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SUBCOMMITTEE ON TOXICOLOGY OF SELENIUM*

Interlaboratory trial on the DETERMINATION OF TOTAL SELENIUM IN LYOPHILIZED HUMAN BLOOD SERUM

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Interlaboratory trial on the determination of total selenium in lyophilized human blood serum

Summary - An interlaboratory cooperative study was conducted, to determine total selenium (Se) in two lots of a commercially-available (Seronorm) lyophilized human blood serum. The main objective was to arrive at consensus concentration values in order to establish control sera for chemical analysis. A secondary goal was to assess the performance of different analytical methods as applied to the analysis of serum specimens with normal concentrations of Se, i.e. about 100 μg/L. Twenty seven laboratories worldwide participated in the trial using six inherently different analytical methods: (1) fluorometry, (2) electrothermal atomization atomic absorption spectrometry with or without sample pretreatment, (3) neutron activation analysis (instrumental or with radiochemical separation), (4) hydride generation atomic absorption spectrometry, (5) isotope dilution mass spectrometry, and (6) X-ray fluorescence spectrometry. For this population of laboratories, methods (2) and (4) exhibited the largest systematic differences in concentration. Based on about 100 analyses from 18 laboratories using techniques (1), (2), (3), (5) and (6) the following mean Se concentrations ± a 68% confidence interval for one future observation were found - Seronorm 102: 91±7 μg/L; Seronorm 103: 93±7 μg/L. It is hoped that this information will lead to the establishment of these sera as quality control materials useful to clinical and other laboratories measuring Se in serum matrices.

INTRODUCTION

Although a great many investigations have been reported on the methodology and applications of a variety of analytical techniques for the estimation of total selenium (Se) in biological tissues and fluids, very few biological materials are available with assigned concentrations of Se for the validation of new methods and for precision or accuracy control. The first such material was a powdered kale preparation (Ref. 1) for which a consensus value for Se was established by means of an interlaboratory cooperative study. Subsequently, the US National Bureau of Standards (NBS) issued several reference materials, orchard leaves, bovine liver, wheat flour and rice flour with certified levels of selenium and maize stalk and kernel with recommended values of the element (Ref. 2). Some other biological materials (fish flesh, copepod, human hair and lyophilized animal blood) are available from the International Atomic Energy Agency (IAEA) (Ref. 3). However, no materials with a serum-like matrix have been characterised for Se concentration, even though the incorporation of such a material into the analytical procedure of the clinical laboratory is really indispensable for monitoring and maintaining analytical data quality. Consequently, an interlaboratory cooperative study was conducted under the auspices of the IUPAC Subcommittee on Selenium, Commission on Toxicology to establish the total Se concentrations in two human blood serum pools. The plan was that a number of experienced laboratories throughout the world should apply their preferred routine or research methods for selenium determination to the analysis of the pools. This would serve both for deriving consensus concentration values, and for assessing the relative merits of different analytical methods as applied to the analysis of normal sera, with Se concentrations in the vicinity of 100 μg/L.

EXPERIMENTAL

The pool materials used in this study were batches (nos. 102 and 103) of lyophilized human serum, designated "Seronorm Protein", provided by Nyegaard and Co. AS Oslo, Norway in vacuum-sealed vials. These pools comprise normal human sera without added preservatives, and are expected to contain endogenous levels of trace elements. Batch 102 has been previously characterized in respect of the concentrations of 12 proteins and total and protein nitrogen (4) and is available commercially for laboratory quality control of such analyses.

A selenium solution to serve as an aqueous unknown for submission to the participating laboratories was prepared from high purity black elemental Se pellets (Canadian Copper Refiners, Montreal, Can) and Aristar nitric acid (BDH Chemicals, Poole, England). One gram of Se,

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accurately weighed to ± 0.0001 g, was dissolved in 10.0 mL HNO_3 by gentle heating and made up to 1000 mL with deionized water to give a stock solution containing 1000 μg Se/mL in 0.1 mol/L HNO_3. A reagent blank using 6.5 mL HNO_3 in a final volume of 1000 mL was similarly prepared to give a 0.1 mol/L HNO_3 blank solution. A portion of the stock solution was diluted to give a "working standard" solution containing 8.40 μg Se/mL and this solution and the reagent blank were packaged in 10 mL flame-sealed borosilicate glass ampoules labelled D and H, respectively. Participants were informed only that D was a Se standard and H a blank solution in 0.1 mol/L HNO_3.

A minimum of six vials of each of the two sera were submitted to participating laboratories with manufacturer's instructions for sample reconstitution with 1.00 mL of pure water. In addition, several laboratories (all in North America, chosen solely on the basis of sample shipping logistics) received one ampoule each of the selenium unknown and blank solutions. Participating laboratories were located throughout Europe (N=12) and North America (N=13) with one each from New Zealand and the Peoples Republic of China. Analytical methods used by the 27 laboratories submitting results are summarized in Table 1.

TABLE 1. Analytical methods used for determination of selenium in human blood serum by a total of 27 laboratories

Method	Code	No. of Laboratories
Acid decomposition-fluorometry	ADF	7
Electrothermal atomization atomic absorption spectrometry	EAAS	6
Instrumental neutron activation analysis	INAA	5
Acid decomposition-hydride generation atomic absorption spectrometry	ADHAAS	4
Acid decomposition, dry ashing - fluorometry	ADDAF	1
Acid decomposition, solvent extraction - electrothermal atomization atomic absorption spectrometry	ADSEEAAS	1
Neutron activation analysis with radiochemical separation	RNAA	1
Acid decomposition - isotope dilution mass spectrometry	ADIDMS	1
Acid decomposition - X-ray fluorescence spectrometry	ADXRF	1

