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

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Exposure to titanium (Ti), via the ingestion of pigment grade Ti dioxide (TiO₂), is commonplace for Westernised populations. It may also occur as a consequence of metal ion leaching in subjects bearing Ti-containing implants. Accurate exposure analysis requires fit-for-purpose analytical methodology, especially for true measures of baseline levels. Inductively coupled plasma (ICP) techniques are, mainly, now used for bio-analysis of Ti. Since whole blood reference materials, certified for natural low levels of Ti, are not currently available, we undertook an inter-laboratory comparison of pooled human blood from fasted volunteers +/- low level (+ ~ 2.5 µg/L) or high level (+ 10-20 µg/L) spikes of soluble Ti or TiO₂ particles. Seven established laboratories were enrolled to analyse the samples using ICP based techniques, which included at least one of ICP optical emission spectrometry (ICP-OES), high resolution ICP mass spectrometry (HR-ICP-MS), triple quadrupole ICP-MS (ICP-MS/MS) or single quadrupole ICP-MS (SQ-ICP-MS). Five laboratories diluted the blood for analysis whilst two performed acid digestion. Overall, we showed that the laboratories could, mostly, quantitatively detect modest levels of spiked Ti in blood. Markedly varying levels of Ti, however, were reported for the same baseline pooled sample (0.4 – 24.6 µg/L) and, in this study, specificity was poor for SQ-ICP-MS. Digestion of samples caused sample contamination compromising limits of detection and accuracy, whilst simple dilution had no such problem, and remained linear in response for spikes with ionic and TiO₂ particles. We conclude that measuring baseline levels of Ti in whole blood is challenging but should be readily achievable down to 0.5 – 1.5 µg/L, if sample preparation avoids contamination and instrument techniques are used that negate polyatomic or isobaric interferences from the sample matrix. We also remind those relying upon Ti bio-analytical data for their experimental outcomes that (a) spiking and recovery experiments provide information only on linearity of detection but not at all on accuracy as this will not detect constant positive errors and that (b) biological standard materials for Ti generally contain high levels of the analyte and tend to mask baseline analytical errors. Caution may be required in interpreting the findings of some published Ti/TiO₂ bio-exposure studies.

Introduction

The commonplace use of titanium (Ti), in the form of Ti dioxide (TiO₂) particles, in a range of consumer products, or as a metal in medical implant materials, makes accurate bio-analysis of this element important.

For Ti-containing medical implants it has been suggested that minute changes to Ti blood levels (in the low µg/L range) may occur and act as an early biomarker for wear debris and potential downstream implant failure¹. However, and more widely applicable to the general population, TiO₂ is a frequent additive in processed foods, pharmaceuticals, nutraceuticals and some toothpastes. In the UK the median daily intake for an adult was found to be 2.5 mg (circa 10¹¹-10¹² particles)² whereas, in the Netherlands, Rompelberg *et al*³ estimated the daily intake of TiO₂ for the Dutch population to be 0.19 mg/kg body weight (13.3mg/70kg adult/day). Almost certainly some must be absorbed but how much this is and whether there are any adverse consequences of such continual exposure and absorption is a matter of current discussion⁴⁻¹¹.

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TiO₂ is so stable that complete dissolution requires either concentrated hot sulphuric acid or hydrofluoric acid¹². During gastrointestinal digestion, whilst there may be some minor leaching of the TiO₂ particle surface, significant dissolution beyond that is not anticipated¹³. Consistent with this it has been shown that TiO₂ particles accumulate over time in immune cells of intestinal tissue, indicating that they do indeed survive gut digestion^{9, 14, 15}. As well as understanding gut exposure there is considerable interest in describing systemic exposure^{16, 17} and one major compartment in this respect is the circulation (i.e. blood stream) as this, except for the local (mesenteric) lymph nodes, connects gut absorption to tissue uptake.

To understand an increase in Ti exposure, using whole blood as a convenient sampling compartment, very accurate baseline levels need to be established. This is exemplified by some simple calculations. If an adult ingested 2.5 mg TiO₂/day, and 100% were simultaneously absorbed into 5 L of blood, then maximum circulating levels would be 500 µg/L as TiO₂. However it is likely that only up to 1% is absorbed¹⁰, so maximum circulatory levels would then be 5 µg/L as TiO₂ (3 µg/L as Ti). In ingestion studies, larger exposures than the typical daily dose may be employed but firstly the 1% absorption figure is likely to reduce with increasing oral doses and secondly *all* of the absorbed analyte will never appear in the circulation simultaneously. Indeed, in one such study, peak Ti levels in blood were only 10.7 µg/L, compared to a baseline of 1.25 µg/L, despite the ingestion of 50 mg TiO₂ (30 mg Ti) by human volunteers as a single bolus dose¹⁶. Notwithstanding, it is important to note that: (a) 5 µg/L of TiO₂, for example, in 5 L of blood, still represents ~ 10⁸– 10⁹ particles, and (b) exposure is daily for many people and that TiO₂ may target specific cell types becoming locally concentrated^{9,10}. In other words, assessing, accurately, even low µg/L levels of Ti in the circulation is worthy of careful consideration.

