TÜBİTAK
ULUSAL METROLOJİ ENSTİTÜSÜ

Certificate of the Reference Material

Name of the Material : Amino acids in lyophilized plasma

Material Code : UME CRM 1314

Issue Date : 31.05.2019

Revision Date : 11.10.2021 (Revision history can be found on the last page)

Validity Period of the Certificate : 12 months from the sales date

Certified Values :

Parameter (CAS No)	Mass Fraction (mg/kg)		Molar Concentration[3] (µmol/L)	
	Certified Value[1]	Uncertainty[2]	Certified Value	Uncertainty
Alanine (56-41-7)	32.6	1.7	383	20
2-Aminoadipic acid (1118-90-7)	5.18	0.92	33.7	6.0
2-Aminobutyric acid (1492-24-6)	1.65	0.23	16.8	2.4
Anserine (584-85-0)	12.2	2.9	53	13
Arginine (74-79-3)	15.13	0.93	90.9	5.6
Asparagine (70-47-3)	12.9	2.5	102	20
Aspartic acid (56-84-8)	2.42	0.28	19.0	2.2

[1] Certified values are the mean of six measurement results obtained from two units (total of 18 measurements with triplicate instrumental repetitions) of the CRM by ID-LC-MS technique. The certified values and the uncertainties are traceable to the International System of Units (SI) through a calibration hierarchy using high purity materials of each parameter that were value-assigned using TÜBİTAK UME qNMR purity assessment procedure.

[2] The expanded uncertainty of certified value includes characterization, homogeneity, stability components and is stated as the standard uncertainty of measurement multiplied by the coverage factor $k = 2$, which for a normal distribution corresponds to a coverage probability of approximately 95%. The standard uncertainty of measurement has been determined in accordance with GUM "Guide to the Expression of Uncertainty in Measurement".

[3] Certified values and the uncertainties in molar concentrations are calculated from the mass fraction (mg/kg) using density of the reconstituted material (mean: 1.04702 g/mL, SD: 0.00481 g/mL, n = 15) measured at 22 °C and molecular weight of the analyte.

TÜBİTAK UME, as a reference material producer, has been accredited by TÜRKAK according to TS EN ISO 17034 with the accreditation number AB-0001-RM.

Sales Date

Dr. Mustafa ÇETİNTAŞ
Director

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Certified Values (*Continued*) :

Parameter (CAS No)	Mass Fraction (mg/kg)		Molar Concentration[3] (µmol/L)	
	Certified Value[1]	Uncertainty[2]	Certified Value	Uncertainty
Beta-alanine (107-95-9)	8.5	1.4	100	17
Citrulline (372-75-8)	15.8	1.2	94.4	7.2
Cystathionine (56-88-2)	2.94	0.11	13.85	0.52
4-Aminobutyric acid (56-12-2)	1.57	0.21	15.9	2.2
Glutamic acid (56-86-0)	12.0	1.9	85	14
Glycine (56-40-6)	21.2	1.8	296	26
Histamine (51-45-6)	1.31	0.15*	12.3	1.5*
Histidine (71-00-1)	17.7	1.2	119.4	8.1
Hydroxyproline (51-35-4)	5.24	0.97	41.8	7.8
Isoleucine (73-32-5)	9.9	1.9	79	16
Leucine (61-90-5)	18.6	1.3	148	11
Lysine (56-87-1)	25.7	3.7	184	27
N-methylhistidine (332-80-9)	7.2	1.2	44.6	7.5
Ornithine (70-26-8)	19.7	1.7	156	14
Phenylalanine (63-91-2)	13.56	0.90	86.0	5.7
Proline (147-85-3)	29.4	2.8	267	26
Serine (56-45-1)	9.73	0.73	96.9	7.3
Threonine (72-19-5)	12.3	0.7	108.1	6.2
Valine (72-18-4)	31.1	1.5	278	14
Ethanolamine (141-43-5)	1.81	0.58	31	10
Creatinine (60-27-5)	12.50	0.67	115.7	6.2
Sarcosine (107-97-1)	0.94	0.12	11.1	1.5

[1] Certified values are the mean of six measurement results obtained from two units (total of 18 measurements with triplicate instrumental repetitions) of the CRM by ID-LC-MS technique. The certified values and the uncertainties are traceable to the International System of Units (SI) through a calibration hierarchy using high purity materials of each parameter that were value-assigned using TÜBİTAK UME qNMR purity assessment procedure.

