Determination of essential and toxic metals in blood by ICP-MS using calibration in a synthetic matrix

Application note

Clinical

Authors

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Introduction

One of the most important improvements in inductively coupled plasma mass spectrometry (ICP-MS) in the last decade has been the introduction of collision/reaction cells (CRCs) to remove polyatomic interferences. However, the accurate determination of some metals in complex matrices like blood or urine can still be challenging by CRC-ICP-MS.

A method used to determine Pb in unknown matrices by isotope dilution mass spectrometry (IDMS) was previously published by NIST [1]. IDMS is considered the most accurate method for total blood metals analyses, mainly because it eliminates the blood's matrix effect [2, 3]. However, the IDMS method is relatively expensive, and cannot be used for mono-isotopic metals such as Mn or As. Alternatively, the use of internal standards (ISTDs) can compensate for matrix effects by correcting the analyte response

proportionally to measured changes in the ISTD response. However, unlike isotope dilution, differences in the chemical compositions of the calibration standards and blood solutions can still lead to analytical errors due to differences in ionization behavior of the ISTD in different matrices. In this work, we demonstrate that by matching the ionic strength of the calibration standards with that of the blood samples (matrix matching), the bias in the internal standard technique can be virtually eliminated resulting in accuracy and precision comparable to IDMS.

Our current method uses an aqueous solution of n-butanol, NH_4OH, H_4EDTA and Triton X-100, plus ISTDs as a blood diluent. This diluent is an excellent blood solvent. In addition, the calibration standards are prepared in the same solution with the addition of sodium and calcium chloride for matrix matching. The use of this synthetic matrix is much simpler and more reliable than the more common use of whole blood for the purpose of matrix matching.

Experimental

Instrumentation

Throughout the following study, an Agilent 7700x with an integrated sample introduction system for discrete sampling (ISIS-DS) and helium collision mode were used. The instrument was equipped with MassHunter Workstation Revision B.01.01 for instrument control and data handling software. The ICP-MS was operated with Ni sampler and skimmer cones, MicroMist glass concentric nebulizer and quartz Scott-type spray chamber. The instrument settings and parameters are detailed in Tables 1 and 2.

Reagents

Type 1 deionized (DI) water was used for preparation of all aqueous solutions. Stock standard solutions of As, Cd, Mn, Pb, Hg, and U, each at a concentration of 1,000 mg/L, and Tl, Re, Ir, Rh, and Ge (ISTDs), each at a concentration of 1,000 mg/L, were obtained from SPEX CertiPrep (Metuchen, NJ, USA). A second source of custom standard solutions containing As, Cd, Mn and Pb at 1,000 mg/L each, and Hg and U at 100 mg/L each, was prepared by Inorganic Ventures (Christiansburg,

Table 1. Agilent 7700x ICP-MS and ISIS-DS operating parameters

ICP-MS	Setting
RF power	1550 W
# of points per peak	6
Carrier gas	1.05 L/min
Dilution mode	On
Dilution gas	0.1 L/min
He gas	4.3 mL/min
ISIS-DS	**Setting**
Load time	10 s
Load speed	1.0 rps
Probe rinse time	23 s
Post rinse time	10 s
Post rinse speed	1.0 rps
Loop length	120 cm
Loop tubing ID	0.8 mm

Table 2. Spectrum acquisition parameters

Mass Element	*Integration Time per Mass
^{55}Mn	1.5 s
^{72}Ge	0.70 s
^{75}As	2.1 s
^{103}Rh	0.70 s
^{111}Cd	2.6 s
^{185}Re	0.70 s
^{193}Ir	0.70 s
^{202}Hg	3.2 s
^{205}Tl	0.70 s
^{206}(Pb)	1.1 s
^{207}(Pb)	1.1 s
^{208}Pb	1.1 s
^{238}U	1.2 s

Acquisition Time: 20.02 s, 3 repetitions, Total acquisition time: 60.06 s, Pb (208)= (208)*1 + (206)*1+ (207)*1

* An independent study was used to determine the optimum integration times shown [4], but in most cases, integration times could be rounded to the nearest 0.1 second.

