Journal of Mass Spectrometry and Advances in the Clinical Lab 27 (2023) 33–39

Contents lists available at ScienceDirect

Journal of Mass Spectrometry and Advances in the Clinical Lab

journal homepage: www.sciencedirect.com/journal/journal-of-mass-spectrometry-and-advances-in-the-clinical-lab

Research Article

Method validation of multi-element panel in whole blood by inductively coupled plasma mass spectrometry (ICP-MS)

Amol O. Bajaj [b,c,*], Rebecca Parker [c], Candice Farnsworth [c], Christian Law [c], Kamisha L. Johnson-Davis [a,b,*]

[a] Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT, United States
[b] ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, United States
[c] ARUP Laboratories, Salt Lake City, UT, United States

ARTICLE INFO

Keywords:
Whole blood
Trace elements
Toxic elements
ICP-MS
Method validation

ABSTRACT

Background: Analytical methods to measure trace and toxic elements are essential to evaluate exposure and nutritional status. A ten-element panel was developed and validated for clinical testing in whole blood. Retrospective data analysis was conducted on patient samples performed at ARUP Laboratories.

Methods: A method was developed and validated to quantify ten elements in whole blood by ICP-MS. Fifty microliters of sample were extracted with 950 µL of diluent containing 1 % ammonium hydroxide, 0.1 % Triton X-100, 1.75 % EDTA along with spiked internal standards. Four calibrators were used for each element and prepared in goat blood to match the patient specimen matrix. Samples were analyzed with an Agilent 7700 ICP-MS with a Cetac MVX 7100 µL Workstation autosampler.

Results: The assay was linear for all elements with inter- and intra-assay imprecision less than or equal to 11% CV at the low end of the analytical measurement range (AMR) and less than or equal to 4% CV at the upper end of the AMR for all elements. Accuracy was checked with a minimum of 40 repeat patient samples, proficiency testing samples, and matrix-matched spikes. The linear slopes for the ten elements ranged from 0.94 to 1.03 with intercepts below the AMR and R^2 ranging from 0.97 to 1.00.

Conclusions: The multi-element panel was developed to analyze ten elements in whole blood to unify the sample preparation and increase batch run efficiency. The improved analytical method utilized matrix-matched calibrators for accurate quantification to meet regulatory requirements. The assay was validated according to guidelines for CLIA-certified clinical laboratories and was suitable for clinical testing to assess nutritional status and toxic exposure.

Introduction

Biological monitoring of trace and toxic elements is imperative to detect nutritional deficiency, acute or chronic exposure, and toxicity [1,2]. Reference laboratories can provide esoteric testing for trace and toxic elements that are not routinely performed in a clinical laboratory. The optimal specimen type for analysis is dependent on several factors, such as the target trace element and the optimal specimen type to detect nutritional deficiency or toxicity. Whole blood can be used as a specimen of choice to assess recent element exposure and to detect heavy metals that distribute into red blood cells [3]. Inductively coupled plasma-mass spectrometry (ICP-MS) is used to measure elements at specific mass-to-charge ratios and is considered the gold standard methodology. There are various published methods for multi-element panels that utilize

Abbreviations: AAPCC, American Association of Poison Control Centers; AMR, Analytical measurement range; As, arsenic; Bi, bismuth; Cd, cadmium; CLIA, Clinical Laboratory Improvement Amendments; CLRW, Clinical Laboratory Reagent Water; Co, cobalt; Hg, mercury; ICP-MS, Inductively coupled plasma-mass spectrometry; IRB, institutional review board; KED, kinetic energy discrimination; LOB, limit of the blank; LOD, limit of detection; LOQ, limit of quantitation; Mn, manganese; NH_4OH, ammonium hydroxide; Pb, lead; SD, standard deviation; Sb, antimony; Tl, thallium; ULOQ, upper limit of quantification; WB, Whole blood; Zn, zinc.

Peer review under responsibility of "MSACL"

* Corresponding authors at: ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, United States.
E-mail addresses: amol.bajaj@aruplab.com (A.O. Bajaj), kamisha.johnson-davis@aruplab.com (K.L. Johnson-Davis).

https://doi.org/10.1016/j.jmsacl.2022.12.005
Received 28 August 2022; Received in revised form 9 December 2022; Accepted 12 December 2022
Available online 17 December 2022

acidic or basic digestion for ICP-MS analysis [4–9]. The purpose of this project was to develop and validate a multi-element panel to quantify ten elements in whole blood to implement for clinical testing at ARUP Laboratories.

