Analyst

Paper

Analysis of soluble or titanium dioxide derived titanium levels in human whole blood: consensus from an inter-laboratory comparison

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Table S 1 Whole blood sample collection; number of vials and estimated collection volume

Date taken	Fasted. Number of vials	Non-fasted. Number of vials	Fasted. Volume (mL)	Non-fasted. Volume. (mL)
13/11/2015	7	-	63	-
16/11/2015	5	-	45	-
18/11/2015	5	5	45	45
27/11/2015	4	-	36	-
30/11/2015	10	-	90	-
01/12/2015	5	-	45	-
02/12/2015	-	5	-	45
			Fasted	Non-fasted
	Total pooled	volume (mL)	324	90

Table S 2 Sample preparation and quality control for L1

L1 – ICP-MS/MS	
Reagents	Ammonia solution 0.2% (NH_3) supplied by Romil Ltd (Cambridge, UK), Triton® X-100 0.01% supplied by Fisher Scientific (Loughborough, UK), EDTA 0.01%. Sc, Ga, Y, Ge at 20ppb supplied by Inorganic Ventures; used as a universal diluent for samples and standards (1 in 15 dilution).
Sample handling	Whole blood samples were stored frozen at -20°C until analysis, thawed and equilibrated to laboratory temperature before sampling. Sample tubes were shaken gently to mix it thoroughly prior to opening.
QC	Custom Whole Blood control manufactured by UTAK Labs USA. Lot No. A5541 3 Levels 3, 15 & 30 µg/L
Calibration type	Custom Aqueous Calibration VWR Lot No. D13872
Standards	Commercial bespoke Calibrators from VWR
Dilution	Standards, samples and QCs materials were all diluted 1:15 fold with the diluent.
Diluent, IS add.	0.01%EDTA, 0.01%Tritonx100, 0.20% NH_3 with internal standards of Sc, Ga, Ge and Y at 20ppb (typically stick with Ga)
Rinse/Wash solution	1% NH_3 sol.
Calibration verification	N/A

Table S 3 Sample preparation and quality control for L2

L2 – ICP-MS/MS	
Reagents	Triton® X-100 supplied by Fisher Scientific (Loughborough, UK). Single standard TraceSELECT 1000 mg/L titanium and germanium were supplied by Fluka Analytical (Buchs, CH). Blank human blood was collected in house under Method development ethics. For nanoparticle spike a TiO2 dispersion from Sigma (7000347) was used.
Sample handling	Whole blood samples were stored frozen at -20°C until analysis. Samples were allowed to reach room temperature while mixed on a roller mixer before analysis. Seronorm TM Trace Elements in Whole Blood level 1, Lot 1103128 (add. approx. value: Ti=14µg/L) and Level 2, Lot 1406264 (add. approx. value: Ti=10.3µg/L) from Sero AS (Billingstad, Norway). Additional QC were spiked Seronorm standards and 3 spiked samples, which were selected at random. All spiked QCs were spiked at 10µg/L and analysed once per batch of 20 samples.
Calibration type	External matrix matched calibration
Standards	Using the 1000 mg/L single standard of titanium a 200 µg/L single standard stock solutions was prepared. Working standard levels: 1, 5,10, 20, 30, 50
Dilution	Standards, samples and QCs materials were all diluted 20 fold with the diluent. All standards were matrix matched with blank human blood.
Diluent, IS add.	Diluent containing 5 µg/L Ge internal standard: 0.005% v/v Triton® X-100
Rinse/Wash solution	0.005% v/v Triton® X-100
Calibration verification	A 10 µg/L calibration standard is analysed after each set of 10 samples.

Table S 4 Sample preparation and quality control for L3

L3 – SQ-ICP-MS	
Reagents	Ammonia solution (NH_3) supplied by Romil Ltd (Cambridge, UK), Triton® X-100 supplied by Fisher Scientific (Loughborough, UK). Single standard BDH ICP-MS grade 1000 mg/L titanium and germanium were supplied from VWR (Lutterworth, UK). Blank horse blood supplied from TCS Biosciences (Buckingham, UK).
Sample handling	Whole blood samples were frozen at -20°C upon receipt until analysis. Samples were allowed to reach room temperature, then mixed on a roller mixer before analysis.
QC	Quality control material was Seronorm TM Trace Elements in Whole Blood level 1, Lot 1103128 from Sero AS (Billingstad, Norway). No certified range, mean concentration of 14 µg/L.
Calibration type	External matrix matched calibration
Standards	Using the 1000 mg/L single standard of titanium a 10 mg/L and 1 mg/L single standard stock solutions were prepared. Working standard levels: 0.5, 2.5, 5, 25, 50, 125, 250, 500 and 1250
Dilution	Standards, samples and QCs materials were all diluted 50 fold with an alkaline diluent. All standards were matrix matched with blank horse blood.
Diluent, IS add.	Diluent containing 800 ng/L Ge internal standard: 1% v/v ammonia and 0.1% v/v Triton® X-100
Rinse/Wash solution	0.025% v/v ammonia solution and 0.05% v/v Triton® X-100.
Calibration verification	A 50 µg/L calibration standard is analysed at the beginning and end of the analytical run in addition to every 10 samples