VA, USA). High purity sodium chloride, calcium chloride, Triton X-100 and H_4EDTA were obtained from Sigma-Aldrich (Milwaukee, WI, USA), and ammonium hydroxide and n-butanol from Fisher Scientific (Pittsburg, PA, USA).

Carrier solution

The carrier solution is used to push the sample from the loop of the ISIS-DS system to the nebulizer during sample measurement. The carrier solution was identical to the calibration blank solution in order to minimize disturbances due to variations in solution chemistry as the sample transitions to the carrier at the nebulizer - NH_4OH - 2% w/v, H_4EDTA - 0.25 % w/v, 7.5 g/L NaCl and 0.5 g/L $CaCl_2$.

ISIS washing solution

The ISIS washing solution provides additional rinsing of the autosampler probe, connection tubing and the ISIS loop/valve between analytical runs. The washing solution was an aqueous solution of NH_4OH - 2% w/v, H_4EDTA - 0.1% w/v and Triton X-100 - 0.1% w/v.

Analytical protocol

Preparation of intermediate standards in the synthetic matrix

The synthetic matrix solution for the preparation of the intermediate standards was an aqueous solution of NH_4OH - 2% w/v, H_4EDTA - 0.25 % w/v, 7.5 g/L NaCl and 0.5 g/L $CaCl_2$. The final concentrations of the metals in the intermediate standards are listed in Table 3. The intermediate standards were stable for at least 1 week when stored at 4°C.

Table 3. Metal concentrations in the Intermediate Standards

Level	As, Mn, Cd, Pb (µg/L)	Hg, U (µg/L)
1	0.00	0.00
2	5.00	0.50
3	25.0	2.50
4	250	25.0
5	500	50.0

Preparation of intermediate standards for calibration without a matrix-match

The preparation of intermediate standard solution for calibration without a matrix match was identical to the solution used for preparation of intermediate standards in the synthetic matrix (SM), but did not contain either NaCl or $CaCl_2$.

Preparation of quality control (QC) samples

The QC samples were prepared by spiking defibrinated sheep blood obtained from Hemostat Laboratories (Dixon, CA, USA) with inorganic stock standard solutions at three levels (QCLow, QCMed and QCHigh). The spikes were preserved with ~1.5 mg/mL of Na_2H_2EDTA. Approximately 1 mL QC aliquots were used to prepare QC working solutions for four to five subsequent analytical runs before the remaining amounts were discarded.

Blood specimens, QC samples and intermediate standards were diluted 50 times with a diluent identical to the carrier solution, but with addition of ISTDs: Ge, Re, Ir, Tl each at 20 µg/L and Rh at 10 µg/L. Before analysis, the solutions were rotated at 10 rpm for ~20 min to assure complete dissolution of the blood or QC samples. The working standards were prepared daily by manually pipetting 100 µL of the four levels of the respective intermediate standards (Table 3) into 4900 µL of the diluent. The blank solution was prepared identically using 100 µL of the synthetic matrix solution.

QC material and blood specimens were typically soluble in the basic diluent immediately after preparation. A number of the blood specimens however contained small to moderate clots, which would make accurate pipetting difficult or impossible. In such cases, 1 to 2 mL of the clotted blood was transferred into a 2 mL cryogenic vial and ultrasonically homogenized for 10 to 20 seconds. This homogenization process not only effectively dispersed the clots, but it also improved the reproducibility of the analytical results, especially for Mn and Cd. Although it is possible that after the

homogenization micro clots may still be present in the blood specimens, low relative percent differences (RPDs) (less than 20%) were consistently observed between duplicate measurements for all analytes. This suggests that there is no significant effect due to any remaining micro clots on the accuracy and precision of the measurements.