However, there is currently no framework, or even consensus from different experiences, of what sort of analytical approaches should facilitate the most accurate analysis. Indeed, in the literature, baseline circulating levels of Ti in humans are reported from consistently <2 µg/L¹⁸ to >45 µg/L¹⁹. If the low figures (< 2 µg/L) are true then common human exposure to Ti/TiO₂ in the circulation can be assessed. If the higher levels are true then such studies would be very difficult and/or inaccurate.

In short, it is advantageous to have fit-for-purpose, accurate analytical techniques for Ti in whole blood to allow monitoring of exposures. As can be seen in Table 1, most commonly, inductively coupled plasma mass spectrometry (ICP-MS) has been used for such measurements^{1,14, 17–64} although ICP optical emission spectrometry (ICP-OES)^{23, 26, 62, 63, 65–69} and non-ICP techniques like graphite furnace atomic absorption spectrometry (GFAAS) have also been applied^{24, 35, 69–73}. In such analyses whole blood may be diluted and analysed directly or digested before analysis albeit, as noted above, only HF and hot sulphuric acid are likely to provide complete

digestion of TiO₂¹². But, even after digestion, a variety of analytical challenges in the form of interferences may hamper accurate analysis and, due to low levels of the analyte, a sensitive technique is still required. Whereby GFAAS and ICP-OES may struggle in reaching the required sensitivity, the three main current options for ICP-MS analysis, in the form of high resolution (HR)-ICP-MS, single quadrupole (SQ)-ICP-MS and triple quadrupole ICP-MS (ICP-MS/MS) have to combat a large variety of polyatomic and isobaric interferences⁵⁰. For all mass spectrometry methods the ultimate goal is the analysis of the major isotope of titanium at mass 48 (abundance=73.98%⁵⁰), which is hampered by both polyatomic and isobaric interferences⁷⁴. HR-ICP-MS, which uses its capability of mass resolution, can remove most polyatomic interferences but not isobaric interferences as they require $m/\Delta m$ resolution of > 10,000, which exceeds HR-ICP-MS capabilities³², thus only the minor isotopes ⁴⁷Ti and ⁴⁹Ti can be used for analysis. SQ-ICP-MS and ICP-MS/MS apply some form of reaction cell to attempt interference free analysis^{21, 26, 28–30, 33–36, 64}, although reaction chemistry options depend upon instrument design and as such complete interference-free analysis cannot be assured without careful characterisation, in particular for ⁴⁸Ti.

In this work seven laboratories with established ICP bio-analytical expertise came together to compare their findings in blood Ti analyses of the same samples. Since reference materials do not exist with natural low levels of Ti in whole blood, we used pooled human blood at natural low baseline levels (i.e. from fasted volunteers) +/- low and high level spikes of ionic Ti or TiO₂ particles. Findings were then compared and a consensus reached by the authors on potential pitfalls and successful approaches to facilitate accurate baseline blood Ti analysis.

Materials and methods

Blood Collection

Whole blood was collected from volunteers by staff of the volunteer suite at MRC-EWL with appropriate ethical approval (Reference 05/Q0108/30, Cambridge 2). Blood was collected by venepuncture, using Safety-Multifly®-needle 21G with a short tube and assembled Multi-Adapter 14.120 (DEHP-free) and 9 mL S-Monovettes® K3E vacutainers (all Sarstedt, Germany). To help minimise contamination (e.g. from the metal needle) the first blood sample of 1–2 mL was discarded and only the subsequent blood draw was retained. Blood samples were frozen at -20°C.

In total, 324 mL of blood were collected from volunteers who had fasted overnight prior to the blood draw, and 90 mL were collected from volunteers who had not fasted (i.e. had eaten breakfast prior to the blood draw) (Table S1, supplementary

information). Once all the blood was collected, the samples were thawed at room temperature with continuous gentle mixing using a rotary mixer and were then pooled according to whether they were collected from fasted or non-fasted individuals.

Table 1. Reported circulating Ti levels in mammals, relevant to implant or TiO₂ exposure research, with analyses via single quadrupole ICP-MS (SQ-ICP-MS), high resolution ICP-MS (HR-ICP-MS), triple quadrupole ICP-MS (ICP-MS/MS), ICP optical emission spectrometry (ICP-OES) or graphite furnace atomic absorption spectroscopy (GFAAS). Analytical ranges and average levels (in parenthesis) are given.

