An Optimized Method to Obtain Human Biomonitoring Data of Al, Bi, Ce, Cr, Ge, La, Li, Nd, Pr, Ti, Te and Y in Whole Blood

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Background

- The availability of biomonitoring data and its use in human health risk assessments have increased over the past 10 years. The use of human biomonitoring data allows for direct and more precise assessment of the distribution of exposure in a given population.
- North American population-level biomonitoring data for many of the remaining Canadian Chemical Management Plan assessment priorities are lacking.
- Integrated within the Canadian Health Measures Survey (CHMS), the biobank is a nationally representative cohort of biospecimens to be utilized in novel health research.

Objective

- Develop an optimised method to obtain human biomonitoring data on priority metals and trace elements in whole blood biobank samples (elements are listed in the table below).

Introduction

Biomarker Selection

- Whole blood is considered to be an acceptable matrix
- Whole blood measures concentrations of elements at the target site (internal dose) compared to urine.
- Comprises all of the blood components (e.g., proteins, erythrocytes, platelets) compared to serum or plasma.

Sample Demographics

In Canada, general population biomonitoring data are collected since 2007, by the Canadian Health Measures Survey (CHMS), a national survey conducted by Statistics Canada in association with Health Canada and the Public Health Agency of Canada. Results are population weighted to represent 96.3% of the Canadian population, aged 3 to 79 years.

CHMS- Cycle 2 collection sites
https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/ewh-semt/alt_formats/pdf/pubs/contaminants/chms-ecms-cycle2/chms-ecms-cycle2-eng.pdf

Atlantic	St. John's, Newfoundland and Labrador , Colchester and Pictou counties, Nova Scotia
Quebec	Laval, South Montérégie, Gaspésie, North Shore Montréal
Ontario	Central and East Ottawa, South of Brantford, Southwest Toronto, East Toronto, Kingston, Oakville
Prairies	Edmonton-Alberta, Winnipeg- Manitoba, Calgary-Alberta
British Colombia	Richmond, Central and East Kootenay, Coquitlam

CHMS-Biobank
(Available biospecimens at present)
https://www.statcan.gc.ca/eng/survey/household/5071x

Matrix	Age range (years)	Sample size per survey cycle	Volume available
Serum	3 – 79	5700	0.5 ml
	3 – 39	3500	0.5 ml
	6 – 79	5100	0.5 ml
	12 – 79	4100	0.5, 1.0 ml
	20 – 79	3100	0.5, 1.0 ml
Plasma	3 – 79	5700	0.5 ml
	6 – 79	5100	0.5 ml
	12 – 79	4100	0.5 ml
Whole blood	6 – 79	5100	1 ml
	12 – 79	4100	1 ml
Urine	3 – 79	5700	1.0, 2.0, 4.5 ml
	6 – 79	5100	1.0, 4.5 ml
DNA	14 – 79	3700	1 µg

Biobank access and project flow

CHMS Biobank Approval
* Funding
* Privacy considerations
* Ethical considerations
* Peer- approved proposal
* Contracts in place

Transport of samples
Approx. 6000 samples shipped from Winnipeg (biorepository) to the laboratory

Sample analysis
At Health Canada laboratory at Longueuil
By Inductively Coupled Plasma Spectrometry (ICP-MS)

Elemental concentrations
Lab exported results via a secure portal to Statistics Canada to compile a master data file

Descriptive data
Statistics Canada created data tables for risk assessments and researchers for their analyses

Methods

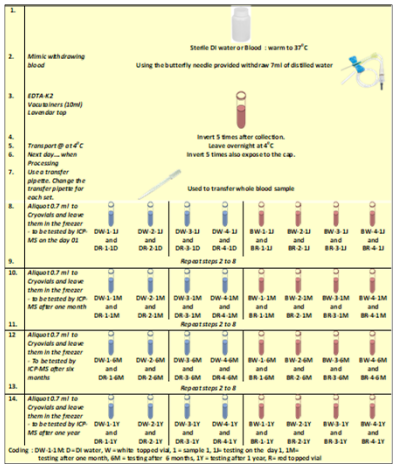
ICP-MS Agilent 7500-Operation Parameters

RF Power:	1500 W
Carrier gas/Auxiliary gas flow rate:	0.90 L/min
Plasma gas flow rate:	14.9 L/min
Make-up (booster) gas flow rate:	0.26 L/min
Nebulizer gas flow rate:	0.90 L/min
Helium gas flow rate (collision cell):	6.2 ml/min
Peristaltic pump flow rate:	0.1 rps
Integration time:	0.9 sec in standard mode, 4.5 sec in high flow He mode
Internal standards:	^{103}Rh, ^{115}In, ^{159}Tb, ^{187}Re
Isotope used for qualification:	Standard mode: ^{27}Al, ^{209}Bi, ^{140}Ce, ^{7}Li, ^{139}La, ^{146}Nd, ^{141}Pr, ^{125}Te and ^{89}Y. Octopole reaction system (ORS): High flow He mode: ^{53}Cr, ^{74}Ge, ^{49}Ti
Polyatomic interferences:	^{53}Cr [ClO,ClOH], ^{74}Ge [FeO, corrected for Se74], ^{115}In [^{115}Sn]

Laboratory method

- *Sample volume used*: 200 µL of whole blood. Analysis Method QLA-MA-0062, optimized by the laboratory.
- *Possibility of leaching/adsorption of metals from the associated materials during blood withdrawal and storage:* Addressed by sequential testing of 2 control sets with de-ionized water and human blood on two types of storage tubes. The procedure from the withdrawal of the blood samples to storage and analysis were mimicked (using a butterfly needle, vacutainer, vials). Blood pool was obtained from Canadian Blood Services.
- *Possible non-homogeneity of the samples during storage/transportation and possible contamination during analysis*: Addressed by conducting accuracy, recovery, reproducibility and blank studies and necessary confirmatory analysis in each analytical batch throughout the project (1.5 years) using Blood reference materials/Control spiked blood samples and Method blanks.