Antimony is found in the Earth's crust and particulates released into the atmosphere, from processes such as volcanic eruptions and forest fires, can travel on the wind [10,11]. Exposure to antimony can occur through inhalation, dermal absorption, and orally. Adverse effect from antimony poisoning include nausea, vomiting, diarrhea, shock, hemoglobinuria, respiratory irritation, cardiac abnormalities, carcinogenic and genotoxic effects [10,11]. Arsenic is present in the environment and can be found in organic forms in seafood (e.g., Arsenobetaine) and inorganic compounds in groundwater, soil, and air (e.g., Arsenic trioxide) [10,12]. Acute arsenic exposure can cause abdominal pain, nausea, vomiting and diarrhea, whereas chronic exposure to arsenic can lead to skin lesions, cardiovascular disease, and cancer [10,12]. Bismuth is used in manufacturing, cosmetics, X-ray contrast media, paints and pharmaceuticals, such as Pepto-Bismol [10]. High concentrations of bismuth can cause abdominal pain, acute renal injury, and neurotoxicity [10,13]. Cadmium is used as an anticorrosive agent for steel and is found in cigarettes. Toxic exposure to cadmium can lead to damage of the kidneys, liver, heart, bone and, in severe cases, lead to death [14]. Cobalt is an essential element and nutritional deficiency can lead to anemia. Exposure to cobalt can come from the release of the metal ion from metal-on-metal joint arthroplasty [15]. Toxic cobalt exposure can lead to cardiomyopathy and cancer [16]. Lead poisoning can occur via the ingestion of lead-based paint, or exposure to lead-contaminated dust or water and may cause anemia, neurotoxicity and organ damage [17]. Manganese is an essential element that supports immune function and cellular growth; however, toxic exposure can manifest in symptoms similar to Parkinson's disease [18]. Mercury is a heavy metal toxin that is present in water, air, and soil in organic, inorganic, and elemental forms. Exposure to mercury can damage the nervous system, immune function and gastrointestinal track [19]. Thallium is a heavy metal found in the environment. Thallium absorption via inhalation, ingestion, and/or dermal exposure can lead to multi-organ damage [20]. Zinc is an essential element cofactor for metalloenzymes and transcription factors. Zinc deficiency negatively impacts immune function, cellular growth, and development, while zinc toxicity causes nausea, vomiting, epigastric pain, lethargy, and fatigue [21,22].

The project was designed to develop and validate a ten-element panel by ICP-MS for analysis of whole blood, in order to quantify antimony (Sb), arsenic (As), bismuth (Bi), cadmium (Cd), cobalt (Co), lead (Pb), manganese (Mn), mercury (Hg), thallium (Tl), and zinc (Zn) for clinical testing to support patient care. The previous in-house method consisted of five ICP-MS assays to analyze ten elements, which had a negative impact on the turnaround time to result when multiple elements were ordered on the same specimen. Transitioning to a multi-element panel approach improved efficiency with laboratory workflow and turnaround time.

Materials and methods

IRB protocol

An approval for the retrospective analysis of clinical samples was obtained from the institutional review board (IRB) of the University of Utah (IRB #00082990).

Specimens

Previously collected whole blood (WB) samples used for proficiency testing, pooled whole blood samples from the de-identified patients spiked with the ten elements, and previously analyzed de-identified patient samples were used in this validation assay as specimens. The patient specimens were transported to ARUP Laboratories for testing and were collected in certified trace-element-free collection tubes. Trace and toxic element testing was performed by using ICP-MS method in a clean room to minimize environmental contamination.

Reagents

Triton X-100 was purchased from VWR (Radnor, PA) and ammonium hydroxide (NH_4OH) was purchased from Sigma-Aldrich (St. Louis, MO). A Barnstead Nanopure Diamond System from Thermo Scientific (Waltham, MA) was used for the clinical laboratory reagent water. Stock solutions at 1000 ppm were purchased from Inorganic Ventures (Christiansburg, VA) for antimony (Sb^{121}), arsenic (As^{75}), bismuth (Bi^{209}), cadmium (Cd^{111}), cobalt (Co^{59}), lead (Pb^{208}), manganese (Mn^{55}), mercury (Hg^{202}), thallium (Tl^{205}), and zinc (Zn^{66}). Gold (Au) was spiked at 1000 μg/L and utilized in the assay to stabilize mercury and to assist with elemental solubility in stock solutions to limit adhesion to the spray chamber surfaces and tubing. Goat blood matrix (Health Research Inc.) was used to prepare the calibration standards for the multi-element panel with every batch of patient specimens. The calibration curve employed the standard addition setting for data analysis, in which the diluent-only reagent blank was subtracted from the calibrator standards, quality control and patient samples. The calibration curve fit was linear and ignored the origin. There was no weighting applied to the calibration curve. Gallium (Ga, at mass 71) was used as the internal standard for Mn, Co, Zn, and As. Indium (In, at mass 115) was used as the internal standard for Cd and Sb. Iridium (Ir, at mass 193) was used as the internal standard for Hg, Tl, Pb, and Bi. The calibrator concentrations for each element are listed in Table 1. Quality control specimens were matrix-matched, produced in-house, and analyzed with every batch of patient samples.

Instrumentation

Supplemental Table S1 provides a comparison between the previous methods and the multi-element panel. The multi-element panel method utilized an Agilent 7700 ICP-MS instrument with a CETAC MVX-7100 μL workstation autosampler and syringe injection system. The MVX-7100 μL Workstation offers highly consistent, syringe driven, low volume and configurable flow rate sample introduction for quadrupole-based, high resolution and multi-collector ICP-MS instrumentation. This technology facilitated the analysis of samples with limited volume and batch analysis of volatile sample types. Helium gas was used in the collision cell to reduce or eliminate the formation of polyatomic species by kinetic energy discrimination (KED).

Operating conditions for the quadrupole ICP-MS instrument settings for the CETAC MVX-7100 μL workstation autosampler and instrument parameters for the Agilent 7700 are listed in Table 2

Performance tune checks were executed before each run to evaluate the gas parameters for KED and to check the manufacturer recommendations for sensitivity, which were >1000 CPS for mass 59, for the tuning solution. The double charged ions were monitored using Cerium (^{140}Ce) and the acceptance criteria was set to <3.0 % for the ratio of mass 70/mass 140. Oxide ions were also monitored using Ce and CeO. The acceptance criteria was set to <1.5 % for the ratio of mass 156 to mass 140.