Technique	species	References	Number of papers	LOD range (average) $\mu\text{g/L}$	plasma/serum levels range (average) $\mu\text{g/L}$	basal blood level range (average) $\mu\text{g/L}$	with implant/after exposure range (average) $\mu\text{g/L}$
SQ-ICP-MS	Rodent + Human	14, 19, 21-43	24	0.02 - 4.00 (0.95)	0.12 - 150 (30.60)	0.4 - 440.0 (63.7)	1.0 -- 770.0 (150.6)
	Rodent	14, 29, 34, 38	4	0.03 - 1.00 (1.00)	(25.0)	0.4 - 50.6 (17.3)	0.4 -- 50.6 (17.3)
	Human	19, 21-28, 30-33, 35-37, 39-43	20	0.02 - 4.00 (1.03)	0.12 - 150 (31.22)	0.5 - 440.0 (70.7)	2.3 -- 770.0 (160.8)
HR-ICP-MS	Rodent + Human	1, 17, 18, 20, 32, 33, 44 -64	27	0.05 - 0.8 (0.2)	0.1 - 4.5 (0.73)	0.5 - 21.0 (2.88)	0.1 - 300.0 (20.8)
	Rodent	44, 52, 53	3	0.5 - 0.8 (0.6)	0.2 - 4.5 (2.4)	2.0 - 4.0 (2.93)	1.2 - 4.2 (2.8)
	Human	1, 17, 18, 20, 32, 33, 45 -51, 54 -64	24	0.05 - 0.3 (0.13)	0.1 - 1.5 (0.54)	0.5 - 21.0 (2.87)	0.1 - 300.0 (23.08)
ICP-MS/MS	Human	65	1		0.3 - 0.8 (0.6)	-	2.0 -6.0 (4.0)
ICP-OES	Rodent + Human	23, 26, 62, 63, 65-69	9	0.9 - 34.1 (9.45)	0.5 - 50.7 (9.2)	1.0 -150.0 (29.5)	1.0 - 795 (19.9)
	Rodent	26	1	-	-	150	150
	Human	23, 62, 63, 65-69	8	0.9 - 34.1 (9.45)	0.5 - 50.7 (9.2)	1.0 -61.2 (12.3)	1.0 - 77.0 (22.1)
GFAAS	Rodent + Human	24, 35, 69-71 - 73	7	2.1 - 9.0 (4.4)	1.0 - 24.0 (6.8)	2.0 - 9.3 (4.6)	2 - 482 (143)
	Rodent	35, 70	2	(9.0)	1.4 - 24 (12.7)	(2.5)	8 - 482 (245)
	Human	24, 69, 71 - 73	5	2.1 - 6.0 (3.3)	1.0 - 9.0 (4.5)	2.0 - 9.3 (5.6)	2 - 320 (92.1)

Polypropylene containers ((500 mL PP WN (BOT3386) and 125 mL heavy duty, wide neck (10728795), Azlon® plastics (UK)) were used for pooling the fasted and non-fasted blood samples, respectively. The bottles were first rinsed five times with ultrapure water followed by an acid soak of 2 days using 5% HNO₃ (Nitric acid puriss. p.a. 2.5 L (84380-2.5 L)). This was followed by an additional five times rinse with ultrapure water before drying the containers in a clean air hood. The thawed blood samples were then pooled into the two groups noted above (i.e. fasted or non-fasted) by pouring from their collection vials directly into the appropriate container within the clean air hood.

The two bottles were closed and mixed continuously on an orbital shaker for about 1 h at room temperature. The fasted

blood pool was then aliquoted by pipetting and weighing 60 mL into a further 4 bottles of 125 mL (heavy duty, wide neck (10728795), Azlon® plastics (UK)), which had been cleaned as above, giving a total of 5 aliquots of fasted whole blood (i.e. in the four 125 mL bottles plus the original).

Soluble (ionic) titanium spike solutions were prepared from a 1000 mg/L titanium stock (12237 SIGMA-ALDRICH, Titanium Standard for ICP, TraceCERT®, 1000 mg/L Ti in nitric acid) by diluting the stock in ultrapure water. Titanium (IV) dioxide was used for particle spiking. This was a commercially available dispersion of rutile and anatase at 33-37 weight % in water with specified particle size being < 150 nm diameter (700347 Sigma Aldrich, USA). Following 5 min sonication of this stock suspension in an ultrasonic bath, more dilute dispersions were

prepared in ultrapure water. To characterise the TiO_2 dispersion used for spiking experiments, its stability over time was first assessed by dynamic light scattering (DLS). The %RSD of the Z-average (85.8 nm diameter) was established to be 3.7% for six measurements of the same dispersion over a 10 day period. The concentration of Ti in the dispersion was assessed by both ICP-OES and ICP-MS/MS, yielding 219.2 mg/L and 219.5 mg/L respectively (= 365.7 mg/L and 366.3 mg/L as TiO_2). This confirmed the original stock dispersion to be 35.45 % $\pm$ SD of 0.45 %.

Four different spiked blood samples, using the fasted blood pool, were then prepared: two with soluble (ionic) Ti and two with particulate TiO_2 . These are referred to as ionic Ti at low level (IL), ionic Ti at high level (IH), particulate TiO_2 at low level (PL) and particulate TiO_2 at high level (PH). To achieve this the four 60 mL aliquots of blood were each spiked with 100 μL of a different diluted spike solution/suspension of Ti/ TiO_2 , followed by mixing as described above, giving final spiked Ti levels as shown in Table 2.