Instrument and run preparation

The instrument operating conditions are listed in Table 1. In this configuration, the instrument's peristaltic pump was pumping the carrier solution at a rate of 0.1 mL/min. The ISIS-DS and the ASX-500 autosampler were configured to perform the following operations:

a. Prewash (valve in "load" position): The autosampler probe goes to position 1 (wash solution) and the pump aspirates the washing solution for 10 s. In this mode, the entire sample introduction path, including the loop, are washed.

b. Sampling: The autosampler probe moves to the sample vial and the ISIS pump aspirates the sample solution for 10 s to fill the loop.

c. The ISIS valve switches to the inject position, directing the sample from the loop to the nebulizer. During this time, the autosampler probe and tubing are rinsed with DI solution for 30 s in the rinse well, and then moved to the 'home" position of the autosampler until the analysis has finished.

d. After allowing 23 s for the sample to reach the nebulizer and the signal to stabilize, data acquisition is started and continues for 60 s.

e. The same process then begins for the next sample.

The total analysis run time for an individual sample can be calculated as follows: 10 s sampling, 10 s prewash, 23 s stabilization time, 60 s acquisition time and 2–5 s for the probe to move positions for total run time of 105–108 s per sample.

Analytical run

A standard analytical run routinely included two replicates for each blood specimen and QC sample. A set of QC samples bracketed the beginning and end of the blood specimens in a run sequence. Additionally, for every 20 blood specimens, at least one QC sample was analyzed. All reported QC and analytical results are averages of two replicate measurements.

Results and discussion

Calibration without matrix-matching

Blood is a complex solution of organic components (red and white blood cells, platelets, etc.) and inorganic ions (Na, K, Ca, Cl, and many others) that define its unique physical characteristics. To make metals analysis by ICP-MS possible, blood specimens are typically diluted 10 to 50 times. Still, the organic components affect the flow rate and nebulization during analytical runs, especially if the analyzed blood specimen is partially coagulated with clots that are not completely solubilized. These fluctuations are observed by changes in analytical response for all analytes present in the solution, and the concept of ISTD addition is one way to compensate for such variations in response. However, the response of analytes and internal standards not only depends on their concentration, but also on the element's first ionization potential (IP). Elements with high first IPs can be suppressed by the presence of easily ionized matrix element(s) at a relatively high concentration such as Na, K and Ca. Our laboratory method called for a substantial dilution of blood samples (50 times) to minimize the matrix effect between our calibration standards, originally without matrix-match components, and blood samples. The Agilent MassHunter software included a useful feature of tracking ISTD stability: a base signal for each ISTD is set during calibration blank analysis, and recoveries of ISTDs for subsequent results are calculated and used as criteria for accepting a run.

Performance of the method was subsequently assessed by the laboratory's participation in five external quality assessments schemas for whole blood metals testing. Although all our results were within acceptable range, a subtle but noticeable trend emerged: almost all results for individual analytes were lower by 1 to 4% compared to the reference values provided by the programs. In addition, ISTDs recoveries were close to 100% for calibration standards and CCBs and CCVs, but were somewhat lower for QC reference materials (Figure 1(a)). While it is assumed that the ISTD responses will accurately correct for suppression effects on the analytes, reducing the relative suppression when possible is the preferred solution. The most common technique for reducing suppression is dilution. However, conventional dilution has the disadvantages of increasing the limits of detection (LOD) as well as the risk of introduction of contaminants, which, when multiplied by the dilution factor further increase the LODs. Agilent's high matrix introduction (HMI) system can use aerosol dilution to avoid the risk of contamination, but since this technology isn't generally available on non-Agilent instruments, a matrix matching technique was evaluated.