Sample preparation

Fifty microliters of calibrator standards, quality controls, and patient samples were aliquoted into a 96-well (deep well) microplate (VWR, Radnor, PA). The samples were diluted with 950 μL of diluent, which contained beryllium (Be), gallium (Ga), indium (In), iridium (Ir) rhodium (Rh), and yttrium (Y), internal standards, gold (Au) as a stabilizer, 0.1 % Triton X-100, 1.75 % EDTA, and 1 % NH4OH, then

Table 1
The concentrations of the calibrators and simple linearity regression equations along with correlation coefficient for the multi-element panel.

Element	Units	AMR	Reference Range (Whole blood)	Standard 1	Standard 2	Standard 3	Standard 4	Linear equation	Correlation Coefficient
Antimony (Sb)	µg/L	1–25	0–6	1	8.75	16.25	25	$y = 1.00x + 0.22$	$R^2 = 0.99$
Arsenic (As)	µg/L	10–250	<=12.0	10	87.5	162.5	250	$y = 0.98x - 2.95$	$R^2 = 0.99$
Bismuth (Bi)	µg/L	1–25	0–5	1	8.75	16.25	25	$y = 1.00x + 0.15$	$R^2 = 0.99$
Cadmium (Cd)	µg/L	1–50	<=5.0	1	17.5	32.5	50	$y = 1.00x - 0.31$	$R^2 = 0.99$
Cobalt (Co)	µg/L	1–50	<=3.9	1	17.5	32.5	50	$y = 0.97x + 0.32$	$R^2 = 0.99$
Lead (Pb)	µg/dL	2–100	<=4.9	2	35	65	100	$y = 1.00x - 0.34$	$R^2 = 0.99$
Manganese (Mn)	µg/L	1–80	4.2–16.5	1	28	52	80	$y = 1.00x - 0.20$	$R^2 = 0.99$
Mercury (Hg)	µg/L	3–80	<=10.0	3	28	52	80	$y = 0.99x + 0.85$	$R^2 = 0.99$
Thallium (Tl)	µg/L	1–50	<=2.0	1	17.5	32.5	50	$y = 1.01x - 0.46$	$R^2 = 0.99$
Zinc (Zn)	µg/dL	50–1500	440–860	50	525	975	1500	$y = 1.00x - 8.64$	$R^2 = 0.99$

Table 2
Optimum operating conditions for the quadrupole ICP-MS instrument settings for the CETAC MVX-7100 µL Workstation autosampler and Instrument parameters for the Agilent 7700.

The quadrupole ICP-MS instrument settings for the CETAC MVX-7100 µL Workstation autosampler	
Parameter	**Optimum operating conditions**
Sample/Loop volume	490 µL
Carrier dispense rate	650 µL/min
Carrier dispense volume	700 µL – to push the sample through the loop
Quick Push	Enabled with a volume of 1000 µL
Extra Rinse	Enabled
Sample Mixing	3 cycles with an uptake volume of 30 µL
Front and back air gaps	used to help isolate the injection sample bolus and decrease stabilization times
Instrument parameters for the Agilent 7700	
Parameter	**Optimum operating conditions**
He gas flow rate	3.0 mL/min
Peak Pattern	1 point
Replicates	3
Sweeps/Replicate	100
Energy Discrimination	5.0 V
Integration Time/Mass	0.3 s for Mn, Co, Zn, Ga, As, Cd, In, Sb, Ir, Hg, Tl, Pb, Bi
Octopole (OctP) Bias	−13.0 V
Radio frequency (RF) Power	1550 W
Sample Depth	8.0 mm
Option Gas and Makeup/Dilution Gas	0
S/C Temp	2C
Extract 1	0 V
Cell Entrance	−30 V
Cell Exit	−70 V
Calibration Mode	Standard Addition. There was no calibration weighting applied

vortexed.

ICP-MS analysis

The diluted WB specimens were aspirated into the ICP-MS using the CETAC MVX-7100 µL Workstation autosampler. The ICP-MS was calibrated for As, Bi, Cd, Co, Hg, Mn, Pb, Sb, Tl, and Zn testing. The aspirant was ionized in argon plasma and atomized in the instrument spray chamber. The argon gas was flowing at atmospheric pressure through a series of concentric glass tubes, referred to as a torch, which generated the argon plasma. The drive coil surrounded the outlet end of the torch, where up to 2.5 kW of radio frequency power was applied. This power sustained a plasma discharge in the argon at a temperature of ~ 6000 K. The ions exited the plasma, passed through the interface of the instrument, then arrived at the entrance of the collision cell where helium gas was introduced to remove the polyatomic interferences. Potential polyatomic interferences for each of the masses/elements in the method are listed in Table 3. All elements were evaluated in gas mode with He at 3.0 mL/min. The main quadrupole filtered the ions from the collision cell. The detector counted and summed the electron abundances for each element. The calibration curve was used to quantify the concentration of the elements in the specimen. The analytical run-time was 2.5 min.