Analysts solicited were generally those currently active in the area of Se measurements in blood and other biological fluids. No constraints were imposed on the analytical methods to be used. The only requirements were that analysts should have previous experience with the chosen method and its application to biological fluids and that the analysts should be satisfied that they and the chosen methods were capable of good performance. Information was provided regarding the identities of the standard and blank solutions and it was indicated to some participants that the Se concentration of each of the sera was about 80 μg/L. One analytical determination on each of the 12 vials of sera was requested. For those laboratories whose methods required volumes larger than 1 mL of reconstituted serum, 12-18 vials of each material were supplied, with the request that the contents of two or three vials of the same batch be pooled prior to analysis in order to provide six independent analyses for each pool. It was requested that an aliquot from each of the selenium unknown and reagent blank ampoules be diluted 100-fold with a diluent appropriate to the measurement technique used and that this be replicated six times. Aliquots of the diluted selenium and blank solutions of size identical to that of the sera samples were to be taken through the entire analytical procedure concurrently with the serum samples so as to give six concentration results (μg/L) for each of the serum pools, for the unknown aqueous selenium solution and the reagent blank. A report form was included with the package of specimens to be completed

with analytical results and information on sample preparation, instrumentation and relevant parameters, methods of calibration and quality control, and estimates of blank levels, precisions and of limit detection. Bartlett's test (Ref. 5) was used to test for heterogeneity of variance (differences in precision) within laboratories using the same analytical method and across all methods. Where more than one laboratory used the same analytical method, analysis of variance (ANOVA) procedures were used to test for differences in mean concentration (relative accuracy) among laboratories. ANOVA procedures were also used to examine differences among methods. In several instances, the statistical analyses were repeated after omitting the data from certain laboratories on the basis of criteria discussed subsequently in the text. Variance components were estimated using the procedure in the Statistical Analysis System (SAS) (Ref. 6).

RESULTS AND DISCUSSION

Analytical results were received from 27 participating laboratories, the majority complying with the request for six analyses per material; one laboratory reported information on 10-12 vials of each serum material. At least one analyst carried out replicate analyses on each of six vials, reporting the mean concentrations for each vial. In all instances, each datum reported was treated as one determination. The majority conducted analyses on volumetric aliquots of the solution reporting μg Se/L. A few used one or other of the following approaches: gravimetric dispensing of serum, converting to volume by measuring the density of the reconstituted solution; analysis of portions of the solid lyophilized serum without reconstitution or analysis of the entire contents of the vial reporting either ng Se/vial or a notional μg Se/L. To achieve comparability of the data, results reported as ng/vial were converted into μg/L [= (ng per vial)/1.07)]. The factor 1.07 is the volume of reconstituted serum calculated from mass/density with mean values for mass 1.0891 g and density 1.021 g/mL independently determined by R. Cornelis (Institute for Nuclear Sciences, Gent Belgium) and Y. Thomassen, respectively.

All individual results for the two serum pools are listed in Tables 2 and 3, respectively; the number of significant figures is as reported by the cooperating laboratories. A summary of the ranges and mean concentrations for these sera is presented in Table 4.

Table 2. Cooperative results for Seronorm Protein, batch 102, μg Se/L

Laboratory no. and method								
1 ADF	2 ADF	3 ADF	4 ADF	5 ADF	6 ADF	7 ADF	8 EAAS	9 EAAS
85.4	91	99.8	78.1	100	90.2	108	57.6	102
80.4	80	99.7	79.7	97	87.9	97	50.1	100
85.0	87	98.5	103.0	96	87.4	97	47.2	99
85.1	84	92.6	84.4	100	90.7	101	49.5	102
89.3	96	92.6	103.0	98	86.5	98	39.2	100
89.3	82	90.3	81.2	97	86.0	100	58.6	100
10 EAAS	11 EAAS	12 EAAS	13 EAAS	14 INNA	15 INAA	16 INNA	17 INAA	18 INAA
84	74	90.6	87	83	100.5	100	93.5	85.5
84	75	90.0	90	65	96.6	94	91.0	88.7
92	74	95.1	-	60	95.3	101	92.7	91.4
89	73	93.0	-	-	87.8	94	-	96.0
87	70	94.7	-	-	87.2	92	-	92.5
85	72	92.4	-	-	86.7	95	-	84.8
19 ADHAAS	20 ADHAAS	21 ADHAAS	22 ADHAAS	23 ADDAF	24 ADSEEAAS	25 RNAA	26 ADIDMS	27 ADXRF
64	72.4 70.1	88.7	68	124	74	85.0	84.9	97
59	72.4 72.4	83.5	64	90	76	83.7	86.2	83
67	72.4 72.4	90.0	68	200	80	94.0	85.0	82
59	72.4 72.4	90.6	72	180	76	83.6	87.3	72
55	70.1 74.8	81.5	68	120	78	95.2	88.1	84
68	72.4 72.4	80.9	76	180	82	85.4	88.1	89

Table 3. Cooperative results for Seronorm Protein, batch 103, μg Se/L

Laboratory no. and method								
1 ADF	2 ADF	3 ADF	4 ADF	5 ADF	6 ADF	7 ADF	8 EAAS	9 EAAS
81.9	89	83.0	76.5	100	91.6	94	46.4	109
78.1	91	92.6	78.1	99	90.0	99	50.2	109
82.8	100	86.9	98.3	100	89.9	94	40.4	107
81.7	98	94.0	96.8	97	87.6	103	38.0	107
85.8	87	95.4	93.6	95	89.8	106	48.4	110
87.0	98	96.9	98.3	97	86.2	103	45.8	107

10 EAAS	11 EAAS	12 EAAS	13 EAAS	14 INNA	15 INAA	16 INNA	17 INAA	18 INAA
95	65	92.9	95	90	91.8	100	96.9	90.4
91	69	95.4	94	72	91.2	101	93.5	88.7
92	68	90.7	-	67	89.9	97	-	79.8
86	68	91.0	-	86	88.3	98	-	94.0
86	73	93.5	-	92	87.7	83	-	88.4
91	70	90.4	-	87	87.5	94	-	85.9

19 ADHAAS	20 ADHAAS		21 ADHAAS	22 ADHAAS	23 ADDAF	24 ADSEEAAS	25 RNAA	26 ADIDMS	27 ADXRF
55	72.4	60.7	91.9	76	190	76	93.3	87.7	96
56	67.8	72.4	87.4	80	150	80	84.7	88.3	90
59	72.4	72.4	90.0	68	180	76	-	89.6	88
64	72.4	72.4	93.2	68	130	76	87.4	90.2	94
51	35.0	74.8	87.4	76	220	74	93.3	95.2	98
14	53.7	72.4	91.3	56	140	64	85.9	89.8	88

TABLE 4. Ranges and mean concentrations of selenium (μg/L) in the sera reported by cooperating laboratories.

