass concentration of selenium (μ g/L) in Batch 105 of blood serum. Numerals beside some points indicate the number of results repesented by the point. Shaded points refer to data excluded from the calculation of a consensus mean concentration according to criteria noted in the footnotes to Tables 7 and 8.



Figure 3 : Estimates of mass concentration of selenium ($\mu g/L$) in Batch 904 of whole blood. Numerals beside some points indicate the number of results represented by the point. Shaded points refer to data excluded from the calculation of a consensus mean concentration according to criteria noted in the footnotes to Tables 7 and 8.

Figure 4 : Estimates of mass concentration of selenium ($\mu g/L$) in Batch 905 of whole blood. Numerals beside some points indicate the number of results represented by the point. Shaded points refer to data excluded from the calculation of a consensus mean concentration according to criteria noted in the footnotes to Tables 7 and 8.

Figure 5 : Estimates of mass concentration of selenium ($\mu g/L$) in Batch 906 of whole blood. Numerals beside some points indicate the number of results represented by the point. Shaded points refer to data excluded from the calculation of a consensus mean concentration according to criteria noted in the footnotes to Tables 7 and 8.

Table 2 : Analytical results for Batch 105 (μ g/L)

					Labor	ratory and	method					
	1	2	3	4	5	6	7	8	9	10	11	12
	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	EAAS	EAAS
	97	91.2	95.4	91.2	102	94 .2	105	76.9	-	80.7	107	104
	98	91.2	97.2	95.3	100	94.2	105	83.3	-	80.7	127	111
	98	91.2	106.2	96.0	98	91.6	102	87.3	-	84.3	115	99
	98	97.2	90.6	96.3	100	97.1	99	75.7	-	86.1	-	-
	-	96.7	95.4	97.4	98	98.7	107	73.0	•	87.0	-	-
	-	93.4	102.8	98.7	-	-	(13	6)* 87.6	-	87.4	-	-
	-	-	•	-	-	-	•	72.2	-	89.8	-	-
	-	-	-	-	-	-	•	74.8	- `	-	-	
Mean	97.8	93.5	97.9	95.8	99 .6	94.4	103.6	78.9	80.	0** 85.2	116.3	104.7
Std.dev.	0.5	2.8	5.6	2.6	1.7	3.2	3.1	6.3	3.5	3.4	10.1	6.0
					Labo	ratory and	method					
	13	14	15	1 6	17	18	19	20	21	22	23	24
	EAAS	EAAS	EAAS	EAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS
	97	86.9	94	102	90.5	-	96.5	100.8	98	93.0	122.6	98
	87	94.8	101	91	90.5	-	90.5	95.3	98	102.2	112.4	98
	88	96.8	102	98	88.9	-	93.5	98.0	103	97 .1	82.6	97
	87	99.9	-	-		-	-	-	-	-	62.8	-
	-	86.8	-	-	-	-	-	-	-	-	-	
	-	90.9	-	-		-	-		-	-	-	-
	-	88.9	-	-	-	-	-	-	-	-	-	-
	-	90.5	-	-		-	-		-	-		-
	-	93.8	-	-			-	-	-	-	-	-
	•	92.9	•	-	-	•	-	-	-	•	-	-
Mean	89.8	92.2	99 .0	97.0	90.0	82.7*	** 93.5	9 8.0	99.7	97.4	95.1	97.7
Std.dev.	4.9	4.3	4.4	5.6	0.9	2.5	3.0	2.8	2.9	4.6	27.4	0.6
Mean Std.dev.	89.8 4.9	92.2 4.3	99.0 4.4	97.0 5.6	90.0 0.9	82.7* 2.5	** 93.5 3.0	98.0 2.8	99.7 2.9	97.4 4.6	95.1 27.4	

