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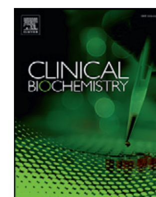

Analytical

Quantification of multiple elements in dried blood spot samples

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ABSTRACT

Background: Dried blood spots (DBS) is a unique matrix that offers advantages compared to conventional blood collection making it increasingly popular in large population studies. We here describe development and validation of a method to determine multiple elements in DBS.

Methods: Elements were extracted from punches and analyzed using inductively coupled plasma-mass spectrometry (ICP-MS). The method was evaluated with quality controls with defined element concentration and blood spiked with elements to assess accuracy and imprecision. DBS element concentrations were compared with concentrations in venous blood. Samples with different hematocrit were spotted onto filter paper to assess hematocrit effect.

Results: The established method was precise and accurate for measurement of most elements in DBS. There was a significant but relatively weak correlation between measurement of the elements Mg, K, Fe, Cu, Zn, As and Se in DBS and venous whole blood. Hematocrit influenced the DBS element measurement, especially for K, Fe and Zn.

Conclusion: Trace elements can be measured with high accuracy and low imprecision in DBS, but contribution of signal from the filter paper influences measurement of some elements present at low concentrations. Simultaneous measurement of K and Fe in DBS extracts may be used to estimate sample hematocrit.

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1. Introduction

Dried blood spots (DBS) are spots of capillary blood from a finger prick dripped onto filter paper, dried and then used for laboratory testing. DBS are commonly used in newborn screening for inborn errors of metabolism and in therapeutic drug monitoring [1], but has become increasingly popular as a sample matrix for biomarkers in population studies as they are easy to obtain, easy to transport, and allows collection in the field with a high response rate. To this can be added that collection of DBS are quick, relatively painless, less invasive than venipuncture and requires minimal field storage capacity. However, the number of validated assays for quantifying biomarkers in DBS samples is still relatively low compared with traditional whole blood, serum or plasma matrices. Reasons for this is the variety of challenges associated with analysis of DBS, e.g. contamination risk, blood spot heterogeneity, hematocrit effect and analyte on-card stability [2,3]. The sample volume is small (5 drops of blood on each card, i.e. approximately 275–375 μL) compared to conventional venipuncture (typical 5 mL of whole blood), and in many population studies and biobanks of e.g. neonatal blood spots collected at birth only one card is obtained per subject. Consequently, samples for analysis are often as small as 3–6 mm punches making the available volume of blood as small as 2–5 μL, which is a challenge in terms of obtaining a sufficient recovery and analyte signal. For some analytical techniques analytes can be quantified or detected directly from DBS punches. However, for the majority of routine analytes, pharmaceutical substances and elements (metals and non-metals) it is necessary to develop an extraction protocol that ensures good recovery rates, minimizes the background contamination from the filter paper and is suitable for the analyte of interest. The ideal procedure enables extraction of a maximum number of analytes from the same punch allowing the remainder of the card to be used for other purposes. Recent development in high-sensitive element analyzers like inductively coupled plasma mass spectrometry (ICP-MS) instruments provides simultaneous measurement of multiple elements at very low concentrations. However, to utilize the ICP-MS technology elements must be extracted from the filter paper, which introduces a dilution factor and a potential loss of sensitivity. Methods for quantification of elements in DBS by ICP-MS have previously been reported [4–7]. These methods all used one-half or a whole intact dried spot with a diameter of 8–13 mm corresponding to a starting blood volume of 20–40 μL blood, and the filter paper was weighed to estimate the amount of blood in each sample.

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Our objective was to develop a method for determination of multiple elements in DBS samples using the smallest possible sample amount with fast throughput and a quality comparable to whole blood and plasma methods. This paper describes the development and validation of a protocol for extraction of a range of elements from two 3.2 mm punches and subsequent quantification by ICP-MS using matrix-matched calibrators.

2. Materials and methods

For comparative analysis element concentrations of Co, Cu, Zn, As, Se, Cd and Pb were compared in a set of 17 matched whole blood samples and capillary blood collected on DBS filter paper, while Na, K and Fe were compared in a set of plasma samples and capillary blood collected on DBS filter paper. Element levels were also compared in matched DBS, whole blood QC samples and samples spiked with elements in order to cover a broader concentration range.

2.1. Subjects and samples

Dried blood spots and venous blood were collected from adult volunteers and approved by The Regional Ethical Committee in Southern Denmark. Capillary blood was obtained from the participant's finger using a microtainer contact-activated lancet (Becton Dickinson, New Jersey, USA), and drops of blood were collected on Whatman 903 protein saver cards (GE Healthcare, Little Chalfont, UK) to fill all five discs. Each card was dried overnight at room temperature and thereafter stored separately in a Zip-Lock® bag with a desiccant at −20 °C for three months until analysis. Prior to analysis, DBS cards were thawed at room temperature and 3.2 mm punches were produced by a semi-automatic DBS puncher (Perkin Elmer, Waltham, MA, USA).

Venous blood was collected by antecubital venipuncture in metal-free tubes containing EDTA (Vacutainer Royal Blue, Becton Dickinson) or Li-heparin (Vacutainer Green, Becton Dickinson). For collection of plasma, whole blood samples were centrifuged at 3600*g* for 10 min at 4 °C, and aliquots of whole blood and plasma were stored at −20 °C for three months until analysis.

For method validation, 75 μL venous EDTA whole blood or whole blood spiked with elemental standard solutions was applied with a precision pipette on the Whatman cards.

