Analysis of Cd, Pb and Hg in blood samples of the reference population group: adults (40-59y) (BMH-Wal3)

METHODOLOGICAL INFORMATION

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1. Introduction

This report describes the methodology used for the analysis Cd, Pb and Hg in blood samples of the reference population group of adults (40-59y) in the context of BMH-Wal3.
Blood samples were collected by ISSeP and transferred for analysis to the trace element laboratory of Sciensano. Samples were kept at -20°C until analysis.

Statistical treatment of the data and calculation of reference values (RV_{95}) was done according to the document 'Méthodologie d'élaboration des valeurs de référence dans le cadre du projet BMH-Wal 1' issued by ISSeP. These results are summarized per element in separate reports. Raw data were transfered to ISSeP in excel files.

2. Materials and Methods

2.1. Standards and chemicals

Nitric acid (Suprapur, SpA 67-69%,) was purchased from Romil (UK), and H_2O_2 (30%, pergydrol, pro analyse) from Merck (Germany). A multi-element standard solution of 1000 mg/kg used for quantification of Cd and Pb was purchased from Analytika (Prague, Czech Republic). A multi-elemental 5 µg L^{-1} tuning solution (Spectropure, Arlington, USA) or 1 µg L^{-1} tuning solution (Thermo Fisher Scientific, Belgium) was used for tuning the ICP-MS. Water used in this study was home produced doubly distilled water (Aquatron, Cole-Parmer, UK).

2.2. Determination of Cd and Pb by ICP-MS

After thawing, ± 1 ml of blood was digested with HNO_3 and H_2O_2 in a DigiPREP heating block system (SCP SCIENCE, Quebec, Canada). Further sample preparation included dilution of the digested samples to a final dilution factor of 12.5. Sample solutions were stored at 4°C until ICP-MS analysis. Total Cd and Pb concentrations of the blood samples were determined by ICP-MS (ICAP RQ; Thermo Fisher Scientific, Belgium), with He as collision gas. Cd was measured on mass 111 (and confirmed on mass 114), Pb was measured on mass 208 (and confirmed on mass 206). Quantification was carried out using an external calibration of the linear type. Calibration standards were in the range 0.005 – 10 µg L^{-1} for Cd and 0.05-10 µg L^{-1} for Pb and prepared by making the appropriate dilutions of the multi element mother stock.

2.3. Determination of Hg by DMA

For determination of Hg in the blood samples an DMA-80 evo direct mercury analyser (Milestone, Italy) was used. 300 µg of blood sample was weight directly into a sampling boat, and quantification was done using the first calibration range of the device (range 0.1- 40 ng Hg).

2.4. Quality control

Each analytical batch included internal quality control measures such as two procedure blanks and a reagent blank as a monitor for possible cross-contamination, a QC standard check every 20 samples to allow verification of potential instrument drift and, a reference material (Seronom-level 1 or 2) to assess trueness and day to day variations. A series of acceptance criteria were applied to each batch, including calibration blank value ≤LOQ/2, procedure blank ≤ LOQ and drift ≤10%.

2.5. Performance characteristics

Limit of Detection - Limit of Quantification – The LOQ of the method is the lowest level that can be determined with an acceptable performance. The LOQ for total As was calculated as 3.3 times the Limit of Detection (LOD = 3 times the standard deviation of 10 blanc samples or 10 pseudoblanc (low spiked) samples for speciation). This resulted in LOQ values in the matrix in the matrix blood of 0.09 µg L^{-1} for Cd (corresponding LOD of 0.0.027 µg L^{-1}) and 2 µg L^{-1} for Pb and 0.2 µg L^{-1} for Hg (corresponding LOD of 0.061 µg L^{-1}) ; after taking into account the dilution factor of the samples.

Trueness –Trueness is a theoretical concept expressing how close the mean of infinite number of results produced by the method is to a reference value. It can be assessed in practice by calculating the relative recovery compared to the reference value (in %). The reference value used in this study is the concentrations of the analyte cited on the certificate of the reference material used. The results are given in Table 1, reference material was added in each measurement and an average trueness was calculated.

Table1: Trueness for Cd, Pb and Hg

CRM or PT samples	Analyte	Reference value (± MU) µg L $^{-1}$	Measured value (± SD) µg L $^{-1}$	Trueness
Seronorm L1 (blood)	Cd	0.28 ± 0.06	0.31 ± 0.02	112%
	Pb	10.0 ± 2.0	11.5 ± 0.3	115%
	Hg	1.63 ± 0.33	1.55 ± 0.07	95%
Seronorm L2 (blood)	Cd	5.1 ± 1.0	5.5 ± 0.4	107%
	Pb	303 ± 31	292 ± 62	96%
Equas 66 7A (blood)	Cd	0.27 ± 0.09	0.24 ± 0.02	88%
	Pb	13.28 ± 2.61	14.8 ± 0.4	111%

Precision –Repeatability standard deviation (s_r = within day variation), between-day standard deviation (s_d) and intermediate precision standard deviation (s_{ip}=within lab reproducibility) were determined based on results of the reference material Seronorm-L1 or L2 (blood) for Cd, Pb and Hg, that were analyzed together with the samples on different days in independent replicates. The values were calculated via one-way analysis of variance using the equations below:

$$s_r = \sqrt{MSW} \quad (1)$$

$$s_d = \sqrt{\frac{MSB - MSW}{n}} \quad (2)$$

$$s_{ip} = \sqrt{s_r^2 + s_d^2} \quad (3)$$

MSW is the mean squares within days, MSB is the mean squares between days, and n is the number of measurements per day in routine (1 replicate). Relative deviations (expressed in %) were obtained by expressing the corresponding standard deviations as a percentage of the mean measured values.

The repeatability relative standard deviation (RSD_r) for Cd, Pb and Hg in blood was respectively 7.6%, 2.7% and 4.8%. The between-day standard deviation (RSD_d) respectively 1.6%, 1.8% and 5.5%. This resulted in a within lab reproducibility (RSD_{ip}) of 7.8%, 3.2 % and 7.3% respectivily.