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# Highly sensitive measurement of whole blood chromium by inductively coupled plasma mass spectrometry

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ABSTRACT

**Objectives:** Chromium (Cr), a trace metal element, is implicated in diabetes and cardiovascular disease. A hypochromic state has been associated with poor blood glucose control and unfavorable lipid metabolism. Sensitive and accurate measurement of blood chromium is very important to assess the chromium nutritional status. However, interferents in biological matrices and contamination make the sensitive analysis challenging. The primary goal of this study was to develop a highly sensitive method for quantification of total Cr in whole blood by inductively coupled plasma mass spectrometry (ICP-MS) and to validate the reference interval in a local healthy population.

**Design and methods:** This method was developed on an ICP-MS with a collision/reaction cell. Interference was minimized using both kinetic energy discrimination between the quadrupole and hexapole and a selective collision gas (helium). Reference interval was validated in whole blood samples ($n = 51$) collected in trace element free EDTA tubes from healthy adults (12 males, 39 females), aged 19–64 years ($38.8 \pm 12.6$), after a minimum of 8 h fasting. Blood samples were aliquoted into cryogenic vials and stored at −70 °C until analysis.

**Results:** The assay linearity was 3.42 to 1446.59 nmol/L with an accuracy of 87.7 to 99.8%. The high sensitivity was achieved by minimization of interference through selective kinetic energy discrimination and selective collision using helium. The reference interval for total Cr using a non-parametric method was verified to be 3.92 to 7.48 nmol/L.

**Conclusion:** This validated ICP-MS methodology is highly sensitive and selective for measuring total Cr in whole blood.



## Introduction

Chromium (Cr), a trace metal element, is implicated in diabetes and cardiovascular disease [1,2]. Cr exists mainly in two oxidative states, Cr (III) and Cr (VI). Cr (III) is biologically active and found in most foods and nutrient supplements [3]. Cr is monitored for occupational exposure [4], metal orthopedic implants [5], and nutritional status [1,2]. There are two major nutritional reasons for monitoring Cr levels. First, is due to Cr's role in diabetes mellitus, cardiovascular disease, and pediatric nutrition [1,2,6]. Second, Cr is routinely added to parenteral nutrition (PN) solutions based on the beneficial effects [7]. There have been several cases of Cr deficiency reported in PN patients [8–10]. Our hospital had followed long-term PN patients since 1976. Our standard practice required measurement of Cr, copper, manganese, selenium, and zinc prior to discharge then every six months while on therapy.

Atomic absorption spectrometry (AAS) is a widely used technique for determination of elements in a variety of matrices. Requirements for better detection limits with less interference drove the development of new technological innovations such as inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS has become the method of choice for most trace metal determinations [11]. Many methods have been described using both AAS and ICP-MS for measuring Cr. ICP-MS used to be plagued with polyatomic interferences for Cr so AAS was favored. However, these interferences have been surmounted by improvements in technology such as magnetic sector filter [12], increased mass spectrometry resolution, and/or reaction cells [13]. The inadequate quantification limits of previous methods also placed barriers to the accurate determination of whole blood Cr reference intervals [14,15]. By magnetic sector ICP-MS, limits of detection (LOD) of 1.15 nmol/L and 3.85 nmol/L were achieved but no further validation was indicated (i.e. limit of quantification (LOQ)) [12,16] and by a dynamic reaction cell ICP-MS the LOQ was 48.08 nmol/L [17].

The primary goal of this work was to develop and validate a high sensitivity ICP-MS method to analyze total Cr in whole blood for

*Abbreviations:* AAS, atomic absorption spectrometry; Cr, chromium; CV, coefficient variation; ICP-MS, inductively coupled plasma mass spectrometry; IS, internal standard; LOD, limit of detection; LOQ, limit of quantification; PN, parenteral nutrition.

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nutritional status studies. To reach the desired sensitivity, it was critical to minimize the polyatomic interferences which are commonly encountered in biological fluids [18]. The key to minimizing these interferences was through refining the collision cell and kinetic energy discrimination parameters. The second goal was to validate a clinically relevant reference interval in a local healthy population utilizing the highly sensitive ICP-MS method.

## Materials and methods

### *Chemical, reagents and solutions*

Triton X-100 and EDTA were from Sigma (St. Louis, MO, USA). Optima Nitric Acid was from Fisher (Pittsburgh, PA, USA). Type 1 water was from a Millipore Synergy System (Billerica, MA, USA). Cr (19.33 mmol/L) and internal standard (IS) mix (286.85 μmol/L gallium and 2.22 mmol/L scandium) were purchased from VHG Labs (Manchester, NH, USA). The Cr concentration was traceable to NIST SRM 3112a, lot# 030730. Both 4.8 ICP grade argon and 6.0 research grade helium were from Praxair (Danbury, CT, USA).

Stocks for Cr were prepared in matrix modifier (0.1% Triton X-100 and 0.1% $NH_4EDTA$ in water). Calibration standards were prepared in 0.5% $HNO_3$ at 5.77 nmol/L, 19.23 nmol/L, 115.39 nmol/L, 288.48 nmol/L, 576.96 nmol/L, and 769.28 nmol/L while IS solution contained 286.85 nmol/L gallium and 2.22 μmol/L scandium in 0.5% $HNO_3$. All solutions were stored at room temperature in acid washed polypropylene containers until use. ClinChek quality controls were purchased from Iris Technologies (Munich, Germany).

