Characterization of a bovine serum reference material for major, minor, and trace elements

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suggested method is supported by the fact that if variable amounts of Py and/or Py5P are added to several samples, the difference in ΔI_f is the same between samples with or without serum if the same amount of Py and/or Py5P has been added, with an accuracy of 1%.

CONCLUSIONS

Three FIA methods which permit the determination of Py and Py5P over a wider concentration range are proposed.

The sequential method has a low determination limit due to the fact that this configuration does not involve splitting of the sample plug, as is the case with the other two configurations, allowing its application to serum samples. This determination has the following features:

The pretreatment of the sample is very simple, in contrast to other nonchromatographic methods in the literature for determination of both compounds (*10*).

The sampling rate (30 samples per hour) is much faster than for other methods suggested for the determination of one of these substances in biological samples (32 samples per day (*12*)).

The recovery is similar to the values found in the literature (*9*, *11*).

Registry No. Pyridoxal, 66-72-8; pyridoxal 5-phosphate, 54-47-7; 4-pyridoxolactone, 4753-19-9; 4-pyridoxic acid 5-phosphate, 954-27-8.

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Characterization of a Bovine Serum Reference Material for Major, Minor, and Trace Elements

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A bovine serum pool was collected, homogenized, and aliquoted under carefully controlled conditions to minimize contamination with trace elements. The composition was established for metals present at high levels (Na, K, Ca, Mg), low levels (Fe, Cu, Zn), and trace levels (Al, Co, Cr, Mn, Mo, Ni, Se, V), by the authors and 12 collaborating laboratories, using several analytical methods. Excellent agreement was obtained in almost all cases, permitting the authors to suggest "recommended" concentrations and estimated uncertainties for these 15 metals. Matrix components, physical properties, and trace element levels of bovine serum are very similar to those of human serum. The Al, Co, and Mn levels are slightly higher, the Mo level substantially higher, and the Se level lower than those usually observed in human serum. This material will be made available to the scientific community through the National Bureau of Standard's Office of Standard Reference Materials as Reference Material 8419.

There is currently a great deal of interest in determining trace elements in biological samples. In addition to the 15 or so trace elements known or believed to be essential to man and animals and thus of interest to nutritionists, medical practitioners, and veterinarians, several others are of interest from the standpoint of toxicology. In investigating the role of trace elements in human nutrition, diseases, and toxicology, one is usually limited to readily accessible samples such as hair, nails, perspiration, saliva, urine, feces, and the various blood components (whole blood, plasma, serum, platelets, red cells, etc.). These various biological materials can present some serious challenges during the analytical procedures. Serum, for example, contains high levels of both proteins and inorganic salts. Often the organic matter must be destroyed (without contaminating the sample) and the remaining salts either removed or compensated for if they cause any matrix effects during the analysis.

Numerous methods and procedures have been used to measure trace elements in these biological materials. However, in many cases, the reported values for the same element in the same substances vary over a wide range. This is often indicative of sample contamination, but in many cases it is also due to inaccuracy of the analytical method. Many reported methods and procedures do not include adequate verification of their accuracy (*1*).

One means of accuracy verification is to analyze the same sample or material for the same element by two independent

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methods. Since many laboratories do not have two independent methods available, an alternate way is to analyze a material (as closely resembling the sample as possible) for which the analyte level has been established by two independent methods. Unfortunately, very few suitable materials exist at the present time, although there is a great deal of activity in this area by several organizations.

We describe here the characterization of a bovine serum material for several elements using independent methods and which will be made available to the scientific community, through the U.S. National Bureau of Standards. NBS will designate this material as RM 8419, in accord with the terminology of the International Organization for Standardization, ISO, which defines a Reference Material, RM, as "a material or substance, one or more properties of which are sufficiently well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials". This serum pool was collected and stored under carefully controlled conditions and was initially to serve as an internal quality control substance for day-to-day determinations of chromium and selenium in serum. This material was subsequently used to investigate the levels of several other trace elements by simultaneous, multielement atomic absorption spectrometry (2). The need to verify the accuracy of these several elements led to collaborative efforts providing at least two independent methods for each element reported.