	Laboratory and method											
	25 ADHAAS	26 PIXE	27 PIXE	28 INAA	29 INAA	30 INAA	31 INAA	32 RNAA	33 RNAA	34 EDXRF	35 ADIDMS	
	91	102	114	83	75	-	94	74	-	80	89	
	90	(66)*	• 104	83	94	-	95	72	-	97	88	
	93	114	106	80	-	-	95	84	-	98	88	
	94	112	113	80	-	-	-	86	-	-	-	
	-	-	111	79	-	-	-	•	-	-	-	
	-	-	113	-	-	-	-	-	-	-	-	
Mean	92.0	109.3	110.2	81.0	84.5	***	94.7	79.0	***	91.7	88.3	
Std.dev.	1.8	6.4	4.2	1.9	13.4	-	0.6	7.0	-	10.1	0.6	

outlying value by Dixon's criterion
 the mean and standard deviation were reported
 no results at all for this laboratory

Table 3 : Analytical results for Batch 904 (μ g/L)

					Labor	atory and	method					
	1	2	3	4	5	6	7	8	9	10	11	12
	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	EAAS	EAAS
	102	98.8	98.6	91.4	94	89.2	109	73.2	100	76.7	168	99
	102	97.2	104.3	91.6	98	87.6	76	75.1	98	77.3	165	95
	103	97.8	110.0	94.7	94	90.0	102	88.5	102	80.4	193	118
	104	94.5	99.2	96.1	96	85.3	(23	9)* 87.7	-	83.7	-	-
	-	94.5	102.7	97.6	92	86.8	52	88.9	-	87.9	-	-
	-	98.3	114.0	98.4	-	95.5	23	75.3	-	90.7	•	-
		-	-	98.9	-	98.7	-	76.9	-	93.1	-	-
	•	-	-	99.9	-	95.5	-	84.3	-	94 .0	-	
Mean	102.8	96.9	104.8	96.1	94.8	91.1	72.4	81.2	100.0	85.5	175.3	104,0
Std.dev.	1.0	1.9	6.1	3.3	2.3	4.9	35.6	6.8	2.0	6.9	15.4	12.3
					Labo	ratory and	method					
	13	14	15	16	17	18	19	20	21	22	23	24
	EAAS	EAAS	EAAS	EAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS
	81	86.8	89	92	94.7	89.0	85.5	96.1	111	100.1	77.7	108
	80	9 4.0	102	98	94 .0	88.0	78.0	97.1	107	99 .6	85.8	102
	86	93.3	98	96	94.7	89.5	96.0	96.8	120	101.2	71.3	107
	79	90.9	-	-	-	-	•	-	-	-	67.4	-
	-	92.2	-	-	-	-	•	-	-	-	•	-
	-	87.0	•	-	-	-	-	-	-	•	-	-
	-	91.1	-	-	-	•	-	-	-	-	-	-
	-	90.3	-	-	-	•	-	-	-	-	-	-
	-	89.7	-	-	-	-	-	-	-	-	-	-
	•	94 .1	-	-	-	-	-	-	-	-	-	-
Mean	81.5	90.9	96.3	95.3	94.5	88.8	86.5	96.7	112.7	100.3	75.6	105.7
Std.dev.	3.1	2.7	6.7	3.1	0.4	0.8	9.0	0.5	6.7	0.8	8.0	3.2
					Labo	ratory and	lmethod	· · · · ·				·
	25	26	27	28	29	30	31	32	33	34	35	
	ADHAAS	PIXE	PIXE	INAA	INAA	INAA	INAA	RNAA	RNAA	EDXRF	ADIDMS	
	93	110	1 99	96	102	97	110	82	. 82	: -	94	ļ
	96	118	184	94	104	93	107	71	64	-	94	ļ
	91	111	171	97	100) 84	107	69	67		. 92	2
	9 1	132	182	92	104					· ·		
	-	-	145	91		• •			• •		. ·	-
	-	-	145		. .						- ·	-
Mean	92.8	117.8	171.0	94.0) 102.5	5 91.3	3 108.0	74.0) 71.() ***	93.3	3
Std.dev.	2.4	10.1	22.0	2.5	5 1.9) 6.7	7 1.7	7.0) 9.0	5	- 1.2	2

Table 4 : Analytical results for Batch 905 (μ g/L)