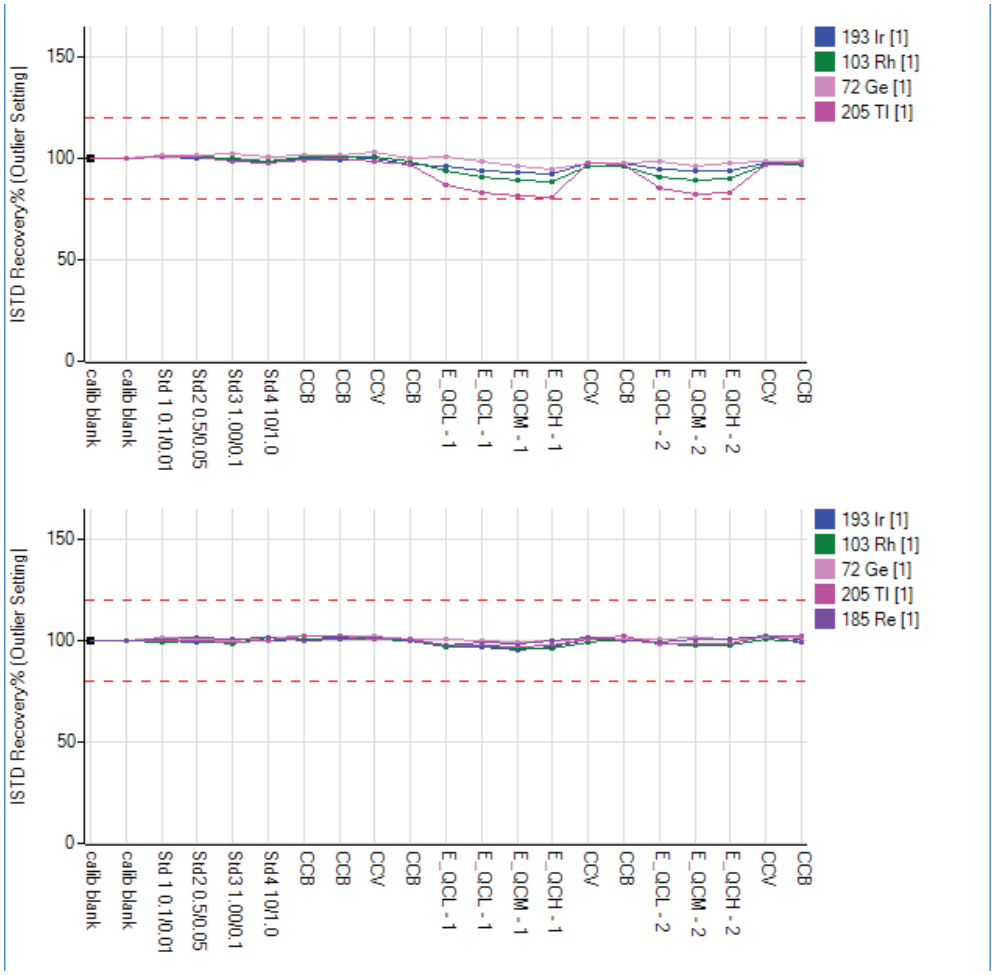

Figure 1. Typical MassHunter ISTD stability graph for QC samples analysis when: **(a)** calibration standards were prepared without a matrix match and **(b)** calibration standards were prepared in the synthetic matrix. In both cases CCBs and CCVs were prepared in the same matrix as the calibration standards.
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Calibration with a matrix-match

The matrix-match approach for calibration standards and samples is based on the principle that the factors affecting the response for all analytes and ISTDs are similar in both calibration standards and sample solutions. Ideally, if all non-analyte components present in blanks and calibration standards were identical or very similar to sample solutions, the analysis would yield unbiased results. A common approach to achieve this matrix-match solution is to add animal blood to the calibration standards [5, 6]. Animal and human blood usually contains both essential and toxic metals, and when this matrix-match method is used, all calibrations for individual metals are adjusted for the presence of the metals in the animal blood calibration blank. Typically, concentrations of toxic metals in blood are low and do not affect respective calibrations, but in the case of essential metals i.e., Mn, which is present in blood at relatively high concentration, correction using a high blank value usually leads to inaccurate calibration.

To improve our results from the preliminary studies, we took another approach to the matrix-matching problem by investigating the use of a synthetic matrix (SM) for blanks and calibration standards and its effects on the analytical results for the inorganic components in blood. The success of this idea depended on the validity of the assumption that analyte and ISTD behavior in the synthetic matrix are very similar to that in whole blood with respect to sample transport efficiency and ionization efficiency. This SM method was tested previously [7] for Pb only, and the results are shown in Figure 1(b). When calibration standards were prepared in the SM, ISTD recoveries were very close to 100% for all samples including calibration standards, CCBs, CCVs and QC reference materials. This study assessed long term stability (259 min) of the method by analyzing 300 samples diluted 50 times. The stability of the ISTDs (Ir and Rh) was consistent throughout all the runs, with no significant drift observed [7].

