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*a Biomineral Research Group, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 0ES, UK and MRC Elsie Widdowson Laboratory (MRC-EWL), 120 Fulbourn Road, Cambridge, CB1 9NL, UK.*

*Email: [dk440@cam.ac.uk](mailto:jjp37@cam.ac.uk)*

*b University Hospital of Wales TRACE ELEMENT LABORATORY, Department of Medical Biochemistry and Immunology, Heath Park, Cardiff,CF14 4XW, UK.*

*c INRS, Unité de Génération d'atmosphères et de Chimie Analytique Toxicologique, Rue du Morvan CS60027, 54519 Vandooeuvre-lès-Nancy, France.*

*d LGC Limited, Inorganic Analysis, Queens Road Teddington, Middlesex, TW11 OLY, UK.*

*e SAS Trace Element Centre, Surrey Research Park, 15 Frederick Sanger Road, Guildford, Surrey, GU2 7YD, UK.*

*f Health and Safety Executive, Biological Monitoring, Harpur Hill, Buxton, SK17 9JN, UK.*

*g Charing Cross Hospital, SAS Trace Element Laboratory, Ground Floor Medical Oncology Block, Fulham Palace Road, London, W6 8RF, UK*

*† Footnotes relating to the title and/or authors should appear here.*

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

D. Koller a, P.Bramhall b, J. Devoy c, H. Goenaga-Infante d, C. F. Harrington e, E. Leese f, J. Morton f, S. Nuñez d , J. Rogersb, B. Sampson g and J. J. Powell a

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 emisison 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 interefrences 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.

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 digestion9, 14, 15. As well as understanding gut exposure there is considerable interest in describing systemic exposure16, 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 concentrated9,10. 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 measurements1,14, 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 applied24, 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 digestionof 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/Δm 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 analysis21, 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 -20o 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) µg/L | plasma/serum levels range (average) µg/L | basal blood level range (average) µg/L) | with implant/after exposure range (average) µg/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 TiO2 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 TiO2). This confirmed the original stock dispersion to be 35.45 % ± 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 TiO2. These are referred to as ionic Ti at low level (IL), ionic Ti at high level (IH), particulate TiO2 at low level (PL) and particulate TiO2 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/TiO2, 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 TiO2 in fasted whole blood, µg/L

|  |  |
| --- | --- |
|  | Final added [Ti] µg/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 -20oC 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 TiO2-spiked fasted bloods and high and low ionic-Ti-spiked fasted bloods). An aliquot of the original Sigma Aldrich TiO2 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 48Ti 🡒150(Ti(NH3)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 47Ti 🡒63(TiO), 48Ti 🡒 64(TiO), 48Ti 🡒 132(Ti(NH2)(NH3)4) 48Ti 🡒150(Ti(NH3)6) and 1 s for the internal standard 72Ge🡒72Ge, with 20 sweeps/replicate with three replicate/sample. The main transition 48Ti 🡒 64(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 49Ti and 0.05 s for the internal standard 73Ge, 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 47Ti were 1 s, and for 49Ti 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 48Ti was used for quantification and 47Ti 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 45Sc🡒61Sc (internal standard) and 46Ti 🡒62(TiO), 47Ti 🡒63(TiO) and 48Ti 🡒64(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% HNO3) plus 3 times the standard deviation of the blank and the instrumental LOQ as the mean blank (20% HNO3) 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 TiO2 particles