	1				Labor	atory and	method					· _
	1	2	3	4	5	6	7	8	9	10	11	12
	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	EAAS	EAAS
	102	09.3	101 6	80.3	97	95 3	76	74.2	104	81 5	217	100
	103	90.5	101.0	89.3	92	86.1	60	75.0	107	83.2	190	107
	105	97.2	110.3	05.6	07 07	84.5	81	70.1	102	87.9	226	113
	104	102.6	00.0	95.0	04	86.9	206	69.1	-	88.8		
	105	102.0	102.5	97.1	94	97.9	28	77.6	-	91.2	-	-
	_	102.6	108.1	97.9		99.5	184	69.4	-	91.6		
	_		-	99.2	-	95.5	-	72.8	-	97.0	-	-
	-	-	-	101.0	-		-	76.3	-	-	-	
Mean	103.8	99.6	103.8	95.8	94.8	91.4	105.8	73.1	102.7	88.7	211.0	106.7
Std.dev.	1.0	2.9	4.4	4.2	2.9	6.3	71.8	3.3	1.2	5.3	18.7	6.5
					Labor	atory and	method					
	13	14	15	16	17	18	19	20	21	22	23	24
	EAAS	EAAS	EAAS	EAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS
	76	74.2	98	101	96.8	84.0	79.5	93.8	97	100.1	75.6	103
	85	63.8	103	112	95.2	85.0	87.0	100.3	107	99.6	89.9	98
	80	79.5	100	105	96.2	86.0	113.0	101.8	91	99.7	51.5	101
	81	69.7	-	-	-	-	-	-	-	-	67.4	-
	-	74.2	-	-	-	-	-		-	-	-	-
	-	74.8	-	-	-	-	-	-	-	-	-	-
	-	84.2	-	-	-	-	-	-	-	-	-	-
	-	79.3	•	-	-	-	-	-	-	-	-	-
	-	79.9	-	-		-	-	· -	-	-	-	-
	-	80.8	-	-	-	-	-	-	-	-	-	
Mean	80.5	76.0	100.3	106.0	96.1	85.0	93.2	. 98.6	98.3	99.8	72.3	100.7
Std.dev.	3.7	6.0	2.5	5.6	0.8	1.0	17.6	i 4.3	8.1	0.3	19.4	2.5
					Labo	ratory and	l method					
	25	26 PIXE	27 PIXE	28 TN A A	29 TNAA	9 30 TNAA) 31 TNAA	32 RNAA	2 33 RNAA	34 EDXRF	35 ADIDMS	

93

91

93

91

94

-

92.4

1.3

92

92

88

93

-

•

91.3

2.2

Mean

Std.dev.

135

113

103

168

•

-

129.8

28.8

188

195

172

187

198

203

190.5

10.9

107

96

104

99

-

-

101.5

4.9

92

95

102

-

-

-

96.3

5.1

94

91

97

-

-

.

94.0

3.0

83

77

77

86

-

-

80.8

4.5

56

85

76

-

-

-

72.7

15.3

91

90

92

-

-

-

91.0

1.0

-

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-

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Table 5 : Analytical results for Batch 906 (μ g/L)