Lab. no.	Method	Seronorm 102 Range	Mean	std. dev.	Seronorm 103 Range	Mean	std. dev.
1	ADF	80.4-89.3	85.8	3.3	78.1-87.0	82.9	3.2
2	ADF	80-96	86.7	6.0	87-100	93.8	5.5
3	ADF	90.3-99.8	95.6	4.2	83.0-96.9	91.5	5.4
4	ADF	78.1-103.0	88.2	11.6	76.5-98.3	90.3	10.2
5	ADF	96-100	98.0	1.7	95-100	98.0	2.0
6	ADF	86.0-90.7	88.1	1.9	86.2-91.6	89.2	1.9
7	ADF	97-108	98.6	1.8	94-106	99.8	5.0
8	EAAS	39.2-58.6	50.4	7.2	38.0-50.2	44.9	4.7
9	EAAS	99-102	100.5	1.2	107-110	108.2	1.3
10	EAAS	84-92	86.8	3.2	86-95	90.2	3.5
11	EAAS	70-75	73.0	1.8	65-73	68.8	2.6
12	EAAS	90.0-95.1	92.6	2.1	90.4-95.4	92.3	2.0
13	EAAS	87-90*	88.5	2.1	94-95*	94.5	0.7
14	INAA	60-83**	69.3	12.1	67-92	82.3	10.3
15	INAA	86.7-100.5	92.4	5.9	87.5-91.8	89.4	1.8
16	INAA	92-101	96.0	3.6	83-101	95.5	6.6
17	INAA	91.0-93.5**	92.4	1.3	93.5-96.9*	95.2	2.4
18	INAA	84.8-96.0	89.8	4.3	79.8-94.0	87.9	4.8
19	ADHAAS	55-68	62.0	5.1	14-64	49.8	18.1
20	ADHAAS	70.1-74.8***	72.2	1.2	35.0-74.8**	66.6	11.7
21	ADHAAS	80.9-90.6	85.9	4.4	87.4-93.2	90.2	2.4
22	ADHAAS	64-76	69.3	4.1	56-80	70.7	8.6
23	ADDAF	90-200	149	44	130-220	168	34
24	ADSEEAAS	74-82	77.7	2.9	64-80	74.3	5.4
25	RNAA	83.6-95.2	87.8	5.3	84.7-93.3	88.9	4.1
26	ADIDMS	84.9-88.1	86.6	1.5	87.7-95.2	90.1	2.7
27	ADXRF	72-97	84.5	8.3	88-98	92.3	4.3

*2 estimates only. **3 estimates only. ***12 estimates.

Performance of methods

In the context of assigning concentrations to these pools, an assessment of method and laboratory performance was a prerequisite. Therefore for each method, the mean concentration and both the between- and within-laboratory standard deviation estimates were calculated (Tables 5 and 6). Plots of the data (Figs. 1 and 2 for Seronorm 102 and 103, respectively) were helpful in interpreting the findings.

From Tables 5 and 6 and Figs. 1 and 2, it is evident that generally parallel results for mean concentrations and variances were obtained for the two sera. The overall ranges of concentration reported were quite broad, 39-200 μg/L and 38-220 μg/L, for Seronorm 102 and 103, respectively. Extremely high concentrations and standard deviations (149±44 and 168±34 μg/L) were reported by laboratory 23; these data were omitted from the calculations. With this exclusion, the concentration ranges were 39-108 and 38-110 μg/L for Seronorm 102 and 103, respectively, narrower but still very broad.

Considering only those methods used by four or more laboratories (Table 5) and using all the data from these laboratories the mean concentrations obtained by ADF and INAA are very similar for each material; lower values were reported from laboratories using EAAS and ADHAAS. Excluding outlying values on the basis of criteria mentioned in the footnote to Table 5 and elaborated subsequently, leading to the exclusion of 2, 1 and all 4 laboratories from EAAS, INAA and ADHAAS, respectively, very good concordance among data from ADF, EAAS and INAA emerged. For Seronorm 103, these means agree reasonably well with means from individual laboratories using RNAA, ADIDMS and ADXRF techniques; however for Seronorm 102, the mean concentrations from laboratories using ADF, EAAS, and INAA were somewhat larger than the mean value reported by each of the other analytical methods, which in turn (except for ADSEEAAS) were in very good agreement. Thus the results from laboratories using methods based on the rather diverse techniques of fluorometry, atomic spectrometry, neutron activation, mass spectrometry and X-ray fluorescence are generally concordant.