Table S 5 Sample preparation and quality control for L4

L4 – HR ICP-MS	
Reagents	TMAH (electronic grade from Alfa Aesar, Ga and Ge internal standard 1000ppm from Alfa ,TritonX-100 from Romil. MiliQ system used for water. Equine horse serum for calibration from Selbourne biologicals with concentration assigned by repeated standard addition calibration.
Sample handling	Samples stored at -20°C until analysed. Samples mixed by repeated inversion and by vortex mixing before analysis.
QC	Seronorm L1 batch 140263 (10.3 µg/L) L2 batch 1103129 (15 µg/L) and L3 batch 1112691 (12.8 µg/L) and ClinCheck (Recipe, Germany) batch 445 with user assigned concentrations of 0.78 (L1), 0.809 L2) and 1.01 (L3) µg/L).
Calibration type	Matrix matched calibration
Standards	Standards prepared in matrix matched serum or whole blood with base concentrations assigned by standard addition analysis
Dilution	Samples , standards and Qc diluted 1:20 in diluent as below
Diluent, IS add.	Diluent contains 0.5% TMAH and 0.005% Triton with Ge and Ga added to 5 µg/L.

Rinse/Wash solution	As sample diluent without IS added.
Calibration verification	A 50 µg/L calibration standard is analysed at the beginning and end of the analytical run in addition to every 10 samples

Table S 6 Sample preparation and quality control for L5

L5 – ICP-OES	
Reagents	Single element stock solutions (1000 mg/L) of Ti and Y traceable to NIST (Certipur, Merck) were used to prepare calibration standards. All solutions were prepared in dilute HNO_3 (0.5 % v/v) prepared using trace element free concentrated acid (Primarplus, Trace Element Grade, Fisher) and reverse osmosis deionised water >18.2 MΩ cm resistivity (Millipore, Synergy System). Prior to the analysis of patient samples, a solution of Zn (2 mg/L) traceable to NIST (Certipur, Merck) was used to check the system performance, as recommended by the instrument manufacturer.
Sample handling	Whole blood samples were frozen at -20 °C upon receipt at the SAS Trace Element Laboratory, Guildford until analysis. Samples were allowed to reach room temperature, then mixed on a roller mixer before analysis.
QC	The IQC materials used were prepared in house and values assigned using high-resolution ICP-MS in HR mode (thanks to Dominique Debellis, Thermo Scientific). The IQC materials were prepared from equine whole blood in EDTA (TCS Biosciences Ltd.) and bovine calf serum (Selbourne Biological Services). Commercially available serum and blood reference materials (Seronorm L2, Sero) and a candidate certified reference material (LGC8276, LGC) were also used.
Calibration type	External calibration. The instrument software generated a linear regression fit for the data using an unweighted linear equation and provided values for the slope, intercept and correlation coefficient.
Standards	Intermediate Ti standards with concentrations of 0, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 µg/L (microgram per litre) were prepared in reverse osmosis water by dilution from an intermediate stock standard (10 mg/L). The calibration standards were made from these by diluting 1:5 with the diluent used for the samples, so that the internal standard concentration was the same in both standards and samples. The blood and serum IQC specimens were also diluted 1:5 with the same diluent.
Dilution	1: 5 in 0.5% v/v HNO_3 with Y as internal standard at 8 µg/L (diluent); the samples were centrifuged to remove precipitate
Diluent, IS add.	0.5% v/v HNO_3 with Y as internal standard at 8 µg/L
Rinse/Wash solution	2% v/v nitric acid and 0.5% v/v Triton® X-100
Calibration verification	The 5th calibration standard (8µg/L, dil.1:5) was analysed at the beginning of the analytical run in addition to every 5 samples

Table S 7 Sample preparation and quality control for L6

L6 – SQ ICP-MS	
Reagents	Certified water was used (Trace Metals 3, RTC, QC1448). All chemicals used in the study were of analytical grade or higher. Nitric acid was used to prepare 2.0 % HNO_3 (v/v) with ultrapure water. All single element stock solutions (1000 mg/L) was delivered by SCP Science and certified for purity and concentration.
Sample handling	The sample was digested in a 50 mL reactor. When digestion finished the sample was transferred in a 50 mL tube. The reactor was washed 3 times with about 10 mL of ultra-pure water. Then, the volume was reduced to 500 µL on a hotplate at 90°C, in order to reduce HF concentration. Finally, the sample was recuperated in a 50 mL tube with 25 mL of ultrapure water.
Digestion	2g od sample were digested with 8 mL HNO_3 and 2 mL HF. Ambient temperature to 170°C in 10 min, then 25 min at 170°C. Finally, 45 min from 170 °C to ambient temperature.
Digestion system	Microwave Assisted Reaction System (MARS) Express
QC	A certified water was used (Trace Metals 3) and diluted at 0.124 µg/L and 1.24 µg/L. About 20 % of deviation were accepted. All the Blank reactors were analysed in order to verify the absence of contamination. Seronorm TM Trace Elements in Whole Blood level 1, Lot 1103128 (add.approx. value: Ti = 14 µg/L) was also used.
Calibration type	external calibration
Standards	Titanium standard solutions for ICP–MS calibration were prepared (at concentration levels of 50 to 10000 ng/L) by diluting a 10 g/L titanium standard stock solution with 2% (v/v) HNO3.
Dilution	2g in 25mL
Diluent, IS add.	An internal standard solution containing 100 µg/L of Ge was prepared by diluting a 1000 mg/L internal standard stock solution with 2% (v/v) HNO3. The internal standard was added to all samples and standard solutions.
Rinse/Wash solution	A 2 % (v/v) HNO_3 was used between two samples, to rinse injection system. All the reactors were soaked in a 20 % (v/v) HNO_3. Then a blank procedure was run in the same conditions than the samples.
Calibration verification	A certified water was used (Trace Metals 3) and diluted at 0.124 µg/L and 1.24 µg/L. About 20 % of deviation were accepted.

