Application Note 43137

Simple, fast and reliable analysis of lead in whole blood using the Thermo Scientific iCAP Q ICP-MS

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Key Words
Blood, Clinical Toxicology, ICP-MS, Lead

Goal
To develop a fully quantitative, high throughput method for the determination of Pb levels in blood by ICP-MS.

Introduction
Man has used the unique properties of Lead (Pb) in a wide variety of ways for hundreds of years. For example the Romans used it for plumbing (lead is plumbum in Latin). More recently, lead was used in gasoline and paints to improve performance before it was phased out in the 1970s when the dangers associated with its use were first recognized. Lead is a neurotoxic metal that affects areas of the brain that regulate behavior and nerve cell development[1]. A series of exposure pathways allow lead to enter the body, including inhalation of particles (e.g. dust from soils exposed to gasoline and paint lead sources) as well as drinking water and (particularly for children) contaminated toys. On entering the body, lead sequesters itself into soft tissue and bone, where it can be released back to the blood. Young children and fetuses are particularly vulnerable since the blood-brain barrier is not yet fully developed and absorption rates are higher than in adults. Lead, even at low concentrations, has been shown to impair cognitive development. Currently, there is no agreed safe concentration of lead in blood; however the United States Environmental Protection Agency (EPA) has defined standards for dangerous levels of lead in dust, paint and soil[2] to safeguard the population. The United States Centers for Disease Control and Prevention (CDC) states that Blood Lead Levels (BLL) >70 µg/dL (700 ng/mL) can cause serious health effects up to and including coma and death. BLL as low as 10 µg/dL (100 ng/mL) are associated with cognitive development, growth, and behavioral issues in children between the ages of 1-5 years. BLL at 10-25 µg/dL do not show clinical symptoms of lead poisoning so it is necessary to screen children. The CDC now designates a reference value for BLL of >5 ug/dL as elevated in children ages 1-5. The Occupational Safety & Health Administration (OSHA) also has guidelines for adult BLLs as it relates to work place exposure. All companies that expose their employees to lead must test employees at least once a year. OSHA guidelines include medical removal from the job if levels are > 60 µg/dL (600 ng/mL), and require follow up testing before returning to work. Because of these requirements, the United States performs millions of Blood Lead Level tests per year. All 50 States have BLL programs and require results to be reported back for statistical purposes.

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Historically, atomic absorption (AA) has been employed for the determination of Pb in whole blood. However, with analysis times often in excess of three minutes, this technique can be quite slow, especially if complicated ashing and atomization programs are used, leading to reduced sample throughput. In addition, AA is not a multi-element approach and often utilizes matrix-modifiers, which can complicate the sample preparation and add cost to the analysis. More recently, ICP-MS has emerged as a rapid, multi-element alternative, with improved detection limits (allowing for reduced sample amounts) and sample throughput over AA. Here, we report a simple, rapid method for BLL by ICP-MS and evaluate between run consistency and sample carry-over.

Sample Preparation

Because of the high instrumental sensitivity of the Thermo Scientific™ iCAP™ Q ICP-MS, samples and reference standards could be diluted 100 times in 0.5% HNO_3 (prior to an additional 2x dilution from on-line addition of an internal standard) while still maintaining the required detection limits. An additional advantage of the relatively high dilution factor used is the improved nebulization of whole blood samples as well as reduced maintenance of the sample introduction system, both leading to longer analysis periods. Calibration was carried out using external aqueous standards in 0.5% HNO_3 with concentrations of 0.001, 0.010, 0.100, 1.00, 2.00, 5.00 and 10.0 ng/mL. The method was validated by measuring the Lyphochek Whole Blood Level Controls (Levels 1, 2 and 3 (561, 562 and 563)) (Bio-Rad Laboratories Inc.).

Instrument Settings

All analyses were made using an iCAP Qc ICP-MS. While the iCAP Qc is equipped with collision cell technology for the suppression of spectral interferences, it was not used in this study and the iCAP Qc ICP-MS was therefore operated in STD mode (Table 1). The standard iCAP Qc sample introduction system consisted of a PFA-ST nebulizer, quartz glass peltier-cooled cyclonic spraychamber and a 2.5 mm ID quartz injector. The 1:100 diluted samples were presented for analysis using a SC-2DX autosampler with integrated 7-port FAST valve from Elemental Scientific Inc (Omaha, NE, USA). A 1 ng/mL Bi (in 0.5% HNO_3) internal standard was added on-line (a further 1:1 dilution) using the 7-port FAST valve, giving a total uptake rate of 400 µL/min (an effective 200x sample dilution). Through the combination of the iCAP Q and the FAST sample introduction system, sample to sample analysis times (including uptake, data acquisition and washout) of < 24 seconds per sample were achieved.

Table 1. iCAP Qc instrument parameters.