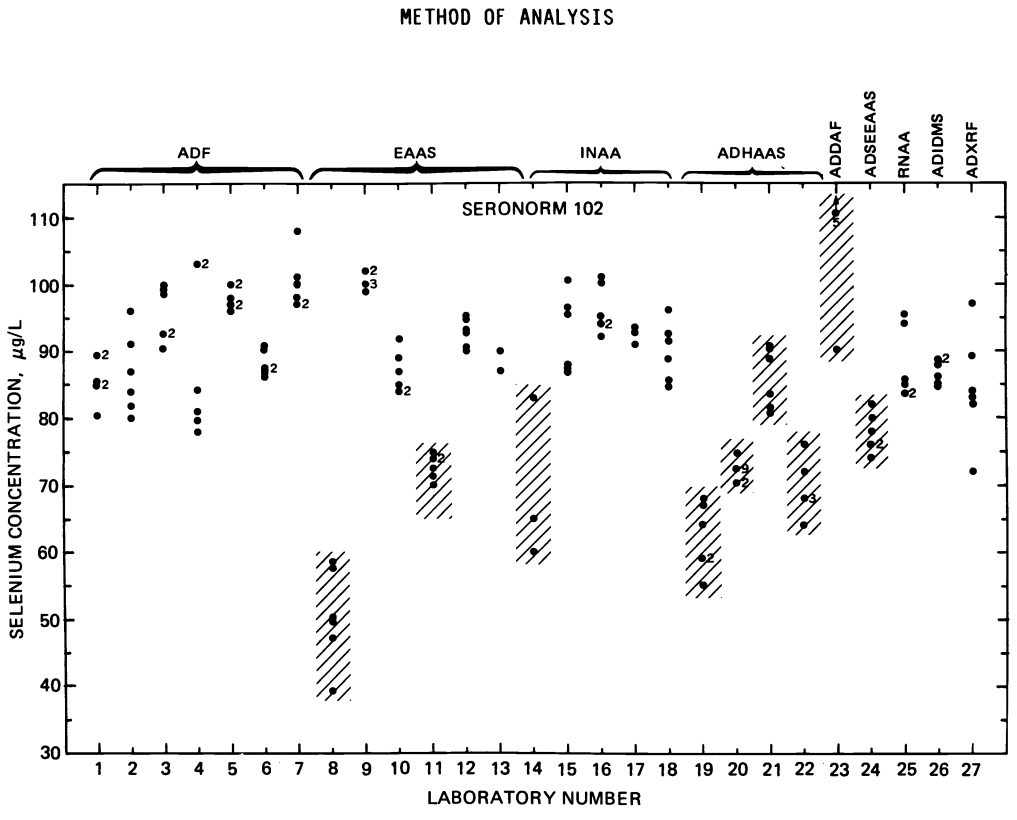

Fig. 1. Individual concentrations of selenium (μg/L) in Seronorm 102 reported by cooperating laboratories. Numerals beside some points indicate the number of results represented by the point. The arrow for laboratory 23 indicates 5 data points beyond the limit of the ordinate scale. Shaded points refer to data excluded from the calculation of a consensus mean concentration according to criteria noted in the footnote to Table 8.

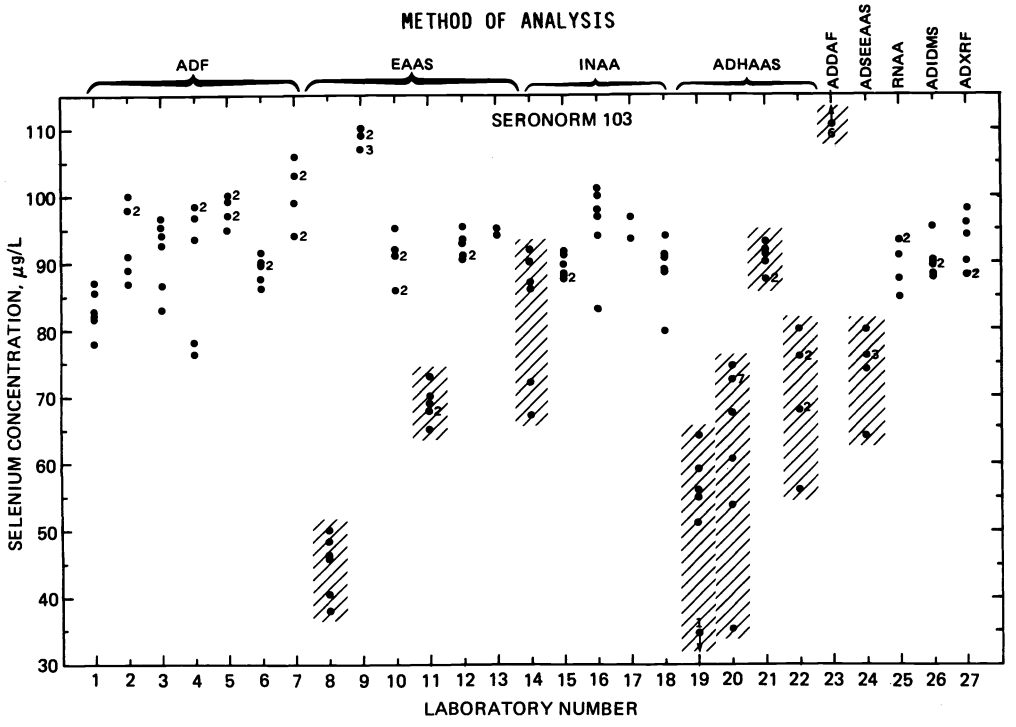

Fig. 2. Individual concentrations of selenium (µg/L) in Seronorm 103 reported by cooperating laboratories. Numerals beside some points indicate the number of results represented by the point. The arrows for 1 datum from laboratory 19 and 6 data from laboratory 23 indicate readings beyond the limits of the ordinate scale. Shaded points refer to data excluded from the calculation of a consensus mean concentration, according to criteria noted in Table 8.

TABLE 5. Relative accuracy of analytical methods, judged by the mean concentrations found for the two serum pools.

