

Certificate of measurement



4005

Frozen Human Serum – Elements and selenomethionine

Certified Reference Material LGC8211

Certified Values

Constituent	Certified value ^{1,2} (µg/kg)	Uncertainty ³ (µg/kg)
Copper	1130	33 ($k=2.07$)
Iron	496	22 ($k=2.05$)
Selenium	64.1	3.0 ($k=2.16$)
Zinc	658	33 ($k=2.09$)
Selenomethionine	25.0	1.6 ($k=2.03$)

Constituent	Certified value ^{1,2} (mg/kg)	Uncertainty ³ (mg/kg)
Calcium	87.0	2.2 ($k=2.00$)
Magnesium	20.28	0.58 ($k=2.10$)
Potassium	142.0	3.7 ($k=2.12$)

Notes:

1. The certified values are mass fractions determined using isotope dilution mass spectrometry (IDMS).
2. The certified values are traceable to the SI through the use of certified or in-house characterised pure standards (see page 3).
3. The quoted uncertainty is the half-width of the expanded uncertainty interval calculated using a coverage factor, k , which gives a level of confidence of approximately 95 %.

This material was released in 2011 as ERM[®]-DA120a. Certification was extended in 2018 to include calcium, iron, magnesium, potassium and selenomethionine and the material was renamed LGC8211.

Date of Issue: October 2018

Signed: _____
Gill Holcombe (Mrs)
for the Government Chemist



Additional Information

Constituent	Concentration ¹ ($\mu\text{mol/L}$)	Uncertainty ² ($\mu\text{mol/L}$)
Copper	18.18	0.53 ($k=2.07$)
Iron	9.08	0.43 ($k=2.05$)
Selenium	0.830	0.038 ($k=2.16$)
Zinc	10.30	0.52 ($k=2.09$)
Selenomethionine	0.1304	0.0086 ($k=2.03$)
Constituent	Concentration ¹ (mmol/L)	Uncertainty ² (mmol/L)
Calcium	2.220	0.071 ($k=2.00$)
Magnesium	0.853	0.031 ($k=2.10$)
Potassium	3.71	0.13 ($k=2.12$)

Notes:

1. The concentration and uncertainty have been calculated using the density of serum: 1.0226 kg/L, expanded uncertainty ($k=2$): 0.0203 kg/L measured at LGC and molar masses of 63.546 g/mol, 78.96 g/mol and 65.38 g/mol for copper, selenium and zinc respectively (G. Audi and A.H. Wapstra, The 1993 atomic mass evaluation, Nucl Phys A565 (1993) 1-65.). The molar masses used for calcium, iron, magnesium, potassium and selenomethionine were 40.078 g/mol, 55.845 g/mol, 24.305 g/mol, 39.0983 g/mol and 196.106 g/mol respectively (IUPAC Atomic Weights of the elements 2011. Pure Appl. Chem., Vol. 85, No. 5, pp. 1047-1078, 2013).
2. The quoted uncertainty is the half-width of the expanded uncertainty interval calculated using a coverage factor, k , which gives a level of confidence of approximately 95 %.

Material Preparation

Human serum from a single donor was prepared from blood obtained at the Royal Surrey County Hospital (Guildford, UK). Five donations were taken, with a minimum of 13 weeks between each donation. The blood was allowed to clot at room temperature, centrifuged, and the serum pipetted into an acid washed container which was then stored frozen at (-80 ± 10) °C. Each batch of serum collected was added directly into the same bottle. The 'clean' serum was mixed thoroughly and then 1.1 mL portions dispensed into 2 mL plastic screw-cap Nalgene® cryovials (Jencons, Leighton Buzzard, UK). Approximately 1000 units of the candidate reference material were produced, which were stored at (-80 ± 10) °C.

Intended Use

The primary intended use of LGC8211 is for instrument calibration and the validation and performance monitoring of methods used for the determination of calcium, copper, iron, magnesium, potassium, selenium, zinc and selenomethionine in human serum samples. It can also be used in the training and evaluation of staff.

This material is clinically relevant since it closely matches the lower level of the normal range for serum selenium in the population.

Homogeneity

The material was tested for homogeneity by analysing randomly selected samples for copper, iron, magnesium, potassium, selenium, zinc and selenomethionine using the characterisation methods described below, and ion chromatography for calcium. The material was judged to be sufficiently homogenous down to a sample size of 0.5 g as the variation between units was not significantly greater than the method variation.

Analytical Methods Used

The approach for characterisation for every property was the use of a single reference measurement procedure at LGC with confirmatory values obtained externally as described in the section Confirmatory data.

Isotope Dilution Inductively Coupled-Plasma Mass Spectrometry (ID-ICP-MS) was used for calcium, copper, iron, magnesium, potassium, selenium and zinc, and Isotope Dilution Species Specific HPLC-ICP-MS was used for selenomethionine. All the methods used were accredited to ISO/IEC 17025.