Method validation experiments:

Accuracy

Accuracy was determined over five different days by evaluation of patient blood specimens (N = 7–95, per element), proficiency testing blood samples (N = 50, per element), and spiked blood samples (N = 12–37, per element). Historical method results were used to compare repeat testing of patient specimens, certified values were used to compare proficiency testing results, and the calculated values were used to compare spikes samples. Comparison plots with correlation coefficient and a relative difference plot were generated for each element. A slope between 0.9 and 1.1 with an intercept less than the LOQ and a correlation coefficient ≥ 0.95 was used as acceptance criteria for each element.

Linearity

Linearity of the assay was determined by preparing samples with high concentrations of elements and specimens with low concentrations of elements in various proportions to achieve the appropriate target concentrations. All ten elements were spiked in the high concentration samples. The low samples were created by diluting the blood pools with CLRW to low endogenous values for all elements. Four replicates, at each desired concentration, were performed on the same day. The acceptance criteria were a slope between 0.9 and 1.1 with a y-intercept less than the LOQ and a correlation coefficient ≥ 0.95.

Table 3
Potential sources of interferences from polyatomic and doubly charged species.

Element	Polyatomic species
^{121}Sb	$^{105}Pd^{16}O^+$
^{75}As	$^{40}Ar^{35}Cl^+$, $^{59}Co^{16}O^+$, $^{36}Ar^{38}Ar^1H^+$, $^{38}Ar^{37}Cl^+$, $^{36}Ar^{39}K$, $^{43}Ca^{16}O_2$, $^{23}Na^{12}C^{40}Ar$, $^{12}C^{31}P^{16}O_2^+$
^{209}Bi	$^{193}Ir^{16}O^+$
^{111}Cd	$^{95}Mo^{16}O^+$, $^{94}Zr^{16}O^1H^+$, $^{39}K_2^{16}O^1_2H^+$
^{59}Co	$^{43}Ca^{16}O^+$, $^{42}Ca^{16}O^1H^+$, $^{24}Mg^{35}Cl^+$, $^{36}Ar^{23}Na^+$, $^{40}Ar^{18}O^1H^+$, $^{40}Ar^{19}F^+$
^{208}Pb	$^{192}Pt^{16}O^+$
^{55}Mn	$^{40}Ar^{14}N^1H^+$, $^{39}K^{16}O^+$, $^{37}Cl^{18}O^+$, $^{40}Ar^{15}N^+$, $^{38}Ar^{17}O^+$, $^{36}Ar^{18}O^1H^+$, $^{38}Ar^{16}O^1H^+$, $^{37}Cl^{17}O^1H^+$, $^{23}Na^{32}S^+$, $^{36}Ar^{19}F^+$
^{202}Hg	$^{186}W^{16}O^+$, $^{184}W^{18}O^+$
^{205}Tl	$^{189}Os^{16}O^+$
^{66}Zn	$^{50}Ti^{16}O^+$, $^{34}S^{16}O_2^+$, $^{33}S^{16}O^1_2H^+$, $^{32}S^{16}O^{18}O^+$, $^{32}S^{17}O_2^+$, $^{33}S^{16}O^{17}O^+$, $^{32}S^{34}S^+$, $^{33}S_2^+$

Sensitivity

The sensitivity was determined by evaluating the limit of the blank (LOB), limit of detection (LOD), and limit of quantitation (LOQ). The LOB was analyzed in four replicates over a period of five days using diluent-only blank samples. The mean and standard deviation (SD) were determined, and the LOB was calculated as the average plus three times the SD. The acceptance criterion for the LOB was a value less than the LOQ for each element. The LOD and LOQ values were determined by analyzing blood pools, n = 4 for five days, with targets at the desired low concentrations. The target for the LOD was half the concentration of the LOQ. The accuracy and %CV for LOQ was set with ≤ 20 % as acceptable.

Imprecision

The intra-run and inter-run imprecision were determined for the assay. Both used blood samples spiked to the desired concentrations. Intra-run imprecision was performed by analyzing n = 20 on the same day of a sample of low and high concentration, separately. Inter-run imprecision was performed by separately analyzing four low concentration and four high concentration samples per day for five days. The acceptability criteria for %CV of each element was set to be ≤ 20 %.

Carryover

Two blood pools sets were prepared for the evaluation of carryover. One pool was spiked to ten times the concentration of the highest calibrator (H) and the other pool was unspiked (L). These pools were run in the pattern of L1, L2, L3, H1, H2, L4, H3, H4, L5, L6, L7, L8, H5, H6, L9, H7, H8, *L*10, H9, *H*10, *L*11. If carryover interferences were present, the low samples, which were immediately following the high samples (L4, L5, L9, *L*10, *L*11) would quantify at a higher concentration than the low samples following the low samples (L2, L3, L6, L7, L8) in the sequence. Percent carryover was calculated using following formula:

$$\text{Percent Carryover} = \frac{(\text{Average of high} - \text{low samples}) - (\text{Average of low} - \text{low samples}) \times 100}{\text{Average of high samples}}$$

The experiment was performed on three different days, with an acceptability cutoff set to 1 %.

Dilution

Evaluation of dilution was performed using a blood pool spiked with all ten elements at a high concentration. The sample was diluted 2-fold, 5-fold, 10-fold, and 20-fold with clinical laboratory reagent water (CLRW). Four replicates at each dilution level were ran along with undiluted sample. The diluted samples were processed and analyzed. The acceptability cutoff was ± 10 % deviation.