Method	Entire lab population: No. of labs	No. of estimates	Mean concn. ± SEM µg/L[1]	After exclusion of labs[2]: No. of labs	No. of estimates	Mean concn. ± SEM µg/L
			Seronorm 102			
ADF	7	42	91.8 ± 2.2	7	42	91.8 ± 2.2
EAAS	6	32	81.2 ± 7.7	4	20	92.8 ± 3.4
INAA	5	24	89.8 ± 4.2	4	21	92.7 ± 1.4
ADHAAS	4	30	72.3 ± 3.9	0	-	- -
ADDAF	1	6	149 ± 18	0	-	- -
ADSEEAAS	1	6	77.7 ± 1.2	0	-	- -
RNAA	1	6	87.8 ± 2.2	1	6	87.8 ± 2.2
ADIDMS	1	6	86.6 ± 0.6	1	6	86.6 ± 0.6
ADXRF	1	6	84.5 ± 3.4	1	6	84.5 ± 3.4
			Seronorm 103			
ADF	7	42	92.2 ± 2.2	7	42	92.2 ± 2.2
EAAS	6	32	81.7 ± 9.7	4	20	96.6 ± 4.8
INAA	5	26	89.3 ± 2.5	4	20	91.4 ± 2.1
ADHAAS	4	30	68.7 ± 6.5	0	-	- -
ADDAF	1	6	168 ± 14	0	-	- -
ADSEEAAS	1	6	74.3 ± 2.2	0	-	- -
RNAA	1	6	88.9 ± 1.7	1	5	88.9 ± 1.7
ADIDMS	1	6	90.1 ± 1.1	1	6	90.1 ± 1.1
ADXRF	1	6	92.3 ± 1.7	1	6	92.3 ± 1.7

(1) SEM: standard error of the mean.
(2) Criterion for exclusion of laboratories ($P<0.05$ in all cases): excessive deviation of means from other laboratories using identical or different methods.

TABLE 6. Precision of analytical methods within- and between-laboratories

Method	Entire lab population					After exclusion of labs[3]				
			std. deviation µg/L[2]					std. deviation µg/L[2]		
	N[1]	n[1]	S_w	S_b	S_x	N[1]	n[1]	S_w	S_b	S_x
					Seronorm 102					
ADF	7	42	5.65[4]	5.48	7.87	7	42	5.65[4]	5.48	7.87
EAAS	6	32	3.70[4]	18.88	19.24	4	20	2.30	6.40	6.80
INAA	5	24	5.75	8.69	10.41	4	21	4.44	1.93	4.84
ADHAAS	4	30	3.56[4]	7.22	8.05	0	-	-	-	-
ADDAF	1	6	43.5	-	43.5	0	-	-	-	-
ADSEEAAS	1	6	2.94	-	2.94	0	-	-	-	-
RNAA	1	6	5.32	-	5.32	1	6	5.32	-	5.32
ADIDMS	1	6	1.46	-	1.46	1	6	1.46	-	1.46
ADXRF	1	6	8.26	-	8.26	1	6	8.26	-	8.26
					Seronorm 103					
ADF	7	42	5.43[4]	5.25	7.55	7	42	5.43[4]	5.25	7.55
EAAS	6	32	3.02	23.68	23.88	4	20	2.39	8.95	9.26
INAA	5	26	6.49[4]	4.63	7.97	4	20	4.71	3.36	5.78
ADHAAS	4	30	11.67[4]	11.66	16.50	0	-	-	-	-
ADDAF	1	6	34.3	-	34.3	0	-	-	-	-
ADSEEAAS	1	6	5.43	-	5.43	0	-	-	-	-
RNAA	1	5	4.11	-	4.11	1	5	4.11	-	4.11
ADIDMS	1	6	2.66	-	2.66	1	6	2.66	-	2.66
ADXRF	1	6	4.27	-	4.27	1	6	4.27	-	4.27

(1) N = Number of laboratories; n = Number of estimates.
(2) S_w, S_b and S_x (= $(S_w^2 + S_b^2)^{\frac{1}{2}}$) are estimates of within-laboratory, between-laboratory and overall standard deviation of a single observation, respectively.
(3) Criteria for exclusion of laboratories ($p<0.05$ in all cases): excessive deviation of mean from means for other laboratories using identical or different methods.
(4) Statistical analyses indicate heterogeneity of within-laboratory variance.

As far as method precision is concerned (Table 6), within-laboratory standard deviation (S_w) from all the laboratories (except 23) ranged from 1.5 to 11.7 µg/L (and was usually <6 µg/L. Statistical analysis indicated that all procedures displayed heterogeneity of within-laboratory variance ($P<.05$), (ie. that S_w was laboratory-dependent) for either or both pools. For the methods which were used in more than one laboratory, EAAS showed the largest between-laboratory standard deviation (S_b) and overall standard deviation (S_x). Exclusion of the outlying data resulted in large decreases in the estimates of S_w and S_b for ADHAAS (Seronorm 103) and in S_b for EAAS. For the multi- laboratory methods, with outlying data removed, EAAS appeared to be the most precise method (smallest S_w) whereas INAA exhibited superior among- laboratory performance. A brief discussion follows of the individual methods used by the participant laboratories.

Acid decomposition-fluorometry (ADF). The ADF method is widely employed for the measurement of Se in a wide variety of biological matrices and in this study, it was used by 7 out of 27 laboratories. The organic matrix in the sample is destroyed by a mixture of oxidizing acids converting Se into Se (IV), which is complexed with diaminonophthalene and quantitated by molecular fluorescence (Ref. 7). Interlaboratory-collaborative testing with foodstuffs (Ref. 8) demonstrated excellent performance leading to the acceptance of ADF by the Association of Official Analytical Chemists (AOAC) as an official method of analysis of foods (Ref. 9a). Earlier independent work using ADF (Ref. 10) led to an AOAC official method for plants (Ref. 9b). In a subsequent study (Ref. 11) there was again excellent agreement among laboratories using ADF and concordance with data by INAA. It is notable that of the four multilaboratory methods used in the present study, ADF was the only one in which the means from the different laboratories were consistent. Mean concentrations of Se in Seronorm 102 and 103 obtained by ADF are in excellent agreement with the respective means obtained by EAAS and INAA (after omission of outliers) as well as by three other single laboratory methods, RNAA, ADIDMS and ADXRF for Seronorm 103. Although performance of laboratory 4 was more variable than that of the other six laboratories (Table 4), It is clear that ADF is a precise technique. Good agreement has also been reported in the literature (Ref. 12) for Se/human serum results obtained by ADF, EAAS and ADHAAS.