In our present study, concentrations of all diluent components were: 2% NH_4OH w/v, 0.1% H_4EDTA w/v, 4% n-butanol w/v and 0.1% Triton X-100 w/v. The concentration of H_4EDTA in the intermediate standard solutions was 0.25% (or 2.5 g/L), enough to fully complex the Ca^{+2} and calibration metals. To solubilize the H_4EDTA, a substantial amount of NH_4OH (2% w/v) was used during solutions preparation. The increase in concentrations of the diluent components in our study reflects the advancement in ICP-MS technology that have occurred during the last 10 years, specifically regarding the increased plasma robustness to withstand such high concentrations of organic and volatile solution components and removal of polyatomic interferences by collision/reaction cells.

The high concentration of n-butanol increased overall sensitivity by about two fold. The Ce oxide ratio ($^{156}CeO/^{140}Ce$) was unchanged at about 1%, and no isobaric interferences were found in the study for any metals.

Analytical results for four levels of NIST 955c are shown in Table 4.

Selection of internal standards

As conventionally done, the selection of the ISTDs for the analytes in this study was based on the common practice of matching the first ionization potential and atomic mass as closely as possible with the analyte. However, Figure 2 demonstrates that when calibration in synthetic matrix is employed, the analytical results are quite similar regardless of which ISTD was selected for an analyte. Additionally, while Ge was evaluated in this work and is commonly used as an internal standard for several elements including arsenic and selenium, caution should be exercised because it has been reported to be present in blood at significant concentrations [9].

Table 4. Analytical results of four levels of NIST 955c SRM using synthetic matrix matched calibration standards. *Based on our laboratory performance in other external quality schemas, we've determined that the NIST 955c L1 reference value for As is likely inaccurate.

Element	ISTD	NIST Level	Conc. Units	NIST 955c SRM		
				In synthetic matrix	Certified value	Reference value
^{55}Mn	^{72}Ge	955c L1	µg/L	15.58 ± 0.26		
		955c L2	µg/L	16.74 ± 0.36		
		955c L3	µg/L	17.89 ± 0.27		
		955c L4	µg/L	18.66 ± 0.21		
^{75}As	^{72}Ge	955c L1	µg/L	0.129 ± 0.014		2.07* ± 0.63
		955c L2	µg/L	20.4 ± 0.4		21.9 ± 1.7
		955c L3	µg/L	52.4 ± 0.7		53.9 ± 3.4
		955c L4	µg/L	78.6 ± 1.0		77.5 ± 4.2
^{111}Cd	^{103}Rh	955c L1	µg/L	0.026 ± 0.012		0.0317 ± 0.0062
		955c L2	µg/L	2.17 ± 0.05	2.14 ± 0.24	
		955c L3	µg/L	5.28 ± 0.07		5.201 ± 0.038
		955c L4	µg/L	10.48 ± 0.15	9.85 ± 0.17	
^{202}Hg	^{193}Ir	955c L1	µg/L	0.039 ± 0.009	0.017 ± 0.011	
		955c L2	µg/L	5.33 ± 0.12		4.95 ± 0.76
		955c L3	µg/L	18.8 ± 0.35	17.8 ± 1.6	
		955c L4	µg/L	34.7 ± 0.64		33.9 ± 2.1
^{208}Pb	^{205}Tl	955c L1	µg/dL	0.423 ± 0.006	0.424 ± 0.011	
		955c L2	µg/dL	13.95 ± 0.15	13.95 ± 0.08	
		955c L3	µg/dL	27.64 ± 0.29	27.76 ± 0.16	
		955c L4	µg/dL	45.56 ± 0.41	45.53 ± 0.23	
^{238}U	^{185}Re	955c L1	µg/L	0.0065 ± 0.0014		
		955c L2	µg/L	0.0073 ± 0.0016		
		955c L3	µg/L	0.0079 ± 0.0008		
		955c L4	µg/L	0.0037 ± 0.0012		