### *Sample preparation*

Sample preparation consisted of adding matrix modifier (4.5 mL) and 0.5 mL of patient whole blood, quality control, or calibration standard into a 15 mL acid-washed polypropylene conical tube. The mixture was vortexed then centrifuged for 5 min at 3488 ×g. The mixture was introduced to the ICP-MS through continuous flow via a 1.0 mL continuous sample loop configured with an Elemental Scientific Autosampler (Omaha, NE) while the IS was added through a high-flow addition tee (0.25 mm i.d.).

### *ICP-MS method*

This method was developed on a Thermo Fisher X Series2 ICP-MS configured with collision/reaction cell technology. Instrument was controlled by PlasmaLab (ver. 2.6.1.355, ThermoFisher). The sample introduction system was an O-ring free, baffled quartz cyclonic spray chamber (Elemental Scientific) fitted with a $PC^3$ Peltier cooler operating at 3 °C and PFA-ST nebulizer (Elemental Scientific). The samples were introduced by an integrated low-flow peristaltic pump operating at 13% of max speed and a SC-4 SDX micro FAST sample introduction system (Elemental Scientific) equipped with a Teflon/carbon fiber probe (150 cm capillary; 1.0 mm i.d.), followed by an Xt interface cone under hot plasma (1400 W) condition and a standard one-piece quartz torch with 1.8 mm sapphire injector tube.

Daily auto-tune and performance characteristics were monitored. A customized instrument tuning was done with the goal of reaching >100,000 counts for indium, a nebulizer gas flow for $Ar_2$ with counts <1500, and a CeO/Ce ratio <0.0200. Helium gas at a flow rate of 3.5–5.0 mL/min was used in the collision cell. The kinetic energy discrimination and standard mass resolution were set at 4.5 V and 0.02 AMU, respectively. Data acquisition was performed in peak jump scan mode with a dwell time of 100.00 ms for scandium and gallium and 1000.00 ms for Cr. Each reading had 20 sweeps with 3 readings per sample.

### *Method validation*

Matrix effect was evaluated by extracting and injecting a candidate dilution matrix solution (0.01% EDTA/0.01% Triton-X-100 spiked with Cr at 19.23 nmol/L), 6 patient samples, and 1:1 mixtures of patient samples with the candidate matrix solution. The criterion for passing was that the 1:1 mixture's measured value had to be within 20% of the theoretical value which was mean of the measured values of the patient specimen and candidate matrix solution. Interference from endogenous ions was investigated at two different Cr concentrations (low and high) in the matrix modifier before and after spiked with calcium, potassium, iron, and chloride at 2.63 mmol/L, 4.99 mmol/L, 25.07 μmol/L, and 110.00 mmol/L, respectively. It was found acceptable if interference containing sample results fell within 20% of the original samples for both levels tested. Linearity was examined in triplicate by serially diluting spiked pooled whole blood using matrix modifier. Analytical recovery and imprecision were evaluated at each concentration level. The lower limit of quantification was determined by the lowest concentration in the linearity study with an accuracy within 100 ± 20% and a coefficient variation (CV) <20%. Carryover was evaluated by running three extractions of the sequence $low_1$–high–$low_2$, where $low_2$ was a reinjection of $low_1$. There would be no significant carryover if $low_2$ was within 20% of $low_1$ and that $low_2$ was within ±3 standard deviations of the $low_1$ value. The standard deviation was determined using $low_1$ values. Precision was evaluated using a modified protocol based on the Clinical Laboratory and Standards Institute EP10-A3 guideline (Wayne, PA, USA), which included running the sequence mid–hi–low–mid–mid–low–low–hi–hi–mid twice a day for 5 days using patient derived samples to determine both intra-assay and total CVs. Method comparison was performed between the new ICP-MS method and a graphite furnace AAS method using 40 leftover patient specimens.

### *Statistics*

Statistics were calculated using Excel (Microsoft, Redmond WA, USA), SigmaStat 3.5 (Systat, San Jose, CA, USA) and EP Evaluator Release 9 (Data Innovations, South Burlington, VT, USA). A precision profile was created from the calibrator and sample results in the precision experiments using EP Evaluator with an acceptable CV of 20%.

### *Reference interval sample collection*

Collection of blood samples for reference interval verification was approved by the Institutional Review Board. In brief, whole blood samples (n = 51) were collected in EDTA tubes free of trace elements from healthy adults (12 males, 39 females), aged 19–64 years (38.8 ± 12.6), after a minimum of 8 h fasting. These samples were collected using stainless steel metal needles after collection of approximately 35 mL of blood for reference interval determination of other analytes. Patient exclusion criteria were [19]: pregnancy; body mass index below 15 or above 30; having a cold, flu, virus or other infection in the past two weeks; a diagnosis of diabetes, malabsorption syndrome or Crohn's disease; gastric or intestinal surgery, or frequent diarrhea; and chemotherapy in the past year, current immunosuppressant therapy. Blood samples were aliquoted into cryogenic vials and stored (<2 years) at −70 °C until analysis.

## Results

### *Interferences*

Determination of Cr in whole blood was a challenge mainly due to matrix effects. Interferences were eliminated by multiple modifications until an acceptable lower LOQ was achieved. First, selective