Electrothermal atomization atomic absorption spectrometry (EAAS). EAAS is attractive because it requires little or no sample preparation apart from addition of a suitable bulk matrix containing a Se-stabilizing element. Saeed et al. (Ref. 13) and Alexander et al. (Ref. 14) have shown that Se in naturally-occurring Se organic compounds in body fluids is quantitatively stabilized at ashing temperatures as high as 1100° or 1200°C by incorporating Ni or Ag, respectively. Thus it is possible to determine Se in serum by a direct electrothermal atomic spectroscopic method without the requirement for sample decomposition or separation of the analyte from the matrix. All analysts in this work used Ni to stabilize Se. Results from three of the six laboratories using EAAS are in excellent agreement with each other and with most of the results from laboratories using ADF and INAA. Data from a fourth laboratory, although higher ($P<0.05$) than results from the other laboratories using EAAS, were similar to those from ADF and INAA laboratories, and were retained for the final statistical analysis. Results from two laboratories (8 and 11) showing large negative biases may have been influenced by the technique of calibration or by analyte loss during the ashing step. Good precision of the EAAS approach and good agreement with results obtained by ADF and ADHAAS has also been demonstrated by the interlaboratory comparison organized among nine Finnish and two American laboratories (Ref. 12).

Instrumental neutron activation analysis (INAA) and neutron activation analysis with radiochemical separation (RNAA). Neutron activation analysis (NAA), entails irradiation of the sample to produce radioactive Se isotopes, the concentrations of which are measured by monitoring γ-radiation of specific energies. The instrumental version, in conjunction with γ-ray spectrometry (INAA), involves no chemical operations and is one of the very few techniques not requiring sample decomposition. The instrumental technique is widely accepted as excellent for the determination of Se in biological materials. With serum samples, little error can be expected from effects due to thermal-neutron self-shielding, or gamma-ray self-absorption. Should matrix activation lead to interferences, or if beta activity is to be monitored, resort can be made to the radiochemical separation version (RNAA). In this study, five laboratories used INAA and one used RNAA. The γ-emission of the 120 day half-life isotope ^{75}Se was counted in four laboratories after 1-3 month cooling periods; two relied on counting the 17 sec half-life of ^{77m}Se. That excellent performance can be realised is confirmed by the very good agreement among four of the five INAA and one RNAA data sets, and also by the very good concordance of these results with values derived by the other techniques.

Acid decomposition hydride generation atomic absorption spectrometry (ADHAAS). Procedures for liberating Se from a digest as gaseous hydrogen selenide, H_2Se, coupled with detection by atomic absorption spectrometry, have been widely reported (Ref. 15). One of the most common approaches is to liberate H_2Se using $NaBH_4$ as a reductant, and sweep the gas with argon into an electrically-heated quartz tube or an argon-hydrogen-entrained-air flame for dissociation into atomic Se. Because this technique is more rapid than the manual fluorometric method, recent years have witnessed increasing interest in hydride generation atomic spectrometry (HAAS). However, in spite of the fact that some laboratories are able to elicit good performance from the technique, serious reservations exist regarding the performance of the method in general. An interlaboratory collaborative study under the auspices of the AOAC (Refs. 16, 17) of the ADHAAS method as applied to a range of food materials and standard solutions provided a solid data base for evaluation. Based on information from 28 laboratories using 16 variants of hydride generation apparatus, the conclusion was that, in general the method suffers from both systematic error and imprecision. Serious imprecision of ADHAAS is also noted in the report (Ref. 12) of an interlaboratory trial with human blood serum. In the current study, low mean concentrations were reported by three of the four laboratories using ADHAAS in comparison with the results from most of the other laboratories. Furthermore, large within-laboratory standard deviations are evident for Seronorm 103 for the same three laboratories. This finding is in line with past experience leading to caution in the interpretation of results from the application of ADHAAS. In estimating the overall Se concentration, data from the all four laboratories using ADHAAS were discarded.

Acid decomposition solvent extraction electrothermal atomization atomic absorption spectrometry (ADSEEAAS). Previous investigations on the measurement of Se using direct electrothermal atomization AAS have indicated serious interference effects of sample matrix constituents. Inorganic constituents of the matrix remaining after acid decomposition of foodstuffs were observed (Refs. 18, 19) to almost totally obliterate the Se signal from the graphite furnace used. Although recent designs of graphite furnace and general improvements in technique make possible the direct determination of Se in biological fluids, some analysts feel that separation of the analyte from interfering components of the matrix is advantageous. This may be effected either by reduction-precipitation of elemental Se from the sample digest or by chelation-solvent extraction (Ref. 20). The latter approach was used by one laboratory in this trial. Following digestion of serum samples with HNO_3-$HClO_4$-H_2SO_4 and reduction to Se(IV), the analyte was chelated with an *o*-diamine, extracted into toluene and quantitated by graphite furnace EAAS. Estimates of the precision (S_w) of the data from this laboratory were similar to those from other acceptable laboratories. The values found for the mean concentrations were between those from all laboratories using

either of the alternative atomic spectrometric techniques (EAAS or ADHAAS). Compared to the results from all the methods, however, the mean concentration based upon ADSEEAAS was approximately 10% lower than most of the other values reported (Table 5).