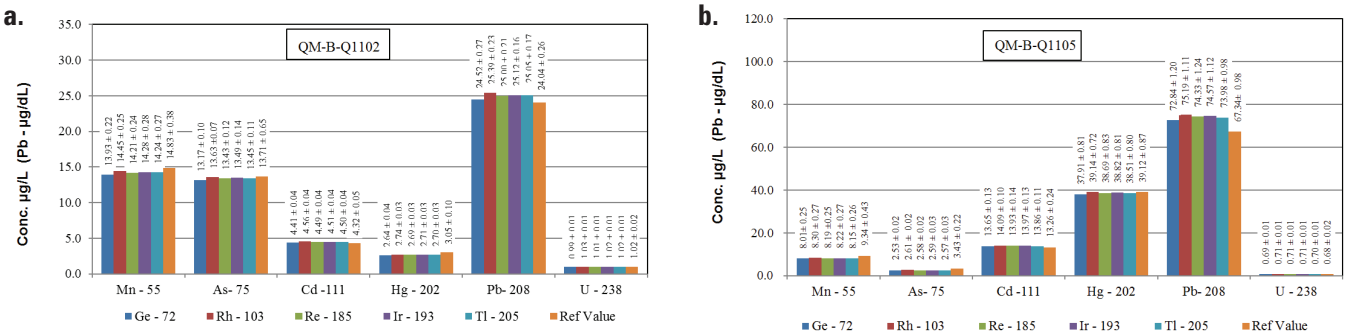

Figure 2. Comparison of analytical results for two samples, (a) QM-B-Q1102 and (b) QM-B-Q1105, from the Quebec Multivalent External Quality Assessment Scheme (QMEQAS) metal panel using calibration in a synthetic matrix. Results for each analyte are based on average of eight runs, with each set of results calculated against five different ISTDs. Results for each analyte are compared to QMEQAS reference values. *Reproduced by permission of The Royal Society of Chemistry (RSC).*

As one of the reference laboratories for the Wisconsin State Laboratory of Hygiene (WSLH) Filter Paper Blood Lead Proficiency Testing Program (FPPTP), we analyze pools of whole blood specimens that WSLH will eventually use to prepare proficiency testing samples for filter paper blood lead spots. During the FPPTP June 2011 event, the same solutions prepared for eleven samples were analyzed twice; first with calibration standards without a SM (Figure 3(a)) and second with calibration standards in a SM (Figure 3(b)). Both analyses were run against 5 different ISTDs.

Near perfect correlations (slopes, intercepts and correlation coefficients) are observed between WSHL Pb means and results determined in the present study when either ^{103}Rh, ^{185}Re, ^{193}Ir or ^{205}Tl were used as ISTDs, and a slight difference existed when ^{72}Ge was used.

The four ISTDs yielded coefficients of variation (CV) ranging 0.46 to 0.79% for each of the eleven PT samples (Figure 3(b)). For calibration without a matrix match however, the respective CV for Pb results were about 4% (Figure 3(a)). The perfect/near perfect correlation coefficients observed for all correlations (Figure 3) indicate that all eleven blood specimens have very similar matrices.

As illustrated in Figures 3(a) and (b), the Pb recoveries using ^{103}Rh, ^{185}Re, ^{193}Ir and ^{205}Tl ISTDs were generally very similar for calibration in a SM, but notably different for calibration without a matrix-match. Although we selected NaCl and $CaCl_2$ as the SM salts for the present study, it is possible that a single salt at the appropriate concentration would be sufficient. We will be studying this and continuing our refinements in further investigations.