					thod	atory and m	Labor					
12	11	10	9	8	7	6	5	4	3	2	1	
EAAS	EAAS	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	ADF	
99	1 65	83.2	102	74.8	74	85.3	98	91.1	96.4	101.5	100	
109	192	84.6	88	70.1	55	86. 9	90	94.3	102.9	102.1	103	
108	163	86.5	100	82.3	85	89.2	90	95.8	110.3	98.3	105	
-	-	88.4	-	79.9	128	88.4	96	96.3	96.4	98.8	105	
-	-	89.3	-	73.2	143	(75.0)	92	96.4	99 .8	103.7	-	
-	-	90.7	•	79.3	184	86.5	-	98.5	111.2	102.1	-	
-	-	91.1	0)* -	(60.	-	95.5	-	99.3	-	-	-	
	-	-	-	72.4	-	-	-	103.2	-	-	•	
105.3	173.3	87.7	96.7	76.0	111.5	87.2	93.2	9 6.9	102.8	101.1	103.3	Mean
5.5	1 6.2	3.0	7.6	4.5	48.6	1.6	3.6	3.6	6.6	2.1	2.4	Std.dev.
	·				ethod	atory and m	Labor					
24	23	22	21	20	19	18	17	16	15	14	13	
ADHAAS	ADHAAS	ADHAAS	ADHAAS	ADHAAS	HAAS	ADHAAS A	ADHAAS	EAAS	EAAS	EAAS	EAAS	
106	63.4	103.2	96	110.8	98.0	79.0	100.0	88	88	74.5	93	
106	67.4	94.5	88	103.7	93.5	79.0	97.3	86	93	74.4	93	
111	69.4	96.1	120	94.8	87.0	82.0	100.0	86	98	76.3	96	
-	71.3	-	-	-	-	-	•	-	-	64.3	97	
-	-	-	-	-	-	-	-	-	•	79.8	-	
-	-	•	-	-	•	-	-	-	•	79.7	-	
-	-	-	-	-	•	-	•	-	-	82.1	-	
-	-	-	-	-	-	-	-	-	-	69.6	-	
-	-	-	-	-	•	-	•	-	•	78.3	-	
-	-	-	-	•	-	•	-	•	-	62.0	•	
107.7	67.9	97.9	101.3	103.1	92.8	80.0	99 .1	86.7	93.0	74.1	94.8	Mean
2.9	3.4	4.6	16.7	8.0	5.5	1.7	1.6	1.2	5.0	6.8	2.1	Std.dev.

Laboratory and method											
	25	26	27	28	29	30	31	32	33	34	35
A	DHAAS	PIXE	PIXE	INAA	INAA	INAA	INAA	RNAA	RNAA	EDXRF	ADIDMS
	95	138	148	92	98	86	111	93	96	-	91
	94	146	176	91	108	99	104	83	80	•	90
	90	(95)*	173	91	104	88	112	80	81	-	91
	91	143	188	95	103	-	-	86	-	-	-
	-	•	186	97	-	•	-	-	-	-	-
	-	-	197	-	-	-	-	-	-	-	-
lean	92.5	142.3	178.0	93.2	103.3	91.0	109.0	85.3	85.7	. ***	90.7
id.dev.	2.4	4.0	171	2.7	4.1	7.0	4.4	6.8	9.0	-	0.6

 Table 6 : Relative performances of analytical methods judged by estimates of the means, "pure" between-laboratory standard deviations, repeatabilities (within laboratory standard deviation) and the 95-% confidence intervals of the mean.