Copper, selenium and zinc quantification:

The analyses were carried out using an Agilent 7500ce ICP-MS operating in gas mode. Helium was the cell gas for copper and zinc; hydrogen was the cell gas for selenium. Each analysis comprised 10 x 100 replicate ratio measurements of the $^{63}\text{Cu}/^{65}\text{Cu}$, $^{78}\text{Se}/^{77}\text{Se}$ and $^{66}\text{Zn}/^{67}\text{Zn}$ ratio.

Primary calibration materials were purchased from NIST: SRM 3114 for copper, SRM 3149 for selenium and SRM 3168a for zinc.

^{65}Cu and ^{77}Se enriched isotope spikes were obtained as metals from AEA Technology plc (Harwell, Didcot, Oxfordshire, UK), and dissolved in nitric acid (HNO_3). A ^{67}Zn spike was obtained as a metal from Oak Ridge National Laboratory (Oak Ridge, Tennessee, USA) and dissolved in HNO_3 . The concentrations of copper, selenium and zinc in the spikes were determined by reverse isotope dilution using the primary standard as calibrant.

Human Serum Certified Reference Material BCR 637 was used as a matrix quality check for selenium and zinc. Seronorm Level 1 was used as a matrix quality check for copper. Single element standard solutions were used as a calibration check: selenium and zinc from Ultra Scientific (Kingstown, RI, USA), copper from Romil (Cambridge, UK).

Ten units of the material were defrosted and equilibrated to room temperature. Each unit was analysed in duplicate with 0.3 g of a mixed spike (^{65}Cu , ^{77}Se , ^{67}Zn) being added to 0.5 g of serum and diluted to 10 g with 1 % HNO_3 .

Calcium, iron, magnesium and potassium quantification:

A ThermoScientific Element sector field ICP-MS (Waltham, Ma, USA) was used for the analysis with a Cetac 500 autosampler (Teledyne CETAC Inc., Omaha, NE, USA).

After an appropriate amount of the enriched material (^{42}Ca , ^{57}Fe , ^{25}Mg and ^{41}K) was added to approximately 0.3 g of serum, the sample was diluted to approximately 10 g with 0.5 % HNO_3 and thoroughly mixed. Blends were equilibrated at room temperature for over 3 hours. Further dilutions were performed volumetrically with 0.5 % HNO_3 prior to analysis.

The primary standards used as calibrants for calcium, iron, magnesium and potassium were SRM 3109a, SRM 3126a, SRM 3131a and SRM 3141a respectively. They were purchased from NIST (Gaithersburg, USA) and diluted gravimetrically to appropriate concentrations. These standards were checked against in-house gravimetrically prepared pure metals/salts, namely; CaCO_3 (Alfa Aesar B16U048), iron metal slugs (Alfa Aesar J13W053), magnesium metal slugs (Alfa Aesar MG 397016PF) and KCl (Alfa Aesar 22325). CaCO_3 and KCl were simply dissolved in 2 % HCl and 1 % HNO_3 (Ultrapure, Romil, UK), respectively. Iron and magnesium were digested on a hot plate using

concentrated HCl and HNO₃ for iron and 20 % HCl for magnesium. The enriched spikes were prepared in the same manner as the natural materials.

Additionally, certified reference materials were used for quality control purposes, namely; ERM[®]-DA251a - Human Serum (for calcium, magnesium and potassium) and NIST SRM 1598a-Animal Serum (calcium and iron).

With every batch, two standard addition blends were prepared using randomly selected vials.

Nine units of the material were analysed in duplicate.

Selenomethionine quantification:

HPLC-ICP-MS measurements were performed using a 1200 HPLC system from Agilent Technologies (Cheshire, UK) for chromatographic separation and an Agilent 7700 ICP-MS for element-specific detection. Reverse phase ion-pairing (RP-IP) HPLC was performed using an Agilent Zorbax Rx C8 column (250 x 4.6 mm i.d. 5 µm particle size) with a 2 % (v/v) methanol mobile phase containing 0.1 % (v/v) trifluoroacetic acid (Sigma Aldrich).

After an appropriate quantity of ⁷⁶SeMet solution was added to approximately 0.3 g of serum, the samples were subject to enzymatic hydrolysis using a mix of protease and lipase in Tris-HCl (pH 7.5) with 0.02 % DL-dithiothreitol (DTT). The addition of DTT helps to protect the free selenomethionine fraction from oxidation during enzymatic extraction. Incubation was carried out at 37 °C for 22 hours in the dark. Following centrifugation, the supernatant was filtered through 0.45 µm PTFE syringe filters and injected directly onto the HPLC column without any further dilution.

Two independent selenomethionine standards with natural isotopic composition were purchased from Sigma-Aldrich (purity ≥99 %, St. Louis, MO, USA) and LGC Mikromol GmbH (purity 99.57 % assay Luckenwalde, Germany). The total selenium concentration of the microwave digested stock calibration standards was measured by ICP-MS with isotope dilution analysis against inorganic calibration standards. In addition, purity and distribution of a concentrated standard solution was checked using HPLC-ICP-MS.

The ⁷⁶Se enriched selenomethionine spike material was purchased from IsoSciences (King of Prussia, PA, USA). The concentration of selenium in the spike was determined by reverse isotope dilution using the natural standard as calibrant.

Within each batch at least two blanks, one blend of an independent standard solution, a quality control serum (BCR-637) and a spiked serum sample were prepared.