Acid decomposition isotope dilution mass spectrometry (ADIDMS). IDMS is a sensitive, precise and accurate technique for determining elements which exist in at least two isotopic forms and for one of which a purified stable isotope preparation is available. A known quantity of this isotope is equilibrated with the sample which is then chemically processed to yield the analyte as a mixture of endogenous and spike isotopes in a form suitable for introduction into a mass spectrometer. Measurement of the ratio of isotope signals at appropriate m/e values leads to concentrations of the original analyte. In this work, one laboratory reported data using this method. After adding a known quantity of ^{82}Se to the contents of each vial, the samples were digested with HNO_3-H_3PO_4-H_2O_2, Se was chelated with 4-nitro-o-phenylenediamine and extracted into chloroform. Separation of the Se-chelate from the matrix by GC preceded mass spectrometry. Within-laboratory precision was very similar to the best results obtained with other methods and the mean concentration found for Seronorm 103 was in excellent agreement with the other accepted values.

Acid decomposition X-ray fluorescence (ADXRF). Most XRF instruments do not have sufficient detectivity for the direct determination of Se in biological fluids; isolation and pre-concentration of the analyte is necessary. The XRF procedure used in this study entailed HNO_3-$HClO_4$ digestion, and pre-concentration on activated charcoal prior to measurement with an energy-dispersive X-ray fluorescence spectrometer. For Seronorm 103 there was acceptable agreement with data from the other methods, but for Seronorm 102 the mean was lower and the S_w was somewhat greater than for the other techniques.

Performance with submitted aqueous unknown solution and reagent blank
The purpose of submitting an aqueous solution of concentration known to the initiating laboratory to some of the participating laboratories was to obtain data which might cast light on the anticipated among-method and among-laboratory variations. The aqueous unknown solution as well as the reagent blank were taken through the complete analytical procedure (i.e. as for serum) by each laboratory receiving these solutions. The data for both the 100-fold diluted unknown solution with a target concentration of 84.0 µg Se/L and a similarly diluted reagent blank are presented in Table 7 and Fig. 3. Out of a total of 65 results from 11 laboratories, 38 results from 9 laboratories were within ± 10% of the target value but only 15 from 6 laboratories were within ± 5%. All of the values from one laboratory were within ± 5% with a mean of 84.5 ± 1.6 µg/L (SD). There appeared to be no clear relationship between the concentrations reported for the sera and for the aqueous unknown solution. For example, the results of laboratory 23 were clearly unacceptable in the main study whereas for the aqueous solution, 4 of the 6 results from this laboratory were within 10% of the target concentration. With respect to the reagent blank solution, reported concentrations were quite heterogeneous, varying from -10.1 to <20 µg/L.

Estimation of selenium concentrations in Seronorm Protein proposed reference materials
A prerequisite to the assignment of the Se concentrations of the two Seronorm Protein pools was the identification and removal of outlying values. An *apriori* decision was made to treat outliers on a laboratory basis, either retaining or rejecting all the data from each laboratory. Criteria for data exclusion were based on statistical and subjective considerations. The three statistical criteria were (A) comparison of within-laboratory variances, S_w, among laboratories using the same analytical method; (B) comparison of means among laboratories using the same method and (C) comparison of means among laboratories using different methods. The fourth criteria (D) was a subjective decision applied to data generated by ADHAAS. A summary of laboratory selection is in Table 8, with the corresponding excluded data designated by the shaded areas in Figs. 1 and 2. In three cases (lab 8, lab 14 and lab 23), exclusion was on the basis of two criteria. No laboratory was excluded solely on the basis of criterion A (ie. data from lab 4 using ADF were retained).

The data generated by ADHAAS and ADSEEAAS warrant further discussion. Overall, results from the four laboratories using the ADHAAS procedure were biased below the means accepted for the final calculations. One of these laboratories (no. 21), however, supplied values with excellent precision and in agreement with the majority of data from the remaining laboratories for both Seronorm 102 and 103. A decision could easily have been made to retain the data for laboratory 21. However, because the majority of laboratories using ADHAAS obtained consistently low values and because previously published reports cast doubt on the adequacy of the ADHAAS technique (Refs. 12, 16, 17) *all* ADHAAS-generated data were excluded in estimating the Se concentrations. Se concentrations reported by the one laboratory which used ADSEEAAS yielded measures of precision and mean concentrations which were in excellent agreement with the results from the two other atomic spectrometric techniques, EAAS and ADHAAS (Tables 5 and 6).

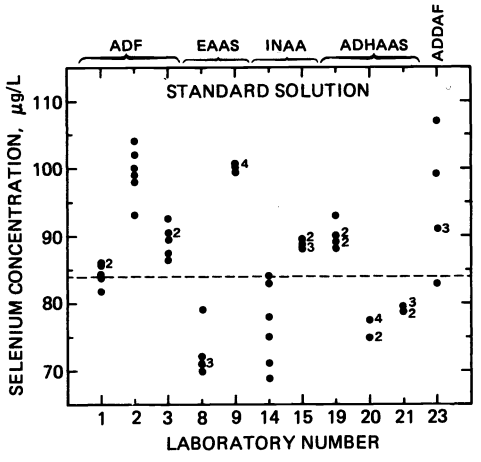

Fig. 3. Concentrations of Se reported for 100-fold diluted unknown solution taken through the entire analytical procedure. Numerals beside some data points refer to the number of results represented by the point. Dashed line represents target value of 84.0 µg/L.

TABLE 7. Cooperative results for aqueous unknown and blank solutions µg Se/L.