Bovine serum was selected because it can be collected in large quantity under conditions that minimize contamination with adventitious trace elements. The composition and physical properties of bovine serum—major, minor, and trace element levels, viscosity, moisture, specific gravity, etc.—are virtually identical with those of human serum. Being a liquid, it can be easily homogenized. Finally, the risks associated with handling human serum, such as hepatitis, AIDS, etc., are minimized.

EXPERIMENTAL SECTION

Bovine Serum Pool 7292. Details of the collection, preparation, and storage of the bovine serum material are described in detail elsewhere (3). Blood was collected in a plastic pail directly from the severed carotid artery of an unconscious dairy cow, then immediately poured into plastic centrifuge bottles. After clotting, the blood was centrifuged and the serum collected and centrifuged again to remove any remaining red blood cells. All of the serum was pooled in a large plastic container, stirred for 1 h, aliquoted by plastic siphon tube into sterile polypropylene tubes (4.2–4.3 mL/tube), and the tubes were capped, labeled, bagged with ice cubes to maintain 100% humidity, and stored at −20 °C. All operations except the initial collection were carried out in a Class 100 area of a clean room facility. Tubes were selected at random throughout the collection run for analysis. A number of empty tubes were tested with both water and 0.1 M HCl for cleanliness by graphite furnace atomic absorption spectrometry for several elements and were found to be free of detectable contamination.

Methods. The various methods used by the authors and collaborators are shown in Table I. Here the methods are arranged by the various techniques used, for the elements shown. It would be impractical to describe the detailed procedures used in each case, so these are referenced, where possible, to the literature. In some instances, unpublished results are indicated, which refer to methods not yet published. Specific details regarding the methodology may be obtained from the respective authors.

RESULTS AND DISCUSSION

A comparison of the reported major (matrix) components of human and bovine serum is made in Table II. Here it can be seen that the two materials are similar. The measured specific gravity of each is identical, as is the moisture content.

The elements measured in this study (Table I) fall into three categories, namely, major (Na, K, Ca, Mg), minor (Cu, Fe, Zn), and trace (Al, Co, Cr, Mn, Mo, Ni, V, Se, As, Rb, Cd, Cs). When most of these were first compared using the SIMAAC technique (2), the results shown in Table III were obtained. Here again, the striking similarity between human and bovine serum is evident. The Co and V values in the bovine serum are slightly higher than the accepted literature values for human serum, but these are only semiquantitative values using the SIMAAC system. The bovine values for Al and Mn are several times higher than human, while Mo is more than an order of magnitude higher. It is not known if this represents contamination or, more likely, the diet and other material ingested by cows. Since cows are ruminants, they may well have absorptions of certain trace elements different from monogastric animals.

Homogeneity. Being a liquid substance, it should be fairly straightforward to obtain homogeneous aliquots of a bovine

Table I. Methodologies Used in Analyzing Bovine Serum Pool 7292

technique	elements	ref
colorimetry (COL)	Ca	4
	Fe	5
	Mg	6, 7
flame atomic absorption spectrometry (FAAS)	Ca, Fe, Mg	8
	Cu	9
	Zn	8, 10, 11
flame atomic emission spectrometry (FAES)	Na, K	12
	Rb	13
graphite furnace atomic absorption spectrometry (GFAAS)	Al	14
	Co, Fe	15
	Cr	16
	Cu	17
	Mn	18
	Ni	19
inductively coupled plasma atomic emission spectrometry (ICP/AES)	Al, Cd, Cu, Fe, Mn, Mo, Ni, V, Zn	20
inductively coupled plasma atomic fluorescence spectrometry (ICP/AFS)	Cu, Fe, Mn, Zn	15, 21
isotope dilution mass spectrometry (IDMS)	Cr	16
	Se	22
ion selective electrode (ISE)	Na, K	4
neutron activation analysis (NAA)	As, Co, Cr, Cs, Cu, Fe, Mn, Mo, Rb, Se, Zn	23
simultaneous multielement atomic absorption spectrometry (flame) (SIMAAC)	Ca, Cu, Fe, K, Mg, Na, Zn	24
SIMAAC (furnace atomization)	Cu, Fe, Zn	25
	Al, Co, Cr, Mn, V, Mo, Ni	2
voltammetry	Co, Cu, Ni, Zn	26
Zeeman atomic absorption spectrometry (ZAAS)	Al, Cu, Mo	11
	Cr, Zn	11, 27
	Mn, Se	11, 28

Table II. Comparison of Major (Matrix) Components in Human and Bovine Sera

component	human[a]	bovine[b]
total protein, g/L	60–80	54–70
albumin, g/L	38–50	34–50
fat, g/L	1.8–4.1	1.9–5.1
glucose, g/L	0.70–1.10	0.9–1.2
Na, mmol/L	135–148	130–142
Cl, mmol/L	98–106	93–112
P(i),[c] mg/L	30–45	31–49

[a] Reference 29. [b] Reference 30. [c] Inorganic phosphorus.