Table 2 Final spike levels of ionic Ti or TiO_2 in fasted whole blood, $\mu\text{g/L}$

	Final added [Ti] $\mu\text{g/L}$
Ionic low (IL)	2.51
Ionic high (IH)	10.97
Particulate low (PL)	2.64
Particulate high (PH)	21.40

The four spiked and the un-spiked aliquots of fasted whole blood, as well as the pool of non-fasted whole blood, were then further aliquoted (1 mL/tube) into 5 mL sterile sample tubes (Tube, Flat Skirted Base, 57mm x 15.3mm, 5 mL, Polypropylene, Screw Cap, Sterile (60.558.001) Sarstedt, used as supplied), labelled with a unique ID, bar coded and stored frozen at -20°C until shipping. Samples were thus analysed blind, including the in-house ones (Cambridge), and the codes only broken post analysis.

For analysis, each participating laboratory was supplied with three aliquots of each of the six samples (i.e. unspiked fasted and non-fasted bloods, high and low TiO_2 -spiked fasted bloods and high and low ionic-Ti-spiked fasted bloods). An aliquot of the original Sigma Aldrich TiO_2 dispersion was also provided to each lab to enable them to perform further in-house spike experiments with the same material, should they wish to do so. Samples were shipped on dry-ice via a courier.

Instrumentation

The seven participating laboratories have been coded as L1 to L7 and each applied its own habitual in-house methodology for Ti analysis (ie no parameters or requirements were established *a priori* for sample preparation or analytical methodology other than knowing what type of instrument would be used). Instrument set ups were as follows:

L1 used an 8800 ICP-MS/MS (Agilent technologies) fitted with an Agilent Integrated Sample Introduction System for discrete sampling using a Micromist nebuliser and quartz double-pass spray chamber for sample introduction. For data acquisition, integration times were 0.1 s for $^{48}\text{Ti} \rightarrow ^{150}(\text{Ti}(\text{NH}_3)_6)$ and 0.1 s for germanium (internal standard; IS), with 100 sweeps/replicate and 3 replicates/sample. The LOQ was defined by taking a high concentration of titanium in matrix and then diluting it down with water using serial dilutions. Each concentration was analysed in triplicate and the concentration where the CV of the triplicates hits 20% was defined as LOQ (also known as a functional LOQ).

L2 operated an 8800 ICP-MS/MS (Agilent technologies) with an Agilent Integrated Sample Introduction System for discrete sampling (ISIS-DS, 1 mL Loop), Micromist nebuliser, quartz double-pass spray chamber and High Matrix-Accessory (HMI, 0.17 mL/min make-up gas). For data acquisition, Integration t were 3 s for $^{47}\text{Ti} \rightarrow ^{63}(\text{TiO})$, $^{48}\text{Ti} \rightarrow ^{64}(\text{TiO})$, $^{48}\text{Ti} \rightarrow ^{132}(\text{Ti}(\text{NH}_2)(\text{NH}_3)_4)$, $^{48}\text{Ti} \rightarrow ^{150}(\text{Ti}(\text{NH}_3)_6)$ and 1 s for the internal standard $^{72}\text{Ge} \rightarrow ^{72}\text{Ge}$, with 20 sweeps/replicate with three replicate/sample. The main transition $^{48}\text{Ti} \rightarrow ^{64}(\text{TiO})$ was used for quantification and comparing data. The other isotopes were used for internal observation only. The instrument LOQ is defined as the mean of a diluent blank plus ten times its standard deviation and the analysis LOQ equals the instrument LOQ times the dilution factor.

L3 employed an iCAP SQ-ICP-MS (Thermo Scientific, Hemel Hempstead, UK) with a PFA-ST nebuliser (Elemental Scientific, Warrington, UK) and a quartz cyclonic spray chamber. The data acquisition parameters were dwell times of 0.1 s for ^{49}Ti and 0.05 s for the internal standard ^{73}Ge , with 100 sweeps/replicate and three replicates/sample. Analysis was undertaken using KED mode using helium as the collision cell gas at a rate of 4 mL/min.

The instrument LOD was calculated as three times the standard deviation of the blank concentration. The LOQ was defined by multiplying the background equivalent concentration (BEC) (diluted horse blood calibration) times the dilution factor.

L4 utilized a HR-ICP-MS, the Element2 (Thermo Scientific, Hemel Hempstead, UK). The sample introduction system consisted of a PFA nebuliser and Helix spray chamber.

The integration times for ^{47}Ti were 1 s, and for ^{49}Ti were 2 s with 5 replicates of 20 sweeps. The LOD was defined as blank plus 3 times the standard deviation of the blank and the LOQ was the blank concentration plus 10 times the standard deviation of the blank.