Retrospective patient data analysis

To evaluate the ranges of patient results in comparison to the reference range for each element: Sb, As, Bi, Cd, Co, Pb, Mn, Hg, Tl and Zn, the retrospective patient results were retrieved from the internal laboratory information system at ARUP laboratories (Salt Lake City, UT). The limited data set did not include patient identifiers and, therefore, it was not possible to remove patients who underwent serial monitoring during the time in which the dataset was generated.

Stability

Element stability was evaluated at ambient RT and 4 °C for 14 days in original collection tube or trace element-free standard transport tube. Analyte stability was evaluated by running a sample pool for all 86 open positions of a plate over three different days. The run was evaluated for drift by determining the percent difference between the average of the first five samples and the average of the last five samples, as well as determining the %CV for each run. Passing criteria were a %CV less than 20 % and a percent difference less than 10 % per repeat for the analyte.

Statistical analysis

The mean, standard deviation, % coefficient of variation, slope and y-intercept and correlation coefficient for the assay validation characteristics were calculated using Microsoft Office Excel (Microsoft, WA, USA).

Results

Accuracy

Between 69 and 182 samples, depending on element tested, were compared to previous test results from the original methods and consisted of patient samples, matrix-matched fortified samples, and certified concentrations from proficiency testing samples. The slopes for the linear regression analyses ranged from 0.94 to 1.03, y-intercepts were below the LOQ, and a R^2 was 0.97–1.00 or each element. The linear regression and bias plots for each element are shown in Fig. 1A, 1B, 1C and 1D. The results for the accuracy for each element was within the acceptance criteria.

Linearity

The linearity data is shown in Table 1. The best linear fit for all ten elements was observed from the lowest calibrator concentration to the highest calibrator concentration. The maximum specimen dilution was 2x for all the elements. The values were reported as > ULOQ if they were quantified above the highest calibrator.

Sensitivity

The LOB was less than 0.4 µg/L for Sb, As, Bi, Cd, Co, Mn, Hg, Pb and Tl. The LOB was 2.07 µg/dL for Zn. The LOQ was established at 0.8–2 µg/L for Sb, Bi, Cd, Co, Mn, Tl with %CVs of 5.4 %, 5.7 %, 7.6 %, 4.2 %, 10.3 % and 2.7 %, respectively. The LOQ for As was 9.45 µg/L with a % CV 1.8 % and the LOQ for Hg was 3.67 µg/L with a %CV of 5.5 %. The LOQ for Pb and Zn was established at 1.75 µg/dL and 43.95 µg/dL with a %CV of 5.4 % and 1.5 %, respectively. The observed LOD and LOQ results for the elements are mentioned in Supplemental Table S2.

Imprecision

The imprecision of the multi-element panel for the lowest and highest concentration is summarized in Table 4. The assay met the analytical criteria for imprecision.

Carryover

The criteria for carryover were set such that there should be less than 1 % contribution from the high concentration sample into sample at the LOQ concentration for each analyte. The contribution from carryover met acceptance criteria and did not cause falsely elevated results for the low concentration patient samples. The observed carryover results for the elements are mentioned in Supplemental Table S3.