Table III. Comparison of Metals in Human and Bovine Sera Using SIMAAC (*2*, *24*, *25*)

metal	human[a]	bovine[b]
Na, mmol/L	142	141
K	4.1	5.1
Ca	2.56	2.51
Mg	0.82	0.87
Cu, mg/L	1.13	0.73
Fe	1.44	1.96
Zn	0.95	1.11
Al, μg/L	3.4	12
Co	0.3[c]	1.2[c]
Cr	0.24	0.34
Mn	0.48	2.2
Mo	0.6[c]	12
Ni	1.7	2.1
V	0.5[c]	1.4[c]

[a] Mean for 30 healthy adults. [b] Mean for 10 repetitive determinations in bovine serum pool 7292 (NBS designation: RM 8419). [c] Semiquantitative results, i.e., between the detection limit and 5 times the detection limit.

Table IV. Summary of Concentrations Obtained for Bovine Serum Pool 7292—Major Elements

	concentration, mmol/L			
method	Na	K	Ca	Mg
SIMAAC (flame)	141	5.1	2.51	0.87
FAAS			2.53	0.87
FAES	140	5.0		
ISE	141	5.1		
COL			2.42	0.70

Table V. Summary of Concentrations Obtained for Bovine Serum Pool 7292—Minor Elements

	concentration, mg/L		
method	Fe	Cu	Zn
SIMAAC (flame)	1.87	0.72	1.09
SIMAAC (furnace)	2.04	0.75	1.13
FAAS	2.04	0.76	1.08
		0.73	1.31
			1.06
			1.01
COL	1.30		
GFAAS	2.0	0.9	
ZAAS		0.64	1.01
ICP/AES	1.5	0.7	0.9
ICP/AFS	2.29	0.74	1.15
NAA	2.29	0.76	1.18
			1.04
voltammetry		0.8	1.03

serum pool. Numerous tubes of bovine serum pool 7292 were selected at random and several of the major, minor, and trace elements determined using SIMAAC and single-element GFAAS. The "within-run" percent relative standard deviation, % RSD (i.e., several tubes run the same day), by SIMAAC and flame atomization for the major elements Na, K, Ca, and Mg were 1.5, 1.8, 1.0, and 1.0, respectively. The "day-to-day" % RSD's (i.e., tubes run on different days) over a period of about $1^1/_2$ years for these same elements were 1.4, 2.7, 1.8, and 4.0, respectively. Since the long- and short-term % RSD's include homogeneity and overall precision of the methodology, the homogeneity can be no worse than these values, indicating a homogeneous material.

Similar results were obtained for the minor elements, Fe, Cu, and Zn, as well. For example, for Cu by SIMAAC with flame atomization, within-run and day-to-day % RSD's of 2.8 and 5.9, respectively, were observed while with furnace atomization, values of 1.6 and 3.0, respectively, were obtained.

Using single-element GFAAS, 10 tubes of bovine serum pool 7292 had a mean value for the trace element Cr of 0.26 μg/L with a standard deviation of only 0.02 μg/L or a % RSD of about 7. All of these results indicate that this material is homogeneous.

Results. The results obtained by the authors and collaborators for the major, minor, and trace elements in bovine serum 7292 are shown in Tables IV–VI, respectively. Each value is the mean for the reporting laboratory using the method indicated as defined in Table I. In some cases, individual values were reported, or just a mean, or just a single value, and in one case, a range.

From Table IV, there appears to be good agreement among the methods used for the major elements. For the minor elements, Fe, Cu, and Zn (Table V), the values for Cu and Zn tend to agree within about 10%, while for Fe the agreement is somewhat poorer, due primarily to two methods having somewhat lower results than the rest.