Quality control (QC) cards were prepared applying 75 μL whole blood QC materials from Sero (Billingstad, Norway), UTAK (Valencia, CA, USA), QMEQAS (Centre de Toxicologie, Quebéc, Canada) and UK NEQAS (Trace elements program, UK NEQAS, Sheffield, UK), respectively, on DBS cards.

2.2. Preparation of DBS samples with different hematocrit

Seven portions of whole blood with hematocrit values between 22.5 and 79.1% were prepared from a single batch of packed washed erythrocytes and a single batch of plasma as described in NBS01-A6 CLSI standard, Appendix C3. The hematocrit value in each portion was measured on a Sysmex XN10 before aliquots of 75 μL were spotted onto filter paper as described. Element concentrations were measured in three replicates from DBS extracts at each hematocrit level.

2.3. Reagents and utensils

Nitric acid 65%, Triton™ X-100 and Tracecert multi-trace elemental and single trace element standards for ICP-MS analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA). Seronorm™ Certified Reference Materials (CRMs) were obtained from Sero (Billingstad, Norway). UTAK whole blood and serum QC were obtained from UTAK (Valencia, CA, USA). CRM for toxic metals in bovine blood was obtained from NIST (Gaithersburg, Maryland, USA), while whole blood external quality control (EQC) samples containing a range of elements in human biological matrices were obtained from CTQ (QMEQAS program, Centre de Toxicologie, Quebéc, Canada) or from UK NEQAS (Trace elements program, UK NEQAS, Sheffield, UK). XN Check hematology control blood was obtained from Sysmex (Kobe, Japan).

Ultrapure water (>18 MΩ cm) from a Milli-Q system (Millipore, Bedford, MA, USA) was used for preparation of all buffers and standards. Clear polystyrene 96-wells flat bottom plates (NUNC, catalogue 439,454) and 12 mL MiniSorp™ tubes (NUNC, catalogue 468,608) was obtained from Thermo Fisher (Waltham, MA, USA). Three different lots of Whatman 903 protein saver cards were included in the method validation: Lots 6833909W082, 6912111W111, and 7018515W141.

2.4. Contamination control

All handling of samples were performed in a dedicated trace element-clean laboratory. DBS were handled with gloved hands under a laminar flow, except during punching with the semi-automatic puncher. All plastic materials in contact with DBS (tubes, plates, tips) were decontaminated with a 5% HNO_3 (v/v) solution overnight and dried under a laminar flow prior to use.

2.5. Instrumentation and ICP-MS conditions

Analysis of isotopes ^{23}Na, ^{24}Mg, ^{39}K, ^{44}Ca, ^{52}Cr, ^{56}Fe, ^{55}Mn, ^{58}Ni, ^{59}Co, ^{63}Cu, ^{64}Zn, ^{75}As ^{78}Se,^{114}Cd and ^{208}Pb in dried blood extracts and whole blood was performed on a iCAP-Qc ICP-MS (Thermo Fisher, Winsford, UK) equipped with collision cell technology with kinetic energy discrimination (CCT^{ED}). A flow of helium with a purity of >99.999% (Strandmøllen, Ejby, Denmark) was introduced into the collision cell. Sample introduction was performed with a PFA-ST microflow nebulizer combined with a quartz cyclonic spray chamber (Trace elemental scientific, Omaha, NE, USA) in all instances. For automation of the analyses the ICP-MS was equipped with a Cetac ASX-520 autosampler (Cetac Technologies, Omaha, NE, USA). Conditions were daily optimized to obtain the highest signal-to-background ratio for ^{7}Li, ^{59}Co, ^{115}In, ^{137}Ba and ^{238}U along with the ratio of $^{140}Ce^{16}O^{+}/^{140}Ce^{+} < 2\%$ for a solution of 1 μg/L of each trace element (Thermo Scientific, Tune B solution). Sample data were acquired in counts per second with three replicate readings and a dwell time of 50 ms.

To overcome potential polyatomic interferences (such as $^{35}Cl^{17}O$ on ^{52}Cr, ^{35} $Cl^{40}Ar$ on ^{75}As and $^{40}Ar^{40}Ar$ on ^{80}Se) three approaches were employed: 1) if possible, selection of interference-free isotopes for analysis; 2) use of collision cell technology; or 3) use of an equation to correct for interferences. Collision cell technology was applied to elements ^{23}Na, ^{24}Mg, ^{39}K, ^{44}Ca, ^{52}Cr, ^{56}Fe, ^{59}Co, and ^{78}Se. For selenium the less abundant isotope ^{78}Se was measured in order to avoid interference of the $^{40}Ar^{2+}$ dimer from the argon plasma instead of the most abundant isotope of selenium (^{80}Se). Mathematical correction was applied to measurement of ^{78}Se by the equation: $I(^{78}Se)-0.03461\ I(^{83}Kr)$. To compensate for uptake variation and analytical drift on the ICP-MS ^{71}Ga (collision cell mode) and ^{208}Bi (without collision cell) were added as internal standard in all tubes prior to analysis. Data were analyzed using the Qtegra™ software (version 2.2.1465.24) from Thermo Scientific (Winsford, UK).

The hematocrit value in whole blood samples were measured on a Sysmex XN10 (Sysmex, Kobe, Japan), while plasma concentrations of Na, Mg, K and Fe were measured on an Architect c8000 (Abbott Laboratories, Illinois, USA).

2.6. Calibration and standards

In order to maximize comparability between calibrators and unknown samples calibrators were by elemental spiking of a pool of artificial control blood that were deposited with 75 μL on "calibration cards" to be punched and extracted in a manner similar to unknown samples. By treating calibrators and samples in the same manner it is possible to

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