Table S 8 Sample preparation and quality control for L7

L7 – ICP-MS/MS	
Reagents	Single standard 984 mg/kg titanium and scandium were supplied by VHG (Manchester, UK). For nanoparticle spike a TiO_2 dispersion from Sigma (7000347) was used.
Sample handling	Whole blood samples were stored frozen at -80°C until analysis, thawed and equilibrated to laboratory temperature before sampling. Sample tubes were shaken gently to mix it thoroughly prior to opening.
Digestion	Approximately 0.3 g of sample was accurately weighed into PTFE microwave digestion vessels, followed by the addition of 0 .6 g HNO_3, 0.12 g H_2O_2 and 0.015 g HF. After digestion, the digest solutions were made up to 3 g with ultrapure water (>18.2 MΩcm-1, Elga Maxima, UK) and analysed without further dilution
Digestion system	Ethos EZ microwave system with micro-sampling inserts (Milestone, Sorisole, Italy)
QC	Seronorm TM Trace Elements in Whole Blood level 2, Lot 1406264 and 1103129 (add. approx. value: Ti=10.3µg/L and Ti=15µg/L) from Sero AS (Billingstad, Norway). In addition Seronorm QC were spiked with Ti ionic and nanoparticles to check for recovery.
Calibration type	External calibration
Standards	Using the 984 mg/kg single standard of titanium a 94 mg/kg single standard stock solutions was prepared in water. From this a second dilution was prepared at 954 µg/kg. From this a third dilution was prepared at a concentration of 9µg/kg in 20% HNO_3 Ti stock, which then was used to prepare standards at 0.1, 0.5, 1, 2, and 5 µg/kg in 20%HNO_3.
Dilution	The digests were analysed directly by ICP-MS/MS with external calibration using 1 µg/kg Sc as internal standard. Dilution: 0.3g in 3g.
Diluent, IS add.	After the digestion, samples were diluted up to 3 g with ultrapure water (> 18.2 MΩcm-1, Elga, Maxima, UK) and 0.1 g of Sc was added as internal standard to have a final concentration of 1 µg/kg Sc
Rinse/Wash solution	5% v/v HNO_3
Calibration verification	A 1 µg/kg calibration standard is analysed after each set of 5 samples and the calibration curve was measured at the beginning and at the end of the run

Table S 9 Instrument settings for Labs 1-7

	Tune 3 - E. discr., V	Tune 3 - Oct bias, V	Tune 2 - E. discr., V	Tune 2 - Oct bias, V	Tune 1 - E. discr., V	Tune 1 - Oct bias, V	RF Power, W	Sample/skimmer cones	Make-up gas, L/min	Nebuliser type , flow rate mL/min	Tune 3 - Reaction gas He, NH_3	Tune 2 - Reaction gas H_2, O_2	Tune 1 - Reaction gas He, O_2
L1	-7	-5					1550	Pt/Ni		Micromist	1mL/min He, 20% NH_3		
L2	-8.0	-5.8	-8.8	-0.8	-8.0	-5.0	1550	Pt/Ni	0.17	Micromist, 0.88	1mL/min He, 3mL/min NH_3	4.5mL/min H_2, 1mL/min O_2	3mL/min He , 1mL/min O_2
L7			-7.0	-5.0			1550		0.11	Micromist, 1.13		10mL/min H_2, 1mL/min O_2	
L3					CCT Foc. Lens, V	Extr., V							KED mode – Coll. gas He
					-3.05	-108	1550	Pt/Ni, HM interface		PFA-ST neb., 1.07			4.75 mL/min
	Mirror L. Right, V	Mirror L. Left, V	Corner L., V	3rd Lens Extr., V	Pole Bias, V	Fringe Bias, V			Sheath gas, L/min				
L6	26	42	-200	-250	-0.5	-3	1400	Ni/Ni	0.19	MicroMist U-Series, 0.92			CRI mode - Coll. gas H_2 (to check)
												110 mL/min	130 mL/min CRI mode - Coll. gas He
L4													Resolution
							1250	Ni					Med. Res. (3000)
L5			Backgrd pixels		Peak pixels	Plasma view							
			2 at higher & lower λ		2	Axial	1250			Low-flow MicroMist, 0.7			