	Enti	re populati	ion of labora	atories			After exlusion of unacceptable results						
Method code	n lab	n rep	x conc μg/L	ST	sĽ	95 % CI	n lab	n rep	x conc μg/L	ST	sL	95 % CI	
	L					Batch 10	5-SERUM						
ADF	10	52	94.1	4.0	8.1	88.5-99.7	6	32	96.5	3.3	1.8	94.6-98.4	
EAAS	6	26	99.8	5.5	8.9	90.9-108.8	4	20	94.5	4.6	3.2	90.1-98.9	
ADHAAS	9	26	95.4	11.5	-	93.1-97.8	7	22	95.5	2.6	3.3	92.6-98.3	
PIXE	2	9	109.8	4.9	-	-	0	-	-	-	-	-	
INAA	3	10	86.7	5.3	6.9	76.5-97.0	1	3	94.7	0.6	-		
RNAA	1	4	79.0	7.0	-	•	0	-	-	-	-		
EDXRF	1	3	91.7	10.1	-	-	0	-	-	-	-	-	
ADIDMS	1	3	88.3	0.6	-	•	1	3	88.3	0.6	-	-	
	<u>.</u>					Batch 90	4-BLOOD						
ADF	10	61	92.6	11.0	8.6	86.3-98.8	8	48	96.5	4.5	6.3	91.7-101.3	
EAAS	6	26	107.3	7.0	30.7	77.1-137.4	4	18	96.6	5.7	5.2	89.7-103.5	
ADHAAS	9	29	94.8	4.9	10.8	87.1-102.5	7	22	95.0	8.7	6.1	89.9-100.1	
PIXE	2	10	144.4	18.5	36.7	83.1-205.6	0	-	-	-	-	-	
INAA	4	15	99 .0	3.5	7.1	91.0-107.0	4	15	99.0	3.5	7.1	91.0-107.0	
RNAA	2	6	72.5	8.4	-	69.2-75.8	0	-	-	-	-	-	
ADIDMS	1	3	93.3	1.2	-	-	1	3	93.3	1.2	-	-	
<u> </u>	_					Batch 90	5-BLOOD)					
ADF	10	60	95.8	23.1	4.6	89.1-102.4	8	46	97.3	4.4	5.5	93.1-101.6	
EAAS	6	26	113.4	7.8	46.8	67.7-159.2	3	9	104.3	5.2	1.8	99.9-108.9	
ADHAAS	9	28	92.8	9.1	7.4	86.6- 99.0	7	22	96.9	7.4	-	94.0-99.7	
PIXE	2	10	160.2	19.6	42.0	91.2-229.1	0	-	-	-	-	-	
INAA	4	95	96.1	3.7	3.8	91.5-100.6	4	15	96.1	3.7	3.8	91.5-100.6	
RNAA	2	7	76.1	10.3	1.4	67.2-86.2	0	-	-	-	-	-	
ADIDMS	1	3	91.0	1.0	-	-	1	3	91.0	1.0	-	-	
	L		<u></u>			Batch 90	6-BLOOD)					
ADF	10	56	94.2	11.6	7.5	88.4-100.0	8	44	96.1	4.0	6.2	91.4-100.8	
EAAS	6	26	104.5	7.3	33.8	71.4-137.7	4	13	94.9	3.7	7.2	86.8-103.1	
ADHAAS	9	29	93.6	6.6	12.5	84.6-102.6	6	19	98.9	4.6	5.4	93.7-104.0	
PIXE	2	9	160.2	14.6	24.2	118.0-202.3	0	-	-	-	-	-	
INAA	4	15	99 .1	4.4	7.8	90.3-108.0	4	15	9 9.1	4.4	7.8	90.3-108.0	
RNAA	2	6	85.5	8.0	-	-	2	6	85.5	8.0	-	-	
ADIDMS	1	3	90.7	0.6	-	-	1	3	90.7	0.6		-	
				_									

n = number of ..., lab = laboratories, rep = replicates; conc = sample concentrations; sr = estimate of with-in laboratory standard deviation or repeatability; sL = estimate of "pure" between lab standard deviation (cfr ref 6); 95 % CI = 95 % confidence interval of the method mean.

Statistical Criterion	A	B 1	B2	с	D	Final decision	n.lab. exclud./ tot.lab.	
Method			lab. N°			lab. N°	lol.iad.	
ADF	•	8	3.8	•	7,8,10	7,8,9°,10	4/10	
EAAS		11	11	11	12	11,12	2/6	
ADH-AAS	-	23	23	23	-	18°,23	2/9	
PIXE	-	26	-	-	26,27	26,27	2/2	
INAA	-	29	29	29	28	28,29	2/3	
RNAA	-	32	-	•	32	32	1/1	
EDXRF	-	34	-	34	-	34	1/1	
ADIDMS	-	•	•	-	-	-	0/1	

Table 7: Laboratories excluded in calculation of mass concentration of Se, Batch 105.

Criteria for exclusion are :

A: outlying mean in comparison with all other laboratories (Dixon's criterion)

B: heterogeneity of variance (Bartlett's test, P<0.05), B1 : with-in laboratory variance significantly different from variances of all other laboratories, B2 : with-in laboratory variance significantly different from variances of laboratories using the same method (Snedecor F test, P<0.05 with regard to the mean variance of remaining laboratories)</p>

C: variance higher than the 3-sigma control limit for standard deviation of replicate measurements

D: difference between successive means higher than 3 %

°: lab having reported only mean and standard deviation.

Table 8 : Laboratories excluded in calculation of mass concentration of Se, Batches 904, 905, 906.