Scheme for Leach testing

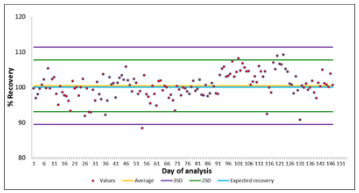


Results

Method reporting limits and QC/ QA of elements

Element	MRL (ug/L)	QC Material	QC Concentration (µg/L)	Correlation Coefficient (r)	Accuracy/ recovery (%)	% CV
Al (Aluminum)	8	SeroNorm L2	68.9	0.9994	96	6
Bi (Bismuth)	0.1	QMBQ1416	1.10	0.9989	92	3
Ce (Cerium)	0.05	QMBQ1416 spk	1.00	0.9998	100	3
		SeroNorm L2	0.086		105	6
Cr (Chromium)	0.7	QMBQ1416	3.21	0.9996	93	5
Ge (Germanium)	1	QMBQ1416 spk	1.00	0.9998	93	11
La (Lanthanum)	0.05	QMBQ1416 spk	1.00	0.9996	100	3
		SeroNorm L2	0.090		112	6
Li (Lithium)	0.4	QMBQ1416 spk	1.00	0.9997	94	11
Nd (Neodymium)	0.05	QMBQ1416 spk	1.00	0.9998	100	4
		SeroNorm L2	0.086		95	7
Pr (Praseodymium)	0.02	QMBQ1416 spk	1.00	0.9996	100	3
		SeroNorm L2	0.021		101	7
Te (Tellurium)	0.4	QMBQ1416	6.86	0.9993	91	7
Ti (Titanium)	10	SeroNorm L2	10.3	0.9956	98	10
Y (Yttrium)	0.06	QMBQ1416 spk	1.00	0.9996	99	3
		SeroNorm L2	0.077		95	9

Recovery studies of QC materials tested throughout the project



Summary data- whole blood elemental concentrations (µg/L) for the Canadian population aged 3 to 79 years (n=5757)

Element	MRL (µg/L)	%<MRL	50th percentile (95% CI)	95th percentile (95% CI)
Aluminum	8.0	97.08	<8.0	<8.0
Bismuth	0.1	95.43	<0.1	<0.1
Cerium	0.05	99.53	<0.05	<0.05
Chromium	0.7	96.63	<0.7	<0.7
Germanium	1.0	100	<1.0	<1.0
Lanthanum	0.05	99.72	<0.05	<0.05
Lithium	0.4	33.58	0.47 (0.43-0.51)	1.3 (1.2 – 1.4)
Neodymium	0.05	99.88	<0.05	<0.05
Praseodymium	0.02	99.91	<0.02	<0.02
Tellurium	0.4	100	<0.4	<0.4
Titanium	10.0	99.97	<10.0	<10.0
Yttrium	0.06	99.88	<0.06	<0.06

- The modified ICP-MS method to detect these metals in whole blood samples gave reproducible results.
- Accessing existing biobanks such as the CHMS are a cost-effective, time efficient way of obtaining biological samples for human biomonitoring data.
- Using the biobank, it was possible to obtain nationally representative data from approximately 6000 Canadians on 12 substances.
- Some of these elements were measured in humans for the first time, filling an important data gap for human health risk assessments.

Discussion

- Resultant concentrations were expected to be at ppt or ppb levels. The MRLs of ICP-MS for the determination of Bi, Ce, Cr, La, Li, Nd, Pr, Te, G and Y are sufficiently low. For Al and Ti, MRLs were higher than desired. In this multi-element analysis, given the diverse group of elements examined, instruments could not be optimized for all elements. Because Al is an ubiquitous element and Ti has inherent polyatomic interferences, ultra-low concentration analyses were not possible.
- High resolution instrumentation or ICP-MSMS equipped with reaction cell may have resulted in lower MRLs. However, this instrumentation was not available for this project. Analysis by such instrumentation would have taken more time and therefore, would have been more expensive.
- Recoveries were in an acceptable range of 70-130%; the majority were between 80-120%. The inter-assay CV% ranged from 3-11%. Accuracy and CV on the reference materials were very good (although SeroNorm concentration for Al was 10x the MRL).
- Results of confirmatory analyses gave Relative Standard Deviations (RSDs), less than or equal to 30%. Mostly, less than or equal to 15%.
- Leach testing showed no contaminations from the storage or associated materials used for analysis of Al, Bi, Ce, Cr, Ge, La, Li, Nd, Pr, Te, Ti and Y at the MRLs. The associated materials tested were made of plastic or glass except for the needles. Although significant contaminations have been reported in other studies for other elements from various associated materials, associated materials tested in this project were not found to contribute significantly to the overall concentration in elements that were analysed.

Conclusions

- High confidence in biomarker data due to the rigor of the QC/QA method.
- Leach testing did not identify any contamination for these elements at the MRLs.
- MRLs for Al and Ti were higher than desired. Multi-element analysis by ICP-MS is a suitable method for determination of many analytes in a large number of samples. However, single quad ICP–MS has limitations, mainly related to polyatomic interferences in the analysis of bio fluids resulting in higher detection limits for these elements. Also, some elements such as aluminum are ubiquitous, ultra-low concentration analyses are difficult because of inadvertent contamination at the instrument, laboratory environment or at the sample collection level.

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