[2] The expanded uncertainty of certified value includes characterization, homogeneity, stability components and is stated as the standard uncertainty of measurement multiplied by the coverage factor $k = 2$ (*except Histamine, $k = 2.32$), which for a normal distribution corresponds to a coverage probability of approximately 95%. The standard uncertainty of measurement has been determined in accordance with GUM “Guide to the Expression of Uncertainty in Measurement”.

[3] Certified values and the uncertainties in molar concentrations are calculated from the mass fraction (mg/kg) using density of the reconstituted material (mean: 1.04702 g/mL, SD: 0.00481 g/mL, $n = 15$) measured at 22 °C and molecular weight of the analyte.

Informative Values

Parameter (CAS No)	Mass Fraction (mg/kg)		Molar Concentration[3] (µmol/L)	
	Assigned Value[1]	Uncertainty[2]	Assigned Value	Uncertainty
Argininosuccinic acid (2387-71-5)	12.8	2.0	46.0	7.3
3-Methylhistidine (368-16-1)	9.6	1.7	59	11
2-Aminopimelic acid (3721-85-5)	1.06	0.21	6.3	1.3
3-Aminoisobutyric acid (144-90-1)	1.78	0.39	18.1	4.0
Methionine (63-68-3)	7.52	0.98	52.8	6.9
Tyrosine (60-18-4)	5.46	0.37	31.6	2.2
Tryptophan (73-22-3)	7.1	2.2	36	12
N-Acetyltyrosine (537-55-3)	45.4	7.3	213	35

[1] Assigned values are the mean of six measurement results obtained from two units (total of 18 measurements with triplicate instrumental repetitions) of the CRM by LC-MS technique.

[2] The expanded uncertainty of assigned value includes characterization, homogeneity, stability components and is stated as the standard uncertainty of measurement multiplied by the coverage factor k = 2, which for a normal distribution corresponds to a coverage probability of approximately 95%. The standard uncertainty of measurement has been determined in accordance with GUM "Guide to the Expression of Uncertainty in Measurement".

[3] Assigned values and the uncertainties in molar concentrations are calculated from the mass fraction (µg/kg) using density of the reconstituted material (mean: 1.04702 g/mL, SD: 0.00481 g/mL, n = 15) measured at 22 °C and molecular weight of the analyte.

Description

The Certified Reference Material (CRM) is a solid material in an amber glass vial with screw cap produced by lyophilization of human plasma. Plasma was obtained from 48 healthy men donors. Additional information is presented in the certification report.

Intended Use

This material is intended to be used for method performance check and validation purposes of amino acid measurements in plasma by LC-MS and LC-MS/MS methods.

The material is not suitable for evaluating the accuracy of amino acid measurements in human plasma using routine *in vitro* diagnostic amino acid kit methods unless the commutability of the material is proven by the user.

Assessment of method performance can be done by comparing the measurement results with the certified values. The procedure for comparison is presented in the certification report.

Instructions for Use

All content of the unit should be reconstituted at once according to the recommended protocol presented below:

- UME CRM 1314 and deionized water should be kept at room temperature at least for one hour in the room with balance before weighing.
- Before opening, the vial should be tapped gently to table or benchtop in order to collect the whole content at the bottom.
- Screw cap is opened. The vial, with the inner cap, is placed to the balance, and tare is pressed. Unit is opened gently at vertical position by balancing the inner pressure with outer pressure. Care should be taken in order not to lose any sample on cap or elsewhere. 2750 µL of water is then added gently with a calibrated pipette into the sample. Then the inner cap is closed.
- Vial, with its content and inner cap is weighed (m).
- The average amount of water added to the analysed units at TÜBİTAK UME was

$$m_{avg} = (2.76682 \pm 0.00562)\ \text{g}\ (k = 2)$$

If m is deviating from m_{avg}, then the corrected value of the analyte concentrations can be calculated as:

$$\text{Corrected analyte concentration} = \text{Certified value} \times \frac{m_{avg}}{m}$$