Parameter	Value
Peristaltic pump speed	32 rpm
Pump tubing	orange/green tubing for both carrier (sample) and internal standard
Nebulizer	PFA-ST
Interface cones	Nickel
RF Power	1550 W
Cool gas flow	14 L/min
Auxiliary gas flow	0.8 L/min
Nebulizer gas flow	1.06 L/min
Injector Type; ID	Quartz; 2.5 mm

Results

The analytical figures of merit for five consecutive runs are listed in Table 2, and an example calibration curve is shown in Figure 1. In order to keep analysis times at a minimum, particularly important when sample sizes are limited as is often the case in clinical analyses, a dwell time of 10 ms was used with only 10 sweeps. The instrument detection limit (IDL) obtained with aqueous calibration standards prepared in 0.5% HNO_3, was ~1 ng/L (0.0001 µg/dL) and the blank equivalent concentration (BEC) was ~2 ng/L. Sensitivity is expressed as $\Sigma^{206}Pb+^{207}Pb+^{208}Pb$ and is half of the normal STD mode level, due to the 1:1 on-line IS addition. The percentage recovery of the internal standard, Bi, over the course of the analysis (Run #3) is shown in Figure 2 and ranged from 94% to 103%. This indicates the analytical robustness of the method and freedom from matrix suppression.

Run #	Analyte	Dwell (sec)	Mode	Sweeps	Sensitivity cps/ng/L	R value	BEC (ng/L)	LOD (ng/L)
1	$^{206+207+208}$Pb	0.010	STD	10	125	0.999996	1.7	1.0
2	$^{206+207+208}$Pb	0.010	STD	10	140	0.999990	1.9	0.9
3	$^{206+207+208}$Pb	0.010	STD	10	142	0.999993	0.8	1.1
4	$^{206+207+208}$Pb	0.010	STD	10	121	0.999950	1.3	0.2
5	$^{206+207+208}$Pb	0.010	STD	10	137	0.999991	1.6	0.8

Table 2. Analytical figures of merit for five consecutive analytical runs.

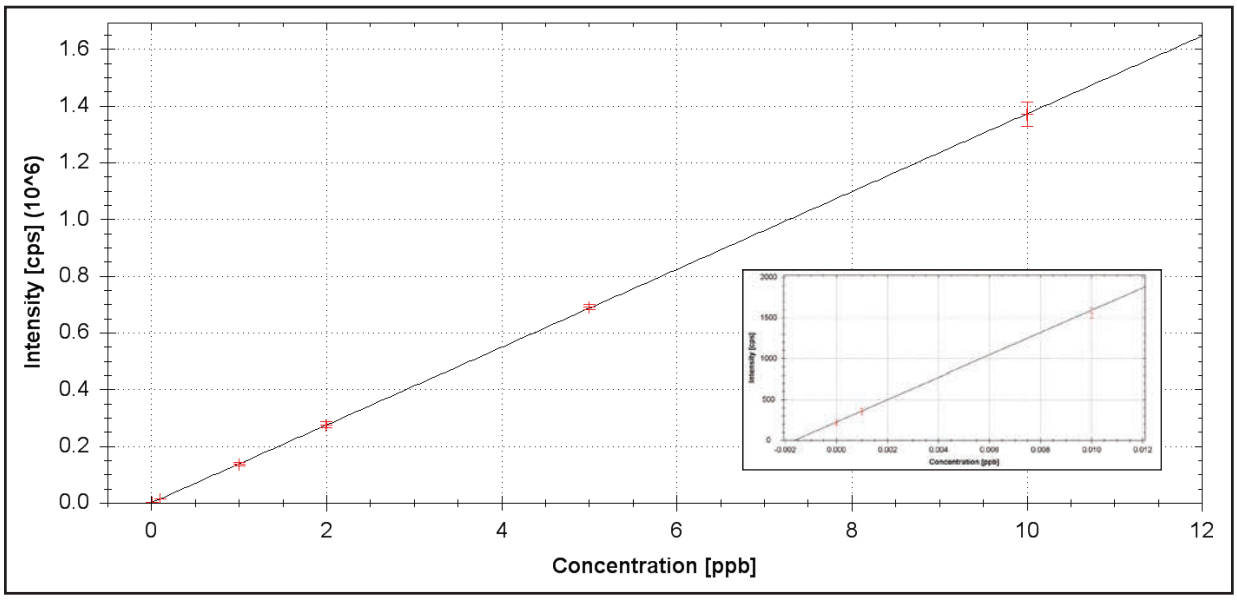

Figure 1. Example calibration curve for $^{206+207+208}$Pb from 0 to 10 ng/mL. The insert shows the same curve with concentrations between 0 and 0.01 ng/mL.