Twelve units of the material were analysed in duplicate.

CONFIRMATORY DATA

The Royal Surrey County Hospital organises a proficiency testing scheme, the Trace Element Quality Assurance Scheme (TEQAS) in which over 100 laboratories participate. Serum ERM[®]-DA120a (now LGC8211) was used for one round of the TEQAS scheme for the determination of copper, selenium and zinc, in which 50 laboratories participated. The results are shown in the table below.

TEQAS results

Constituent	Number of results	PT Assigned value		Standard deviation	
		$\mu\text{mol/L}$	$\mu\text{g/kg}$	$\mu\text{mol/L}$	$\mu\text{g/kg}$
Copper	47	17.6	1096	1.5	93
Selenium	33	0.794	61.3	0.076	5.9
Zinc	46	9.9	632	2.0	128

The certified values obtained at LGC for calcium, iron, magnesium, potassium and selenomethionine agreed with those obtained by National Institute of Metrology of China (NIM) within their respective expanded uncertainties as can be seen in the table below.

NIM results

Constituent	Results - NIM	Units	Standard uncertainty	Expanded uncertainty	k
Calcium	87	mg/kg	0.7	1.4	2
Iron	500	$\mu\text{g/kg}$	14	28	2
Magnesium	20.6	mg/kg	0.2	0.4	2
Potassium	142.9	mg/kg	1.5	3	2
Selenomethionine	26.4	$\mu\text{g/kg}$	0.7	1.4	2

Stability

Due to their stable chemical nature, no changes were expected in the concentration of total calcium, copper, iron, magnesium, potassium, selenium and zinc during the life of the material when stored under the recommended conditions (see Storage section). Regarding selenomethionine, a long-term stability study was conducted over six months that showed no evidence of temperature-mediated instability. Moreover, no significant change in its content was observed in tests carried out two years after the certified value had been assigned. The material will continue to be monitored under the post-certification stability monitoring programme in place at LGC. This material is shipped with dry ice to ensure it remains frozen during transit.

Analytical Method Used for the Determination of Density

The density of the serum was determined in order to convert the certified values to molar concentrations. High purity water and 10 serum vials were placed in a water bath at $(20 \pm 0.02) ^\circ\text{C}$ for 2 hours. An Eppendorf Multipipette was calibrated, using the high purity water and a calibrated balance, to dispense 0.5 mL. The weight of 0.5 mL of serum was determined by dispensing 0.5 mL portions into a pre-weighed empty container. This process was repeated 10 times for water and 10 times for the serum (each from a different serum vial).

Commutability

The commutability of LGC8211 was evaluated for calcium, iron, magnesium and potassium, according to guidelines published by Clinical and Laboratory Standards Institute (CLSI) in Guide EP30-A.

The concentration of those elements was determined by nine clinical laboratories using routine methods, and by LGC using the ID-ICP-MS reference method on 3 units of LGC8211 and on twenty pooled patient samples used in a Proficiency Testing (PT) scheme run by WEQAS (Cardiff, UK).

The material was found to be commutable for the determination of calcium, iron, magnesium and potassium for the following methods:

- Ca: Arsenazo III, indirect ion-selective electrode, NM-BAPTA and CPC
- Fe: Ferrozine
- Mg: Calmagite and Xylidyl blue
- K: Indirect ion-selective electrode

Safety Information

The material is of human origin and should be handled with adequate care. The material was tested for HIV ab, Hep B Surface Ag and Hep C. All were found to be negative.

For further details refer to the safety data sheet.

Storage Conditions and Shelf Life

The material should be stored at $(-20 \pm 5) ^\circ\text{C}$ in the original closed vial before use.

This certificate is valid for 3 months from the date of shipment provided the sample is stored under the recommended conditions.

Instructions for Use

Prior to use, the material should be thoroughly thawed by equilibration at room temperature for at least 2 hours, and mixed by inverting the vial several times. LGC has no data on the effect of further freezing and thawing of the material before use, and therefore it is recommended that, after opening, the material is stored at refrigerator temperature $(5 \pm 4) ^\circ\text{C}$, and used within 1 month.

The minimum amount of sample to be used is 0.5 g.

Metrological Traceability

The certified values for total copper, selenium and zinc are traceable to the SI through the use of NIST standards SRM 3114, SRM 3149 and SRM 3168a, respectively.

The certified values for total calcium, iron, magnesium and potassium are traceable to the SI through the use of NIST standards SRM 3109a, SRM 3126a, SRM 3131a and SRM 3141a respectively.

The certified value for selenomethionine is traceable to the SI through the use of an in-house characterised pure standard. The in-house assigned purity value is traceable to the SI through the use of SRM 3149 and through appropriate calibration and control of the instrumentation used in accordance with the requirements of ISO/IEC 17025 for calibration. LGC's capability to produce SI traceable selenium speciation measurements has been verified by participation in key comparison studies organised by the Inorganic Analysis Working Group of the Consultative Committee for Amount of Substance (CCQM, <http://www.bipm.org/metrology/chemistry-biology/>).

Unit Number

Date of Shipment

Legal Notice

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