Comparing the results obtained for the trace elements (Table VI), the difficulties of measuring analytes in the microgram per liter range and below, in such a complex matrix, become more apparent. Quite acceptable agreement between the values for Co, Cr, Mn, Ni, and Se by the various methods is observed. For Al and Mo, the agreement is somewhat poorer, being about a factor of 2 between the lowest and highest value. For V, values of 1.4, 1.2 and 4.0 μg/L were reported. While the SIMAAC and ICP/AES values appear to be in good agreement, they are both semiquantitative, that is, less than 5 times the detection limit of these two methods. The 4.0 value is a little suspect, since if it were in fact this high, the other two methods would have been able to quantitate their results. This value, and the two highest Mo values, could result from the fact that the samples were only partially

Table VI. Summary of Concentrations Obtained for Bovine Serum Pool 7292—Trace Elements

	concentration, μg/L							
method	Al	Co	Cr	Mn	Mo	Ni	Se	V
SIMAAC (furnace)	12	1.2[a]	0.34	2.2	12	2.1		1.4[a]
ICP/AES	12			2.5	7.6	<4		1.2[a]
					20			
ICP/AFS				2.4				
GFAAS	20	0.9	0.28	2.9	25	2.2	23	4.0
	9			2.7		1.2		
ZAAS	9.9		0.2	3.3	17.6		17.7	
			0.31	2.5			14–18	
IDMS			0.27				15.0	
NAA		1.3	0.33	2.5	15.8		15.3	
		1.1						
voltammetry		1.47				1.61		

[a] Semiquantitative results.

Table VII. Recommended Values and Estimated Uncertainties for Bovine Serum Pool 7292 Reference Material

element	recommended value ± estimate of uncertainty
Na	141 ± 2 mmol/L
K	5.1 ± 0.2 mmol/L
Ca	2.5 ± 0.1 mmol/L
Mg	0.85 ± 0.1 mmol/L
Fe	2.0 ± 0.4 mg/L
Cu	0.75 ± 0.1 mg/L
Zn	1.1 ± 0.1 mg/L
Al	13 ± 5 μg/L
Co	1.2 ± 0.3 μg/L
Cr	0.30 ± 0.05 μg/L
Mn	2.6 ± 0.5 μg/L
Mo	16 ± 4 μg/L
Ni	1.8 ± 0.6 μg/L
Se	16 ± 2 μg/L
V	<2 μg/L

digested prior to analysis, and run against aqueous standards.

Recommended Concentrations. Considering all of the values, and what is known about the laboratories, analysts and techniques used, recommended concentrations, and estimated uncertainties can be assigned to bovine serum pool 7292. These are values with which the authors feel reasonably confortable. Naturally, these are subject to scrutiny and revision by the scientific community but form a reasonable starting point. These recommended values are indicated in Table VII. Criteria used in assigning the estimates of uncertainty are based primarily on ranges encompassing values the authors are reasonably confident of, centered near the mean and/or near the values the authors have the greatest confidence in. Reasonable experimental reproducibilities and standard deviations of the means are also considered. True, this is not a statistically meaningful way to select recommendations, but an insufficient quantity of data was available for a more statistically valid assessment.

Other Elements. Arsenic, rubidium, and cesium values by NAA were also reported. For As, two determinations gave values of 0.32 and 0.37 μg/L, for Rb, 120 and 120 μg/L, and for Cs, 0.26 and 0.17 μg/L. For Rb, a confirming value of 113 μg/L was obtained by FAES using the method of additions. Since these determinations were made by only one or two methods, we cannot recommend a value for these. These values are for information only. Cadmium was also measured by only one technique, ICP/AES. A fairly large range was observed, but it would appear that the Cd level is probably well below the microgram per liter level.

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Registry No. Na, 7440-23-5; K, 7440-09-7; Ca, 7440-70-2; Mg, 7439-95-4; Fe, 7439-89-6; Cu, 7440-50-8; Zn, 7440-66-6; Al, 7429-90-5; Co, 7440-48-4; Cr, 7440-47-3; Mn, 7439-96-5; Mo, 7439-98-7; Ni, 7440-02-0; Se, 7782-49-2; V, 7440-62-2.

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