				Statistical criterion								
	A	Е		B 1	B2	С	D		F1	F2	Final	nbr.lab.
METHODS	s —			• · · · ·						<u></u>	deci-	excluded
											sion	/ total
	Lab Nº		Batch		Lab Nº			Batch	La	δN°	Lab Nº	nbr.lab.
ADF	- -	-	904	3,7,8,10	7	7	8	904/5	7,8	7,8	7,8	2/10
			905	6,7,10	6,7	7	8	905/6	8		7,8	2/10
			906	3,7,9	3,7,9	7	8	904/6	7,8	7	7,8	2/10
EAAS	11	11	904	11,12,15	11,12,15	11	13	904/5	12,13,14	14,16	11,13	2/6
			905	11,12,14,16	15	11	13,14	905/6	12,14	13,16	11,13,14	3/6
			906	11,14	11,14	11	14	904/6	14	13,14	11,14	2/6
ADHAAS		23	904	19,21,23	19,21,23	•	21	904/5	21	21	21,23	2/9
			905	19,21,23	19,21,23	23	18	905/6	18,24	-	18,23	2/9
			906	20,21	21	21	18	904/ 6	18,21,24	18,21	18,21,23	3/9
PIXE	27	26,27	904	26,27	•	27	•	904/5	•	•	26,27	2/2
			905	26,27	-	26	-	905/6	-	•	26,27	2/2
			906	27	-	27	•	904/6	-	•	26,27	2/2
INAA	-		904	30	•	-	-	904/5	. .	31	-	0/4
			905	-	-	-	-	905/6	31	31	•	0/4
			906	30	•	•	-	904/6	31	-	•	0/4
RNAA	-		904	32,33	•	-	32,33	904/5	32,33	•	32,33	2/2
			905	33	-	-	32,33	905/6	32,33	33	32,33	2/2
			906	32,33	-	-	-	904/6	32,33	32,33	•	0/2
ADIDMS	-	-	904	-	•	-	-	904/5	· ·		-	0/1
			905	-	-	•	-	905/6	-	-	-	0/1
			906	-	•	-	-	904/6	-	-	-	0/1

Criteria for exclusion are those of Table 7 and the following : E; deviation of means for the three batches from other laboratories (Ranking order of laboratory.); F; deviation from the two-sample plots; Fl; outlying laboratory means; F2; outlying deviation from the mean value (outside the 95 -% confidence circle).

			1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Serum 105	Blood 904	Blood 905	Blood 906
5	5	5	6
19	24	23	25
14	10	11	9
80	106	95	100
95.2	96.4	97.5	96.0
3.5	4.4	5.1	4.4
3.7 %	4.6 %	5.2 %	4.6 %
9.8	12.3	14.3	12.3
2.9	5.7	4.7	6.4
0.8	1.4	1.2	1.4
93.6-96.8	93.6-99.2	95.2-99.9	93.2-98.8
4.5	7.1	6.9	7.8
4.7 %	7.4 %	7.1 %	8.1 %
12.6	19.9	19.3	21.8
	Serum 105 5 19 14 80 95.2 3.5 3.7 % 9.8 2.9 0.8 93.6-96.8 4.5 4.7 % 12.6	Serum 105 Blood 904 5 5 19 24 14 10 80 106 95.2 96.4 3.5 4.4 3.7 % 4.6 % 9.8 12.3 2.9 5.7 0.8 1.4 93.6-96.8 93.6-99.2 4.5 7.1 4.7 % 7.4 % 12.6 19.9	Serum 105 Blood 904 Blood 905 5 5 5 19 24 23 14 10 11 80 106 95 95.2 96.4 97.5 3.5 4.4 5.1 3.7% 4.6% 5.2% 9.8 12.3 14.3 2.9 5.7 4.7 0.8 1.4 1.2 93.6-96.8 93.6-99.2 95.2-99.9 4.5 7.1 6.9 4.7% 7.4% 7.1% 12.6 19.9 19.3

Table 9: Mass concentration of Se assigned to the batches of serum and whole blood together with summary statistical report.

Srep = estimate of within-laboratory standard deviation

 \underline{S}_{L} = estimate of pure between lab. standard deviation

<u>S</u> = standard deviation of the mean = $V(\underline{s}^2, \pm \underline{s}^2)$

SEM = $V(\underline{s}^2, /\underline{n} + \underline{s}^2, /\underline{n})$ = standard error of the mean.

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