L5 deployed an iCAP ICP-OES (Thermo Scientific, Hemel Hempstead, UK) using the wavelength of 336.1 nm for Ti and 371.0 nm for Y (internal standard). The sample introduction system consisted of CETAC ASX-510 autosampler, a Micromist nebuliser and a cyclonic spray chamber. The LOD/LOQ was determined by replicate (n=8) analysis of a low concentration

standard (0.2 µg/L) and multiplying the standard deviation by 3 (LOD) and 10 (LOQ). The number of sample replicates was three.

L6 used a Varian 820-MS (SQ-ICP-MS) with an optimized sample introduction system, a low (400 µL/min) glass concentric nebulizer, Peltier-cooled spray chamber with variable temperature control and a Collision Reaction Interface. The data acquisition times selected were 5 replicates of 20 scans of 10 000 µs. The signal given by ^{48}Ti was used for quantification and ^{47}Ti for confirmation. The LOQ was estimated by taking 30 repeat measurements of the blank in the same series. The mean and standard deviation of these 30 measurements were expressed in concentration and the LOQ was calculated as the sum of the mean blank concentration plus ten times the standard deviations³⁵.

L7 employed an 8800 ICP-MS/MS (Agilent technologies) combined with a Micromist nebuliser, quartz double-pass Spray chamber and High Matrix-Accessory (HMI, 0.11 mL/min make-up gas). An Integration time of 1s/isotope was applied for $^{45}\text{Sc} \rightarrow ^{61}\text{Sc}$ (internal standard) and $^{46}\text{Ti} \rightarrow ^{62}(\text{TiO})$, $^{47}\text{Ti} \rightarrow ^{63}(\text{TiO})$ and $^{48}\text{Ti} \rightarrow ^{64}(\text{TiO})$, whereby the 46 and 48 transitions were used for monitoring purposes (e.g. interferences) and the 47 transition for quantification. The LOD was calculated as the mean blank concentration (20% HNO_3) plus 3 times the standard deviation of the blank and the instrumental LOQ as the mean blank (20% HNO_3) plus 10 times the standard deviation. The procedural LOQ referred to the digestion blank and included the dilution factor of 10. Three to five replicates per sample were prepared and analysed.

Sample preparation and analytical approaches of the participating laboratories are summarised in Table 3 and described in detail in Supplementary Tables S2-S8. For the three ICP-MS/MS the difference in chosen reaction chemistry, which is defined by gas combination ratios and tuning, enables different approaches of dealing with interferences yielding different signal intensities and signal to noise ratios. A comprehensive list of instrument settings is given in Supplementary Table S9.

Results

The sample analysis results of all seven laboratories are given in Table 4 and the recoveries of spiked Ti, in the two different forms (soluble and particulate), are shown in Figure 1. Laboratories L1-L6 provided data in µg/L and L7 in µg/g. As the density of blood is close to one, the data were compared directly.

Overall, we considered the analytical data, shown in Table 4 and Figure 1, in four different ways:

Baseline Ti Levels

Different sample preparation methods, instrument type and analytical approaches, gave rise to differences in the results for pooled baseline blood samples.

The range of baseline blood levels are reported in Table 4, and ranged from <LOQ to 21.8 µg/L for non-fasted samples and <LOQ to 24.62 µg/L for fasted samples.

Response to spiked ionic Ti

The concentration of the low spike was below the LOQ for L7, and showed only 40% recovered for L3 (as a result of being close to the LOQ). However the five remaining laboratories reported percentage recoveries ranging from 88 – 110%. For the high spike, all seven laboratories reported percentage recoveries ranging from 87 – 119%. Thus, in cases where the limit of quantification was above 5 µg/L the recovery of the low spike was outside the expected +/- 20% range. However, in all other cases, the laboratories reported apparent, appropriate proportional increases in whole blood Ti levels when baseline samples were spiked with low (+ 2.5 µg/L) or high (+11 µg/L) levels of soluble Ti (Table 4, Fig 1).

Response to spiked TiO_2 particles

For the spiked TiO_2 particles, the concentration of the low spike was below the LOQ for both L3 and L7, whilst the other five laboratories achieved between 71 – 102% recoveries. For the high spike, all seven laboratories reported percentage recoveries ranging from 95 – 128 %. These results very much mirrored the pattern of results for the spiked soluble Ti in that, throughout, all laboratories reported appropriate proportional increases for particulate TiO_2 spikes (see Figure 1) bar two laboratories (L3&7) which could not adequately quantify or, in one case, even detect the low level spike.

Deviation from linearity of response

Within the range of concentrations studied, which represented potential 'real life' values, from baseline to high, all laboratories showed true linearity of response although some were more tightly clustered to the expected line of linearity (based upon spike values) than others, suggesting (anticipated) minor variance in calibration between laboratories, see Figure1.