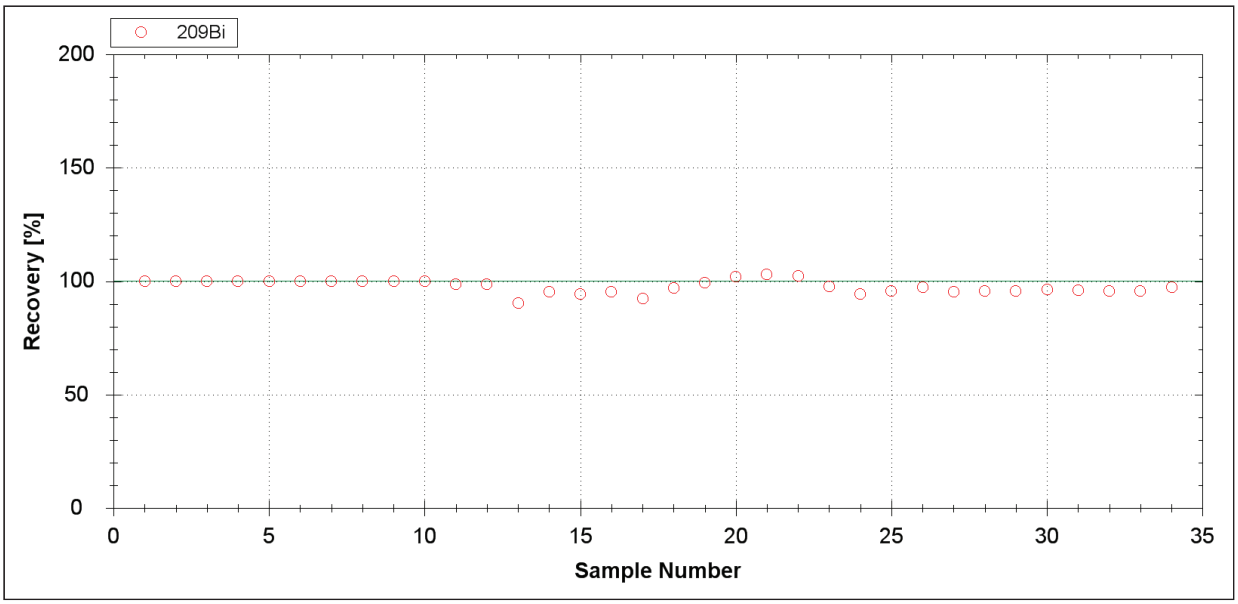

Figure 2. Screenshot from the Thermo Scientific Qtegra software showing the percentage recovery of the internal standard, Bi, during Run #3.

To determine the ability of the iCAP Q to accurately achieve low level BLL measurements, an additional 10x dilution was performed on the low level control (Table 3). The excellent agreement (97-105 %) with the reference values indicates that the method could easily support dilution factors in excess of the 200x used in this work.

Table 3. Serial dilution results for the low level Bio-Rad control (561).

Dilution Factor	Pb Meas. (µg/L)	D.F. Corr. (µg/dL)	Certified Value (ug/dL)	% Recovery
1000	0.085	8.51	8.14	105
100	0.810	8.10	8.14	100
1000	0.079	7.89	8.14	97
100	0.854	8.54	8.14	105
1000	0.083	8.28	8.14	102
100	0.794	7.94	8.14	98

Due to the clinical implications of this test, it is also important to assess the washout characteristics of the sample introduction system, and the likelihood that a false positive could be generated by inadequate washout from a previously analyzed sample. To assess this, a blank was repeatedly analyzed after five measurements of the high control standard (Figure 3). Using a FAST washout of two seconds, the lead background was reduced by >2 orders of magnitude. This equates to a BLL of ~0.001 µg/dL and is, therefore, a more accurate measure of the effective detection limit of the method described.

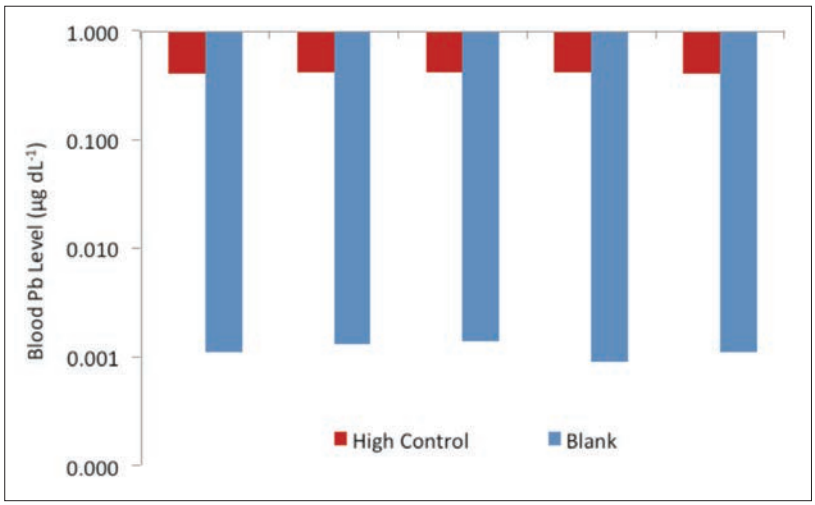

Figure 3. Washout results from five analyses of the high (Level 3) Bio-Rad control followed by five blank measurements.