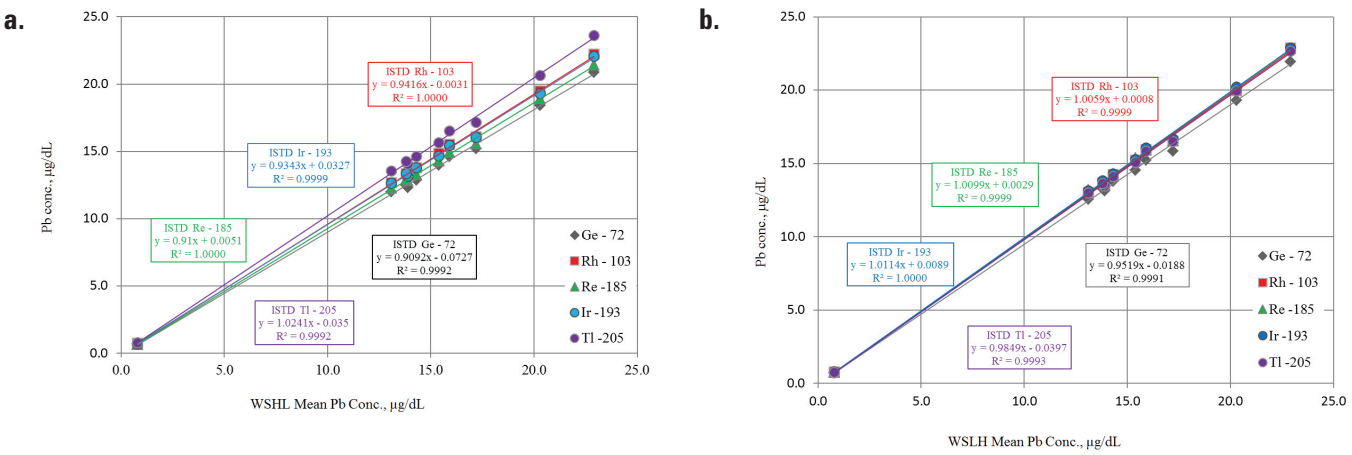

Figure 3 (a) Wisconsin State Laboratory of Hygiene Filter Paper Blood Lead Proficiency Testing (FPPTP – June 2011 event) referee laboratory value assignment for whole blood lead vs. program mean results for various ISTDs using calibration without a matrix match. The analyzed whole blood specimens were used by the Wisconsin State Laboratory of Hygiene to prepare filter paper blood lead spots proficiency testing samples. The perfect or near perfect correlation coefficients observed for all correlations indicate that all eleven blood specimens have very similar matrices. **(b)**The same sample solutions used to generate results for (a) correlations were reanalyzed shortly after using calibration in synthetic matrix. *Reproduced by permission of The Royal Society of Chemistry (RSC)*

Conclusions

A substantial dilution (50 times) of blood samples is not enough to completely eliminate the matrix effect observed in blood metal analyses. The addition of NaCl and $CaCl_2$ to calibration standards created a synthetic matrix (SM) that promoted excellent accuracy for all metals results in the study, comparable only to the isotopic dilution technique. Our study demonstrated that under matrix match conditions a number of ISTDs are acceptable for a given analyte, regardless of first ionization potential or atomic mass. This SM approach eliminates the need for the use of multiple ISTDs to correct instrument run instabilities, and may potentially offer a universal technique to improve the accuracy of ICP-MS results for metal analyses in complex matrices.

The developed method is uniquely qualified for use in biomonitoring studies, where accuracy and precision of analytical results are essential in achieving meaningful correlations and comparisons to results from varying study populations. In August 2011, the calibration standard preparation using the SM became an approved method for the determination of essential and toxic metals in blood for the California Biomonitoring program, and is also used for routine blood lead analysis for patients being treated for lead poisoning. Since the method validation in March 2009, our laboratory has performed blood metals analyses for the following Biomonitoring studies: CYGNET (Cohort Study of Young Girls' Nutrition, Environment, and Transitions), FOX (Firefighter Occupational Exposures), and MIEEP (Maternal and Infant Environmental Exposure Project). Study results from the CYGNET demographic study were recently presented [4, 8]. As the method continued to advance with the matrix match technique, the improved method is currently underway for applications to the BEST study (Biomonitoring Exposures Study).

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Disclaimer

Results and conclusions in this report are those of the authors and do not necessarily represent the views of the California Department of Public Health.

More information

For a full account of this application see publication: Determination of essential and toxic metals in blood by ICP-MS with calibration in synthetic matrix, Ryszard Gajek, Frank Barley and Jianwen She, Anal. Methods, 2013, 5, 2193

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