Discussion

Undoubtedly the most pivotal and yet frequently overlooked aspect of understanding our exposure to Ti/ TiO_2 is having a precise and accurate measurement tool for bioanalysis, especially for baseline levels of Ti. This is because, as previously mentioned, TiO_2 absorption from the gut or Ti release from implants is followed by rapid and high bio-dilution (e.g. into 5L of blood) so small analytical increases

above baseline must be observable if exposure is to be properly monitored. Indeed, ours is not the first study to question the accuracy of low level bio-analytical data for Ti determination. For example, a four-way inter-laboratory comparison in 2014⁷⁵ indicated that the results compared well only when concentrations were > 4 µg Ti/g tissue and that the measurement of lower levels may be more critical and, for

which, further investigation would be required⁵¹. However, ours is the first comparative study with human whole blood - a fundamental matrix to most readily understand human exposure to Ti-containing materials.

Table 3 Summary of methodology used for participating laboratories.

L1 to L7 = Participating Laboratory 1 to 7, LOQ = limit of quantitation, BEC = background equivalent concentration, LOD = limit of detection, cps = counts per second, R value = correlation coefficient for calibration line; NH3 = ammonia mode, O2H2 = oxygen – hydrogen mode

Laboratory ID	L1	L2	L3	L4	L5	L6	L7
technique, used	ICP-MS/MS	ICP-MS/MS	SQ-ICP-MS	HR-ICP-MS	ICP-OES	SQ-ICP-MS	ICP-MS/MS
sample introduction	direct	direct	direct	direct	direct	Digestion	digestion
dilution	1:15	1:20	1:50	1:20	1:5	1:12.5	1:10
Analyte	⁴⁸ Ti -> ¹⁵⁰ Ti [NH ₃]	⁴⁸ Ti -> ⁶⁴ Ti [O ₂ H ₂] *	⁴⁹ Ti	⁴⁹ Ti	Ti, 336.1nm	⁴⁹ Ti**	⁴⁸ Ti-> ⁶⁴ Ti [O ₂ H ₂]
LOQ, µg/L	1.56	0.256	8.77	0.96	1.9	1.6	7
Intercept, cps	34	89	44.16	10	0.008	14.213	0.00045
BEC, µg/L	1.24	0.013	0.175	0.09		0.201	0.0088
Instrument LOD, µg/L	0.664	0.001	2.17	0.29	0.6	0.025	0.008
R value	0.999	0.9992	0.99949	>0.999	0.9994	0.99997	0.9997
Intensity, cps/µg/L	62	63662	59	20	0.0011	273	7476

* ⁴⁸Ti-> ¹⁵⁰Ti[NH₃], to confirm results from O₂H₂ mode

** ⁴⁷Ti, to confirm results from ⁴⁹Ti

Table 4 Summary of Participating laboratory results in $\mu\text{g/L}$ and %recovery. Values in parenthesis are $> \text{LOD}$ but $< \text{LOQ}$.

			L1	L2	L3	L4	L5	L6	L7
			MS/MS, direct	MS/MS, direct	sq MS, direct	HR MS, direct	OES, direct	sq MS, digestion	MS/MS, digestion
baseline	WB,nf	$\bar{X}$, $\mu\text{g/L}$	(0.81)	0.49	(6.20)	(0.7)	(0.6)	21.80	(7)
		SD, $\mu\text{g/L}$	(0.04)	0.08	(0.48)	(0.29)	na	4.25	na
		CV, %	4.9	16.3	na	41.4	na	19.5	na
	WB,f	$\bar{X}$, $\mu\text{g/L}$	(1.34)	0.43	(8.18)	(0.9)	(0.6)	24.62	(7)
		SD, $\mu\text{g/L}$	(0.75)	0.28	(2.82)	(0.13)	na	1.49	na
		CV, %	56.0	65.1	na	14.4	na	65.1	na
ionic spike	WB,f + $2.51\mu\text{g.kg}^{-1}$	$\bar{X}$, $\mu\text{g/L}$	4.11	2.63	9.19	3.36	3.22	27.04	(7)
		SD, $\mu\text{g/L}$	0.35	0.46	0.43	0.66	0.12	3.92	na
		CV, %	8.5	17.5	4.7	19.6	3.7	14.5	na
		%recovery	110	88	40	98	104	97	na
	WB,f + $10.97\mu\text{g.kg}^{-1}$	$\bar{X}$, $\mu\text{g/L}$	14.41	10.08	18.17	12.05	13.13	36.54	16.57
		SD, $\mu\text{g/L}$	1.42	0.42	0.33	1.60	0.53	2.29	2.17
		CV, %	9.9	4.2	1.8	13.3	4.0	6.3	13.1
		%recovery	119	88	91	102	114	109	87
nano spike	WB,f + $2.64\mu\text{g.kg}^{-1}$	$\bar{X}$, $\mu\text{g/L}$	3.24	3.12	(8.22)	3.16	2.47	26.87	(7)
		SD, $\mu\text{g/L}$	0.37	0.368	(0.22)	1.00	0.12	4.7	na
		CV, %	11.4	11.8	na	31.6	4.9	17.5	na
		%recovery	72	102	na	86	71	85	na
	WB,f + $21.40\mu\text{g.kg}^{-1}$	$\bar{X}$, $\mu\text{g/L}$	28.77	21.65	28.71	24.7	21.38	45.00	28.91
		SD, $\mu\text{g/L}$	1.41	1.68	1.59	1.12	0.30	4.25	2.97
		CV, %	4.9	7.8	5.5	4.5	1.4	9.4	10.3
		%recovery	128	99	96	111	97	95	102