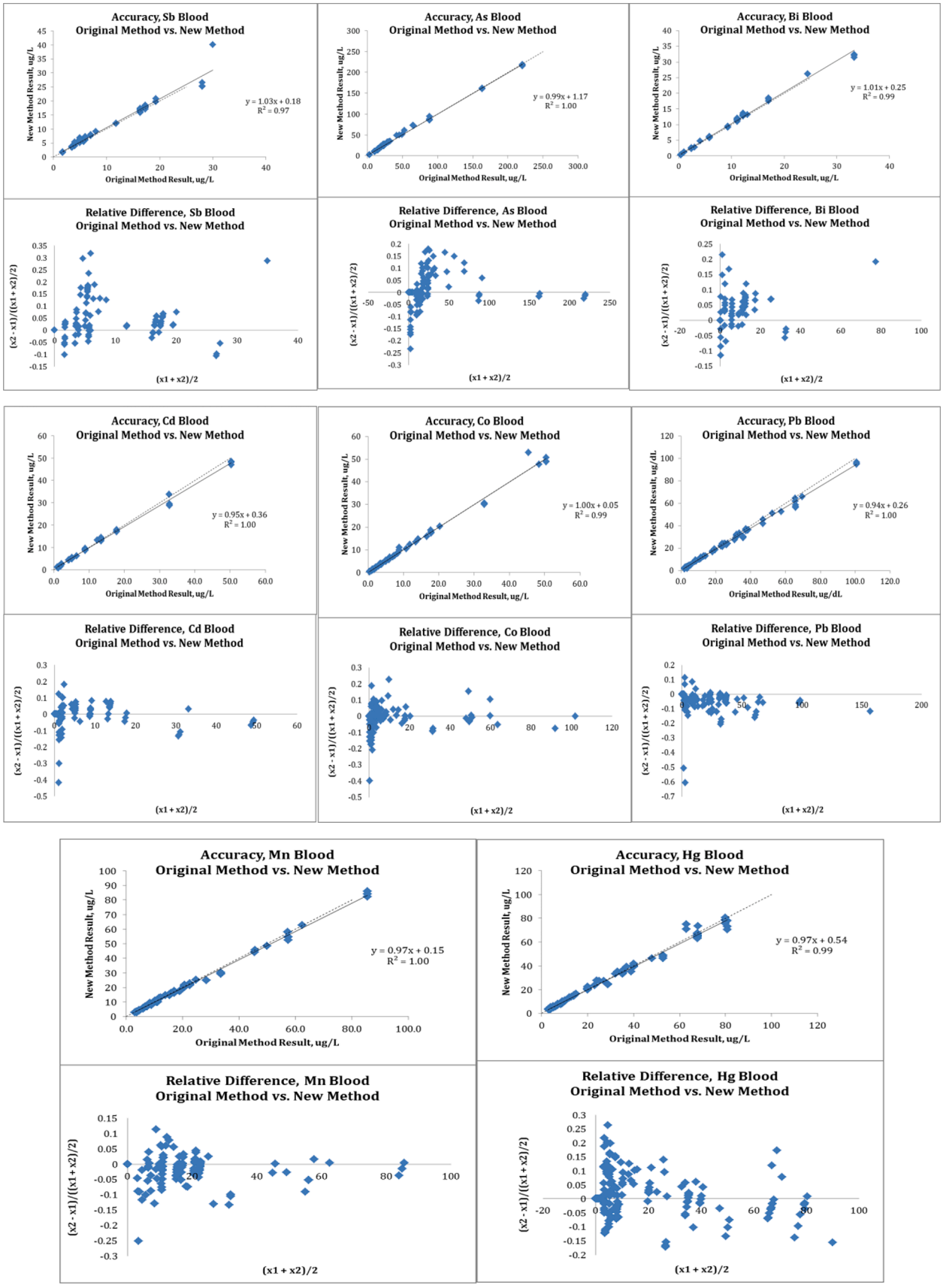

Fig. 1. The simple linear regression and Bias plots for Sb, As, Bi (A), Cd, Co, Pb (B), Mn, Hg (C) and Tl, Zn (D) analyzed for evaluation of accuracy of the method.

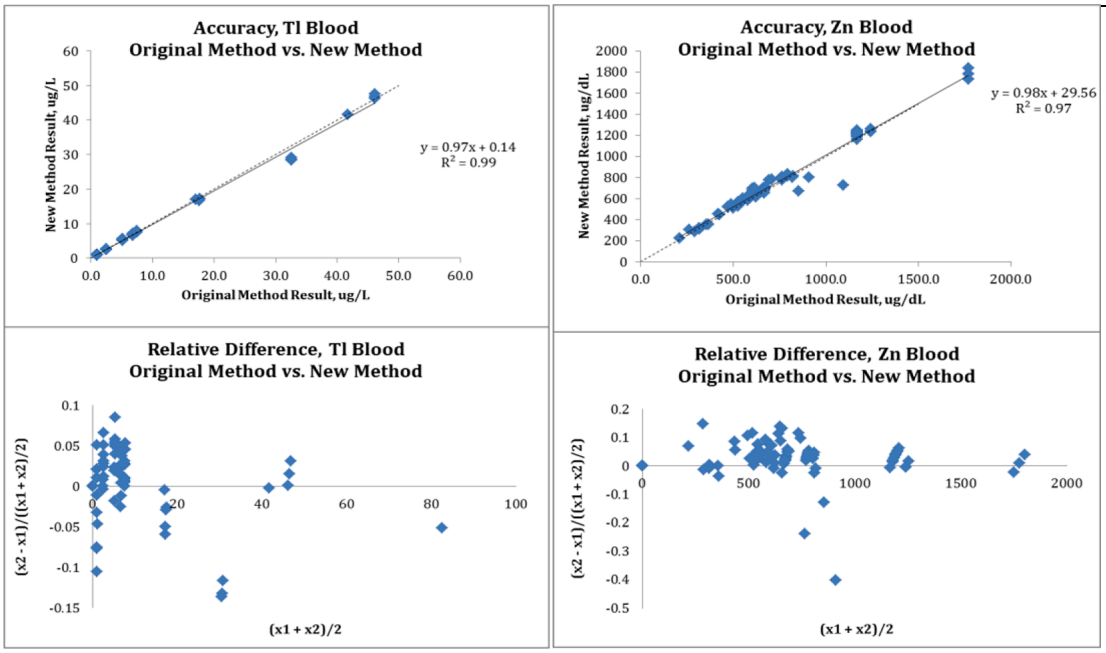

Fig. 1. (*continued*)

Table 4
The low QC and high QC total Imprecision (%CV) for the multi-element panel.

Element	Low QC concentration	Low % CV	High QC concentration	High % CV
Antimony (Sb)	1.4 µg/L	4.1 %	22.25 µg/L	2.7 %
Arsenic (As)	11.69 µg/L	2.9 %	217.54 µg/L	2.9 %
Bismuth (Bi)	1.17 µg/L	2.8 %	21.97 µg/L	2.2 %
Cadmium (Cd)	1.18 µg/L	9.0 %	44.0 µg/L	3.0 %
Cobalt (Co)	1.16 µg/L	4.8 %	44.99 µg/L	2.5 %
Lead (Pb)	2.05 µg/L	2.6 %	80.23 µg/L	3.0 %
Manganese (Mn)	1.27 µg/L	9.0 %	67.87 µg/L	2.6 %
Mercury (Hg)	3.3 µg/L	4.7 %	74.25 µg/L	3.1 %
Thallium (Tl)	1.08 µg/L	3.0 %	43.04 µg/L	3.2 %
Zinc (Zn)	55.58 µg/L	2.4 %	1237.75 µg/L	2.8 %

Reference ranges

The reference ranges for the ten elements were not established by the laboratory, but were adopted from resources, such as the Agency for Toxic Substances & Disease Registry, Centers for Disease Control, American Conference of Governmental Industrial Hygienists, and clinical publications for biological monitoring.