Laboratory number and method										
Unknown solution-target concentration 84.0 µg/L in 100-fold diluted solution										
1 ADF	2 ADF	3 ADF	8 EAAS	9 EAAS	14 INAA	15 INAA	19 ADHAAS	20 ADHAAS	21 ADHAAS	23 ADDAF
85.6	99	92.6	72	100.5	71	89.5	89	77.5	78.9	91
81.8	104	86.5	71	100.8	78	89.5	88	77.5	79.5	107
83.9	98	90.6	79	100.2	69	88.5	90	75.0	79.5	91
84.2	100	87.4	71	99.2	75	88.0	90	75.0	79.5	91
85.9	102	89.4	70	100.9	84	88.0	89	77.5	78.9	99
86.0	93	90.6	71	100.8	83	87.9	93	77.5	-	83
Reagent blank										
4.0	9	-10.1	6	0	<18	<1.9	0.006	<0.1	1.3	18
1.5	9	1.0	0	0	<18	<1.9	0.020	<0.1	1.6	10
1.4	12	- 1.2	0	0	<20	<2.0	0.002	<0.1	1.6	10
0.0	12	3.4	0	0	<19	<2.0	0.038	<0.1	1.6	10
2.0	9	- 0.4	4	0	<18	<2.0	0.058	<0.1	1.6	10
0.1	12	0.7	0	0	<19	<2.0	0.060	<0.1	1.3	18

Table 8. Selection of laboratories for calculation of selenium concentrations in Seronorm materials.

Method	Total no. of laboratories	No. of laboratories excluded	Criteria for exclusion[1]
ADF	7	0	-
EAAS	6	2	(A,B)-8;B-11
INAA	5	1	(A,B)-14
ADHAAS	4	4	C-(19,20,22);D-21
ADDAF	1	1	(A,C)-23
ADSEEAAS	1	1	C-24
RNAA	1	0	-
ADIDMS	1	0	-
ADXRF	1	0	-

(1) Criteria for exclusion are: A-within-laboratory variance is significantly different (P<.05) from variances of other laboratories using the same method; B-mean for the laboratory differs (P<0.05) from mean over the remaining laboratories using the same method; C-mean for the laboratory differs significantly from mean over all remaining laboratories remaining at the time of calculation; D-laboratory excluded in order to exclude all laboratories using the ADHAAS method. Figures refer to laboratory number.

Table 9. Assigned Concentrations of selenium in Seronorm pools 102 and 103[1]

Sample	No. of labs.	No. of methods	No. of analyses	Mean concn. ± SEM µg/L	Range of obser. µg/L	Mean ± SD µg/L[2]
Seronorm 102	18	6	101	91.2 ± 1.3[3]	72.0 - 103.0	91.2 ± 7.0
Seronorm 103	18	6	99	92.6 ± 1.5[3]	76.5 - 110.0	92.6 ± 6.7

(1) Data from 18 laboratories conducting analysis on 125 and 123 vials of Seronorm 102 and 103, respectively by the following methods (No. of laboratories): ADF (7), EAAS (4), INAA (4), RNAA (1), ADIDMS (1), and ADXRF (1).
(2) Mean ± 68% confidence interval for one future observation.
(3) Means are significantly different ($p<0.05$) based on a pairwise within-laboratory comparison. Standard errors shown in the table include between-laboratory variation.

Upon exclusion of EAAS and ADHAAS outliers, however, the ADSEEAAS results exhibited a negative bias with respect to the other atomic spectrometric techniques and with respect to data from other methods and were also omitted from final calculations.

Estimates of mean Se concentrations and uncertainties are summarized in Table 9. The correlation among Seronorm 102 and 103 laboratory means was 0.97 ($P<0.001$) for all laboratories and 0.75 $P<0.001$) for the 18 retained laboratories. For both pools, analysis of the results from the 18 laboratories retained indicated no significant differences in Se concentrations among methods. Although Se levels in both materials were very similar, in the vicinity of 90 µg/L, there was a significant ($P<0.05$) difference between the mean values of 91.2±1.3 and 92.6±1.5 µg/L (SEM) for Seronorm 102 and 103, respectively based on a pairwise within-laboratory comparison. The 68% confidence intervals for one future observation are 91±7 and 93±7 µg/L for Seronorm Protein 102 and 103, respectively.

These values are comparable to the concentration range of 95-140 µg Se/L reported by Saeed et al. (Ref. 13) in sera of individuals not occupationally exposed to Se, and 42-109 µg Se/L reported by Westermarck et al. (Ref. 21) in sera of individuals from different parts of Finland. The data are in excellent accord with the mean values of 83-90 µg/L obtained by Kumpulainen and Koivistoinen (Ref. 12) in an interlaboratory trial on pooled human serum from Finnish residents, and slightly in excess of the mean 81 (±14) µg Se/L established for sera from a large number of healthy individuals by Oster and Prellwitz (Ref. 22) using EAAS and ADHAAS. The purpose of these comparisons are solely to indicate that Se concentrations in the two serum pools are in the *vicinity* of values expected in normal human serum. As diet and geographical location are important influences on serum Se levels, the Seronorm concentration data reflect only these reference materials and are not to be construed as reference values for human serum.

The fact that widely differing analytical methods involving five independent measurement principles led to a consistent data set supports the proposed assignments of concentrations. In this regard it is important to note that sample pretreatment by acid decomposition was not used in two methods, thus indicating that loss of the element *via* volatilization during digestion did not occur. Furthermore, the total lack of sample pretreatment in methods based on neutron activation also substantiates the validity of the assigned values.

CONCLUSIONS

An interlaboratory trial on the determination of total selenium in lyophilized human blood serum pools has been successfully completed. Concentrations of the element have been assigned and some information has been obtained regarding the performance of several methods of analysis. On the basis of approximately 100 analyses by 18 laboratories using five independent methods of analysis - fluorometry, electrothermal atomization atomic absorption spectrometry, neutron activation analysis (instrumental and with radiochemical separation), isotope dilution mass spectrometry, and X-ray fluorescence spectrometry - mean concentrations of total Se ± one standard deviation (68% confidence intervals for one future observation) were found to be 91 ± 7 and 93 ± 7 µg/L for the reconstituted pools Seronorm 102 and 103, respectively. In this study, analytical procedures based on atomic absorption spectrometry (and in particular those employing hydride generation) seemed to be prone to negative biases. It is hoped that these findings will stimulate the establishment of these sera as control materials for the use in clinical laboratories.

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