Figure 1 Spike recoveries for Labs L1-L7; soluble spike = ionic Ti spikes at 2.51 and 10.97 $\mu\text{g/L}$; nano spike = spiked TiO_2 particles at 2.64 and 21.41 $\mu\text{g/L}$. Values in parenthesis are > LOD but < LOQ

It is important to note that this study was not intended to be a comprehensive analysis of all permutations in sample preparation, instrument type, instrument set up and analytical approach to deliver an 'ultimate' methodology. Instead, it drew upon the experience of a cross section of specialist laboratories in the field, undertaking bio-analysis of Ti, to develop an inter-author consensus on what type of approaches may be fruitful, or may be challenging, in achieving fit-for-purpose analyses of whole blood Ti levels. Our conclusions follow (the more general of which are established in analytical methodology *per se* but herein serve to re-emphasise the point for low level Ti bio-analysis):

- Overall, we showed that the methods used for Ti analysis of whole blood, in established laboratories,

can mostly detect low levels of spiked Ti and that they do so quantitatively. The linearity and general sensitivity of the techniques lead us to conclude that, logically, this should hold true for low (baseline levels) of Ti as well. However, the findings also illustrate that in some cases specificity is poor so, as well as detecting the Ti signal correctly and quantitatively, signals caused by the matrix were additionally 'mis-read' as Ti. The lower the true Ti level, the more an interfering matrix-derived signal will impact accuracy.

- As much as the spike-recovery results for this inter-laboratory study were encouraging they do not say anything about specificity, because any 'misread' signal will simply appear as a positive constant error

- in all analyses, demonstrating that any inaccuracy cannot be revealed through spiking experiments.
- Currently available certified reference materials tend to have high levels of Ti (> 10ppb) compared to real life samples. So these, also, may not reveal a relatively small but perhaps important background contribution from the matrix that is incorrectly attributed to a Ti signal (i.e. a large, non-physiological Ti signal could mask a small constant error).
 - It was noteworthy that the results for un-spiked human whole blood revealed low levels of Ti (< 1.5µg/L) when using HR-ICP-MS, ICP-MS/MS and ICP-OES but were elevated when using single quadrupole ICP-MS instruments in direct analysis mode, probably due to the presence of unresolved matrix interferences.
 - In addition to matrix issues for some analyses, it was also apparent that the background Ti levels were elevated when including a digestion step. Although this can be accounted for by an average subtraction (i.e. sample – average blank) it clearly reduces sensitivity for the analyte in the sample and will affect precision at low levels of Ti. The inclusion of such digestion steps for whole blood, by two of the participating laboratories, rather than the more simple 'dilute and shoot' as used by the other participants, was motivated by the fact that TiO₂ particles are extremely resistant to dissolution, and are only quantitatively dissolved by HF or hot concentrated H₂SO₄¹². However, the down side of such diligent preparation is that acids themselves have an inevitable background level of metal ions, which can increase as the acid strips ions from vessels used in the sample preparation process.
 - At least for TiO₂ concentrations up to 37.5 µg/L TiO₂ (21.4 µg/L Ti) in whole blood, there is no requirement for digestion such that, when blood is simply diluted, the particles appear to be efficiently nebulised and destroyed in the ICP's plasma, quantitatively (see Figure 1). However, this is clearly not always the case as other work has shown that sample preparations that destroy animal tissue but not the TiO₂ (e.g. HNO₃ ± H₂O₂) may not quantitatively recover Ti and may suffer with high variance of analysis³⁵. Indeed, although numbers are too small for formal analysis, it is noteworthy that in this work the recovery of the nano-spike (TiO₂) was, generally, just slightly lower than for the low ionic spike (Ti_{ionic}). This potential trend was not sufficient for us to conclude against dilution as the best approach for the samples described in this work but, clearly, each analytical scenario must be considered and characterised on its merits. In other words, at least for human whole blood carrying ≤ 21.4 µg/L Ti as TiO₂, it appears that simple dilution for analysis is generally fit for purpose and preferable to digestion. How critical the extent of dilution is merits further research in the area.