Retrospective data analysis

Retrospective patient data was evaluated from the date that the new method went live in production. Table 5 describes the analysis of results and provides information on the age of the patients, the reference range per element, the total number of patients and the percentage of patient results that were within or outside of the reference range. For all the ten elements, 75.7–99.9 % of patient results were within the reference range.

Stability

For all elements except mercury, if the specimen is drawn and stored in the appropriate container, the trace element values do not change with time. For mercury, as it is volatile, concentrations may decrease after seven or more days of storage. Analyte stability data is shown in Supplemental Table S4.

Discussion

Historically, our laboratory used five separate methods to measure a combined ten elements in whole blood, which required a minimum of five minutes per patient, 500 µL of patient sample, and 4800 µL of diluent. The elements included were arsenic (As), bismuth (Bi), cadmium (Cd), cobalt (Co), mercury (Hg), manganese (Mn), lead (Pb),

Table 5
The retrospective patient data analysis from a National Reference Laboratory.

Elements (Whole Blood)	Age Range (Years)	Reference Interval (Whole blood)	LOQ	Total Volume (N)	% Patient within the Reference Interval	% Patient below the Reference Interval	% Patient above the Reference Interval
Antimony	0–89	0–6 ug/L	1.26	271	88.93 % (N = 241)	N/A	10.70 % (N = 29)
Arsenic	0–97	<=12.0 ug/L	9.45	8633	98.22 % (N = 8479)	N/A	1.76 % (N = 152)
Bismuth	7–89	0–5 ug/L	1.03	752	98.40 % (N = 740)	N/A	1.59 % (N = 12)
Cadmium	0–100	<=5.0 ug/L	0.86	9483	99.66 % (N = 9451)	N/A	0.34 % (N = 32)
Cobalt	0–95	<=3.9 ug/L	1.03	4448	75.76 % (N = 3370)	N/A	24.24 % (N = 1078)
Lead	0–107	<=4.9 ug/L	1.75	267,479	94.34 % (N = 252330)	N/A	5.66 % (N = 15148)
Manganese	0–99	4.2–16.5 ug/L	1.24	6177	79.47 % (N = 4909)	1.93 % (N = 119)	18.60 % (N = 1149)
Mercury	0–103	<=10.0 ug/L	3.67	46,722	89.20 % (N = 41675)	N/A	10.80 % (N = 5047)
Thallium	1–98	<=2.0 ug/L	0.94	996	99.90 % (N = 995)	N/A	0.10 % (N = 1)
Zinc	0–96	440–860 ug/L	43.95	6687	86.50 % (N = 5784)	13.06 % (N = 873)	0.45 % (N = 30)

antimony (Sb), thallium (Tl), and zinc (Zn). The impact to the workflow was that the laboratory had to pre-sort samples, prioritize testing for patient samples, route samples for testing on all five methods and monitor run times to accommodate laboratory test volumes. The development of a whole blood multi-element panel affords a single assay to increase batch run efficiency using a unified workflow for all of the elements. The benefit of implementing a multi-element panel was to automate sample preparation on the liquid handler, decrease the specimen volume required for testing, by a factor of ten, and reduce the analytical run-time by ~ 55 %. The multi-element blood panel was developed utilizing matrix-matched calibrators and quality controls samples to improve analytical accuracy for all elements and assay performance was also monitored by an external proficiency testing program.

Retrospective patient data were analyzed to compare the distribution of patient results to the reference interval. Retrospective data analysis for element testing in whole blood, over the past ~ 3 years, demonstrated that > 98 % patients were within the reference range for As, Bi, Cd, Tl and Zn, based on the reference ranges in Table 5. However, 5.6 – 24.2 % of patients were above the reference range for Sb, Co, Pb, Mn and Hg. Data from the American Association of Poison Control Centers (AAPCC) documents the number of case exposures in 2019. For heavy metal exposure (applicable to our in-house multi-element panel in whole blood), the 2019 report documents As (676 cases), Cd (77 cases), Pb (2467 cases), Mn (50 cases), Hg (1369 cases), Tl (64 cases) and unknown heavy metal (2730 cases) [23]. Pb and Hg were the highest individual heavy metal exposures in that year [23]. Retrospective data analysis from ARUP Laboratories identified patients that had elevated concentrations of Sb, Co, and Mn exposures. Elevated antimony concentrations may come from unintentional skin contamination and contamination from blood collection tube/device. Elevated Sb results should be confirmed with another specimen collection from a Sb-free collection tube/device. High concentrations of Mn could come from total parenteral nutrition and patients on this supplement should be monitored to minimize the risk for toxicity. Elevated results for Co could occur from occupational exposure, metal ion release from joint replacement, and skin or blood collection related contamination. Therefore, confirmation testing should be performed using a metal-free collection device.