Finally, an assessment of the accuracy of the proposed method was carried out by analyzing the control standards during each of the five analytical runs. The results for the BioRad Level 1-3 controls are given in Table 4.

Table 4. BLL (µg/dL) measured as the sum of the stable Pb isotopes 206, 207 and 208 for the Bio-Rad Control Levels 1-3. The % RSD for each measurement is also shown.

Run #	Analyte	Level 1	% RSD	Level 2	% RSD	Level 3	% RSD
Range (AA)	$^{206+207+208}$Pb	6.28-9.42		20.4-30.6		34.2-51.3	
Value (AA)	$^{206+207+208}$Pb	7.85		25.5		42.8	
Value (ID-ICP-MS)	$^{206+207+208}$Pb	8.14		25.2		43.2	
Run 1							
1a	$^{206+207+208}$Pb	7.98	4.8	25.2	1.9	41.9	1.9
Run 2	$^{206+207+208}$Pb						
2a	$^{206+207+208}$Pb	8.22	1.3	25.7	2.0	42.2	1.6
2b	$^{206+207+208}$Pb	8.16	2.6	25.5	1.2	42.1	0.8
2c	$^{206+207+208}$Pb	8.09	2.7	25.7	1.7	41.8	2.1
	Mean ± SD	8.16 ± 0.065		25.6 ± 0.12		42.0 ± 0.21	
	% RSD	0.80		0.45		0.50	
Run 3							
3a	$^{206+207+208}$Pb	8.10	1.4	25.7	0.4	43.5	0.1
3b	$^{206+207+208}$Pb	8.32	1.2	25.5	1.6	43.0	2.0
3c	$^{206+207+208}$Pb	8.16	0.7	25.1	1.1	42.4	1.3
	Mean ± STD	8.19 ± 0.11		25.4 ± 0.31		43.0 ± 0.55	
	% RSD	1.4		1.2		1.3	
Run 4							
4a	$^{206+207+208}$Pb	8.54	3.8	25.8	1.3	40.6	3.1
4b	$^{206+207+208}$Pb	8.40	0.6	26.5	2.9	41.2	1.9
4c	$^{206+207+208}$Pb	8.43	2.9	26.0	0.8	41.3	2.5
4d	$^{206+207+208}$Pb	8.29	3.3	26.1	3.0	41.5	0.9
	Mean ± STD	8.42 ± 0.10		26.1 ± 0.29		41.2 ± 0.39	
	% RSD	1.2		1.1		0.94	
Run 5							
5a	$^{206+207+208}$Pb	7.94	0.6	25.0	1.1	42.5	1.3
5b	$^{206+207+208}$Pb	8.15	2.8	25.9	1.3	42.4	2.6
5c	$^{206+207+208}$Pb	8.19	1.3	25.1	1.0	41.6	2.3
5d	$^{206+207+208}$Pb	8.25	2.2	25.0	1.5	43.0	2.4
5e	$^{206+207+208}$Pb	8.07	1.2	24.5	2.2	42.5	1.4
	Mean ± STD	8.12 ± 0.12		25.1 ± 0.50		42.4 ± 0.50	
	% RSD	1.5		2.0		1.2	
Run 1 – 5							
	Mean ± STD	8.21 ± 0.16		25.5 ± 0.51		42.1 ± 0.75	
	% RSD	2.0		2.0		1.8	

Conclusions

A fully quantitative method for the determination of blood Pb by ICP-MS has been described. Calibration using aqueous calibration standards is possible and the accuracy was assessed by the measurement of control samples. The proposed method, with an instrumental detection limit of 0.0001 µg/dL (and effective method detection limit of 0.001 µg/dL due to sample carry-over) can be used for the analysis of real samples with dilution factors of ≤1000 as demonstrated by recoveries of the low level control. Due to the high sensitivity of the Thermo Scientific iCAP Q ICP-MS, this increased dilution factor would further decrease system maintenance, enabling high volume clinical laboratories to process samples at a rate of 2.5 per minute.

References

1. NRC (National Research Council). 1993. Measuring lead exposure in infants, children, and other sensitive populations. Washington, DC: National Academies Press.
2. United States Federal Register, Part III, Environmental Protection Agency, 40 CFR Part 745; Lead: Identification of Dangerous Levels of Lead; Final Rule.

Disclaimer

The method presented is intended for research purposes only.