For the spiked TiO2 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 TiO2 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/TiO2 is having a precise and accurate measurement tool for bioanalysis, especially for baseline levels of Ti. This is because, as previously mentioned, TiO2 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 -> 150Ti [NH3] | 48Ti -> 64Ti [O2H2 ] \* | 49 Ti | 49Ti | Ti, 336.1nm | 49 Ti\*\* | 48 Ti-> 64Ti [O2H2] |
| 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 µg/L and %recovery. Values in parenthesis are > LOD but < 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 | , µg/L | (0.81) | 0.49 | (6.20) | (0.7) | (0.6) | 21.80 | (7) |
| SD, µg/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 | , µg/L | (1.34) | 0.43 | (8.18) | (0.9) | ( 0.6) | 24.62 | (7) |
| SD, µg/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 + 2.51µg.kg-1 | , µg/L | 4.11 | 2.63 | 9.19 | 3.36 | 3.22 | 27.04 | (7) |
| SD, µg/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 + 10.97µg.kg-1 | , µg/L | 14.41 | 10.08 | 18.17 | 12.05 | 13.13 | 36.54 | 16.57 |
| SD, µg/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+ 2.64µg.kg-1 | , µg/L | 3.24 | 3.12 | (8.22) | 3.16 | 2.47 | 26.87 | ( 7) |
| SD, µg/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 + 21.40µg.kg-1 | , µg/L | 28.77 | 21.65 | 28.71 | 24.7 | 21.38 | 45.00 | 28.91 |
| SD, µg/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 |

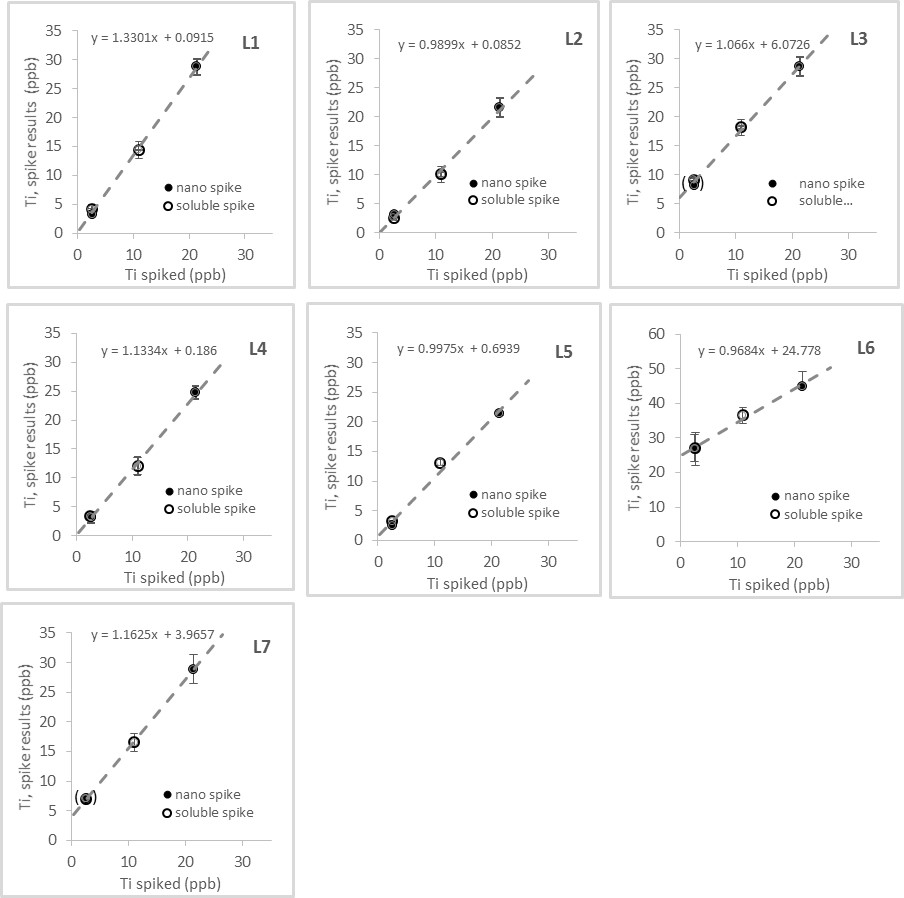


Figure 1 Spike recoveries for Labs L1-L7; soluble spike = ionic Ti spikes at 2.51 and 10.97 µg/L; nano spike = spiked TiO2 particles at 2.64 and 21.41 µg/L., Values in parenthesis are > LOD but < LOQ

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 mostlydetect low levels of spiked Ti and that they do so quantitatively. The linearity and general sensitiviy 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 (Tiionic). 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 basslines. 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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