The efficiencies gained from a single combined method include reduced diluent volume and analysis time, improved imprecision and accuracy, improved reproducibility, and the capability of testing in a 96-well format. The limitation of this study was that retrospective patient data did not contain patient clinical history, diagnosis, treatment and the rationale for evaluating these elements in blood in the laboratory information system. The panel assay by ICP-MS met all validation criteria for clinical biological monitoring of trace and toxic elements in whole blood specimens to support patient care. [24].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors want to thank the staff of the Trace and Toxic Element section at ARUP Laboratories for validation support to transition the assay into the clinical lab. We also thank the ARUP Institute for Clinical and Experimental Pathology and a report developer, David Davis, for de-identification of the retrospective data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmsacl.2022.12.005.

References

[1] M.G. Bornhorst JA, Trace and toxic elemental testing in the clinical laboratory, Lab medicine 37(11) (2006) 690-695.
[2] J. Jin, L. Mulesa, M. Carrilero Rouillet, Trace Elements in Parenteral Nutrition: Considerations for the Prescribing Clinician, Nutrients 9 (5) (2017).
[3] B.L. Strathmann FG, Toxic elements, in: H.A.a.W.C. Rifai N (Ed.), Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th ed, Elsevier2018, pp. 888-924.
[4] M.A. Velez-Quinones, H. Xu, N. Vo, J.M. Gaitens, M.A. McDiarmid, M.R. Lewin-Smith, F.G. Strathmann, Determination of Thirteen Trace and Toxic Elements in Urine Using Inductively Coupled Mass Spectrometry, Curr. Protoc. Toxicol. 78 (1) (2018) e58.
[5] R. Rahil-Khazen, B.J. Bolann, A. Myking, R.J. Ulvik, Multi-element analysis of trace element levels in human autopsy tissues by using inductively coupled atomic emission spectrometry technique (ICP-AES), J. Trace Elements Med. Biol. 16 (1) (2002) 15–25.
[6] K.L. Johnson-Davis, C. Farnsworth, C. Law, R. Parker, Method validation for a multi-element panel in serum by inductively coupled plasma mass spectrometry (ICP-MS), Clin. Biochem. 82 (2020) 90–98.
[7] B. Xu, Y. Zhang, Y. Chen, M. Zeng, J. Feng, J. Tang, L. Yu, Simultaneous multielement analysis by ICP-MS with simple whole blood sample dilution and its application to uremic patients undergoing long-term hemodialysis, Scand. J. Clin. Lab. Invest. 80 (3) (2020) 247–255.
[8] M. Krachler, K.J. Irgolic, The potential of inductively coupled plasma mass spectrometry (ICP-MS) for the simultaneous determination of trace elements in whole blood, plasma and serum, J. Trace Elements Med. Biol. 13 (3) (1999) 157–169.
[9] C. Richardson, E. Roberts, S. Nelms, N.B. Roberts, Optimisation of whole blood and plasma manganese assay by ICP-MS without use of a collision cell, Clin. Chem. Lab. Med. 50 (2) (2011) 317–323.
[10] F.B. Nordberg GF, Nordberg M, Handbook on the Toxicology of Metals, Fourth edition, Elsevier, Waltham, MA., 2015.
[11] S. Sundar, J. Chakravarty, Antimony toxicity, Int. J. Environ. Res. Public Health 7 (12) (2010) 4267–4277.
[12] M. Peana, A. Pelucelli, S. Medici, R. Cappai, V.M. Nurchi, M.A. Zoroddu, Metal Toxicity and Speciation: A Review, Curr. Med. Chem. (2021).
[13] K. Kendel, R. Kabiri, S. Schäffer, Chronic bismuth poisoning with encephalopathy and dementia, Dtsch Med Wochenschr 118 (7) (1993) 221–224.
[14] M. Rafati Rahimzadeh, M. Rafati Rahimzadeh, S. Kazemi, A.a., Moghadamnia, Cadmium toxicity and treatment: An update, Caspian, J Intern Med 8 (3) (2017) 135–145.
[15] A.W. Schaffer, A. Pilger, C. Engelhardt, K. Zweymueller, H.W. Ruediger, Increased blood cobalt and chromium after total hip replacement, J. Toxicol. Clin. Toxicol. 37 (7) (1999) 839–844.
[16] D.G. Barceloux, Cobalt, J. Toxicol. Clin. Toxicol. 37 (2) (1999) 201–206.
[17] A.L. Wani, A. Ara, J.A. Usmani, Lead toxicity: a review, Interdiscip Toxicol 8 (2) (2015) 55–64.
[18] G.R. Evans, L.N. Masullo, Manganese Toxicity, StatPearls, StatPearls Publishing.
[19] Copyright © 2021, StatPearls Publishing LLC., Treasure Island (FL), 2021.
[20] R.A. Bernhoft, Mercury toxicity and treatment: a review of the literature, J Environ Public Health 2012 (2012), 460508.
[21] A. Saddique, C.D. Peterson, Thallium poisoning: a review, Vet Hum Toxicol 25 (1) (1983) 16–22.
[22] L.M. Plum, L. Rink, H. Haase, The essential toxin: impact of zinc on human health, Int. J. Environ. Res. Public Health 7 (4) (2010) 1342–1365.
[23] G.J. Fosmire, Zinc toxicity, Am. J. Clinical Nutrition 51 (2) (1990) 225–227.
[24] D.D. Gummin, J.B. Mowry, M.C. Beuhler, D.A. Spyker, D.E. Brooks, K.W. Dibert, L. J. Rivers, N.P.T. Pham, M.L. Ryan, 2019 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 37th Annual Report, Clinical toxicology (Philadelphia, Pa.) 58(12) (2020) 1360-1541.