The vial with water should be gently shaken with its cap and inner cap closed. If material is stacked to the inner cap, user can gently rotate the vial upside down several times to dissolve those particles. Solid particles should be inspected and mixing should be continued until all of the content is dissolved. User should perform the measurement on the dissolved material as quickly as possible. If it is necessary to keep the dissolved material for later measurements, it should be portioned into smaller volumes and stored at temperatures equal to or lower than -20 °C in dark conditions. Subsamples should be stored in the vials resistive to evaporation and sun light.

The **minimum sample intake** suggested for the reconstituted sample is **100 µL**.

Storage Conditions

The material should be stored at temperatures lower than -20 °C in a dry and dark place. This material can be safely dispatched under conditions where the temperature do not exceed 45 °C for up to one week, *i.e.* at ambient temperature without applying any cooling elements.

TÜBİTAK UME cannot be held responsible for changes that might happen to the material at customer's premises due to noncompliance with the instructions for use, and the storage conditions given in the certificate.

Safety Information

Raw material: Frozen human plasma (origin: Turkey) was tested by the manufacturer (Türk Kızılay) for Anti HCV (-), Anti HIV (-), HBsAg (-), HBV DNA (-), HSV RNA (-), HIV RNA (-), Sifilis (-). However, no known test method can offer complete assurance that the reported or any other infectious agents are absent from this material. As a matter of fact, the material should be treated as a biohazard capable of transmitting infectious disease. This human blood-based product should be handled at the Biosafety Level 2 or higher as recommended for any potentially infectious human plasma or blood specimen. The material is suitable for *in-vitro* use only. Safety Data Sheet (SDS) should be read before use.

Participants

The laboratory that participated to the characterization study is given in the table below.

Laboratory	Address
TÜBİTAK UME	TÜBİTAK Gebze Yerleşkesi, Barış Mahallesi, Dr. Zeki Acar Caddesi No.1, 41470 Gebze - Kocaeli / Türkiye

Methods and/or Techniques Used for the Determination of the Certified Values

Method and technique used in characterization study is given below.

Method/Technique	Parameter
Isotope Dilution Liquid Chromatography Mass Spectrometry (ID-LC-MS)	Alanine, 2-aminoadipic acid, 2-aminobutyric acid, Anserine, Arginine, Asparagine, Aspartic acid, Beta-alanine, Citrulline, Cystathionine, Gamma-aminobutyric acid, Glutamic acid, Glycine, Histamine, Histidine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, *N*-methylhistidine, Ornithine, Phenylalanine, Proline, Serine, Threonine, Tyrosine, Valine, Ethanolamine, Creatinine and Sarcosine.

Commutability

The intended use of this reference material is to check method performance and validation of amino acid measurements in plasma with LC-MS and LC-MS/MS methods.

Commutability of the material, with routine in vitro diagnostic amino acid kit methods, has not been assessed. The user would need to assess the commutability, if UME CRM 1314 is going to be used for evaluating the accuracy of routine *in vitro* diagnostic amino acid kit methods.

Revision History

Date	Remarks
31.05.2019	First issue.
12.02.2021	Intended use and commutability information is revised. Anserine, Asparagine, Glutamic acid, Leucine, Serine and *N*-Acetyltyrosine assigned values and uncertainties are revised. U_{CRM} for histamine is recalculated for k = 2.32. Argininosuccinic acid is moved from Certified Values to the Informative Values section.
28.05.2021	CRM uncertainty values are revised. Methionine and Tyrosine are moved from certified values to the Informative values section.
11.10.2021	Certificate is updated due to changes in the format of certificate for reference materials.