- Even for the four laboratories without obvious analytical issues that would prevent an assessment of the baseline blood level of Ti, there was still marked variation (up to ~ three fold) with reported concentrations in the same sample: namely, 0.43 (L2), < 0.6 (L5), 0.9 (L4) and 1.34 (L1) µg/L, but we note that although the latter three were above the LOD they were below the LOQ, so should only be considered approximate. Whether these differences are only the result of 'noise' near the detection limit or yet more subtle variances in matrix effects is not clear. Whilst we recognise that these are low levels it underlines the lengths/effort that analytical laboratories must go to if they really wish to report credible baseline levels of Ti in bio-analysis.

Conclusion

We conclude that measuring baseline levels of Ti in whole blood is challenging, but necessary if experiments are ever to make accurate exposure assessments (currently an important issue with TiO₂ ingestion or monitoring wear debris from Ti-containing implants). Usual baseline human blood Ti levels are low (likely less than 1.5 ppb). The use of sample digestion or analytical techniques other than HR-ICP-MS, triple quadrupole ICP-MS/MS or ICP-OES do not guarantee a failure to detect, accurately, low circulating levels of Ti but they certainly enhance the risk and special attention would be advisable in data validation. Spiking experiments, and the use of certified reference materials, are important tools in the analyst's tool box but, generally, will not reveal inaccuracies in baseline assessment of blood Ti levels when the presence of unresolved interferences elevate the baseline results or the analyte levels in certified reference materials are given at much higher levels compared to naturally occurring baselines. Cross validation with a second technique should be considered. Finally, based upon our findings, it seems likely that the analytical results of the many published 'exposure' studies reporting levels of baseline circulating Ti above a few ppb in unexposed humans/animals should be viewed with caution. Although the average reported baseline levels from these studies is much lower with high resolution ICP-MS and ICP-MS/MS than single quadrupole ICP-MS, the ranges suggest that none of these techniques are immune from over reporting (Table 1) and, indeed, as we show, sample preparation plays a significant role in determining final values. In our limited experience with digestion methods, the 'gains' (dissolving Ti) were outweighed by the 'disadvantages' (contamination) and, instead, dilution may be better, the extent of which should be validated for matrix type. We hope that this work can help provide a framework for more accurate blood Ti analyses going forward.

Conflicts of interest

There are no conflicts to declare.

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Paper

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

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Supplementary Information for:

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

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

ARTICLE

Journal Name

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 TiO ₂ 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. Seronom 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 Seronom 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 Seronom 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	Seronom 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 (Seronom 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. Seronom 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) HNO ₃ .
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) HNO ₃ . 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 ($>18.2 \text{ M}\Omega\text{cm}^{-1}$, 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: $\text{Ti}=10.3 \mu\text{g/L}$ and $\text{Ti}=15 \mu\text{g/L}$) 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 $954 \mu\text{g/kg}$. From this a third dilution was prepared at a concentration of $9 \mu\text{g/kg}$ in 20% HNO_3 Ti stock, which then was used to prepare standards at 0.1, 0.5, 1, 2, and $5 \mu\text{g/kg}$ in 20% HNO_3 .
Dilution	The digests were analysed directly by ICP-MS/MS with external calibration using $1 \mu\text{g/kg}$ 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 ($> 18.2 \text{ M}\Omega\text{cm}^{-1}$, Elga, Maxima, UK) and 0.1 g of Sc was added as internal standard to have a final concentration of $1 \mu\text{g/kg}$ Sc
Rinse/Wash solution	5% v/v HNO_3
Calibration verification	A $1 \mu\text{g/kg}$ 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

										L1	L2	L7	L3			L6	L4		L5		
Tune 3 - E. Tune 3 - Oct Tune 2 - E. Tune 2 - Oct Tune 1 - E. Tune 1 - Oct 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						
-7	-5	-8.0	-8.8	-7.0	-5.0	-8.0	-5.0	-108	-3.05	Pt/Ni	0.17	Micromist, 0.88	1mL/min He, 3mL/min NH ₃	Micromist	1mL/min He, 20% NH ₃	4.5mL/min H ₂ , 1mL/min O ₂	3mL/min He, 1mL/min O ₂	10mL/min H ₂ , 1mL/min O ₂	KED mode - Coll. gas He	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					Pt/Ni, HM interface	Sheath gas, L/min	CRI mode - Coll. gas H ₂ (to check)	MicroMist U-Series, 0.92	110 mL/min	130 mL/min	Resolution	Med. Res. (3000)	Low-flow MicroMist, 0.7	2 at higher & lower λ	Axial	
26	42	-200	-250	-3	-0.5	-108	-3.05	-108	-3.05	Pt/Ni, HM interface	0.19	PFA-ST neb., 1.07	10mL/min H ₂ , 1mL/min O ₂	Micromist, 1.13	1mL/min He, 3mL/min NH ₃	4.5mL/min H ₂ , 1mL/min O ₂	3mL/min He, 1mL/min O ₂	10mL/min H ₂ , 1mL/min O ₂	KED mode - Coll. gas He	4.75 mL/min	
										CCT Foc. Lens, V	Extr., V										
										Backgrd pixels		Peak pixels	Plasma view								