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TECHNICAL NOTE, ANALYTICAL METHODOLOGY

Impact of Ficoll density gradient centrifugation on major and trace element concentrations in erythrocytes and blood plasma

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ABSTRACT

Project: Ficoll density gradient centrifugation is widely used to separate cellular components of human blood. We evaluated the suitability to use erythrocytes and blood plasma obtained from Ficoll centrifugation for assessment of elemental concentrations.
Procedure: We determined 22 elements (from Li to U) in erythrocytes and blood plasma separated by direct or Ficoll density gradient centrifugation, using inductively coupled plasma mass spectrometry.
Results: Compared with erythrocytes and blood plasma separated by direct centrifugation, those separated by Ficoll had highly elevated iodine and Ba concentration, due to the contamination from the Ficoll-Paque medium, and about twice as high concentrations of Sr and Mo in erythrocytes. On the other hand, the concentrations of Ca in erythrocytes and plasma were markedly reduced by the Ficoll separation, to some extent also Li, Co, Cu, and U. The reduced concentrations were probably due to EDTA, a chelator present in the Ficoll medium. Arsenic concentrations seemed to be lowered by Ficoll, probably in a species-specific manner. The concentrations of Mg, P, S, K, Fe, Zn, Se, Rb, and Cs were not affected in the erythrocytes, but decreased in plasma. Concentrations of Mn, Cd, and Pb were not affected in erythrocytes, but in plasma affected by EDTA and/or pre-analytical contamination.
Conclusions: Ficoll separation changed the concentrations of Li, Ca, Co, Cu, As, Mo, I, Ba, and U in erythrocytes and blood plasma, Sr in erythrocytes, and Mg, P, S, K, Fe, Zn, Se, Rb and Cs in blood plasma, to an extent that will invalidate evaluation of deficiencies or excess intakes.

Introduction

Evaluation of human health effects, nutritional status, or exposure to toxic agents is often based on specific biomarkers, e.g., concentrations in blood or blood fraction, like plasma, erythrocytes or lymphocytes. Ficoll density gradient centrifugation is one of the most commonly used method for isolation and enrichment of human mononuclear cells, particularly from peripheral blood and other biological fluids, e.g., umbilical cord blood and bone marrow [1,2]. For Ficoll separation of blood components, an aliquot of anticoagulant-treated whole blood is layered on top of the Ficoll then subject to centrifugation. Low density lymphocytes, together with other slowly sedimenting cells, such as platelets and monocytes, are retained at the interface between the Ficoll layer and the plasma, and the latter remains on the top. The erythrocytes sediment to the bottom of the centrifuge tube. The mononuclear cells (lymphocytes and monocytes), often the main target of Ficoll separation, and the plasma, are frequently used in various biochemical analyses. The Ficoll-separated erythrocytes are mostly used for the purification of granulocytes [3], but also useful for studies of exposure to essential [4,5] and toxic [6–9] elements. Because the concentrations of some essential elements in erythrocytes may be used as a maker of status [5,10], and also most of the blood concentrations of several toxic elements are localised to the erythrocytes [8,9].

Ficoll is a neutral, highly branched, high-mass polysaccharide, which readily dissolves in aqueous solutions. An appropriate density of Ficoll medium must be chosen for the specific blood under study (e.g., human or rodent cells) [2,11], thus, sodium diatrizoate, a high-osmolality contrast agent [2], is often added into the medium to achieve a desired density of Ficoll medium. For example, the Ficoll-Paque™ PREMIUM aqueous solution, consisting of Ficoll™ PM400 (high molecular weight sucrose polymer), sodium diatrizoate and calcium disodium EDTA (ethylenediamine-tetraacetic acid), is optimised to a density of 1.077 g/mL for the preparation of human mononuclear cells. The manufacturer provides no specification as to the content of impurities in the Ficoll-Paque medium; therefore, the suitability of Ficoll-separated blood fractions for

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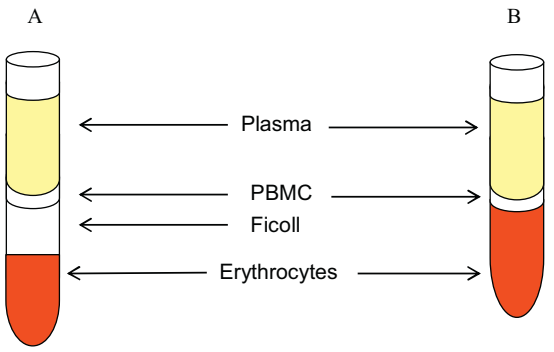

Fig. 1. Separation of peripheral blood mononuclear cells (PBMC) (A) with and (B) without Ficoll-Paque (referred as "Ficoll") density gradient.

specific analyses needs to be established. In particular, the potential impact of Ficoll density centrifugation on the concentrations of major and trace elements in isolated erythrocytes and blood plasma has not been systematically evaluated. The aim of this study was to elucidate the possibility to use erythrocyte and plasma fractions from Ficoll density centrifugation (Ficoll separation) for the assessment of concentrations of major and trace (essential and toxic) elements.

Materials and methods

Blood samples, and erythrocyte and blood plasma fraction

Venous blood was collected (8 mL) in sodium heparin tubes (Vacuette® for trace element analysis; Greiner bio-one, Kremsmünster, Austria) from 11 healthy volunteers living in Stockholm, Sweden. Ficoll-Paque™ PREMIUM (GE Healthcare Bio-sciences AB, Uppsala, Sweden) was brought into room temperature before use. An aliquot of blood (4 mL) was slowly layered on top of an equal volume of Ficoll-Paque PREMIUM solution in a 15 mL polypropylene centrifuge tube (Sarstedt, Nümbrecht, Germany). The tube was put into a swinging bucket centrifuge at 500 × *g* for 30 min at room temperature (EconoSpin, Sorvall Instruments, DuPont, Wilmington (Delaware), USA), to achieve the separation of erythrocytes and plasma (Fig. 1A). The upper layer containing plasma, essentially free of cells, was transferred by polypropylene pipette tip (acid-washed, rinsed with de-ionised water and then dried) into a polypropylene tube (Sarstedt, Nümbrecht, Germany), leaving the layer of mononuclear cells undisturbed at the interface. We then carefully removed the layer of mononuclear cells and the Ficoll-Paque, and kept the bottom layer, which mostly contains erythrocytes, in the centrifuge tube.

For comparison, the remaining 4 mL blood from the same blood collection tube was directly centrifuged without Ficoll-Paque medium (Fig. 1B), using the same centrifuge. Great care was taken to obtain pure plasma and erythrocyte samples. All erythrocyte and plasma samples were obtained within 4 h after blood sampling, and stored at −20 °C until ICP-MS analysis.

In order to check if Ficoll-separation had diluted the plasma, we measured the osmolality in plasma samples using a digital cryoscopic osmometer (OSMOMAT® 030, Gonotec Gesellschaft für Meß-und Regeltechnik mbH, Berlin, Germany), and the specific gravity using a digital refractometer (EUROMEX RD 712 clinical refractometer; EROMEX, Arnhem, Holland).

Determination of elements in erythrocytes and blood plasma by ICP-MS

We measured 22 elements using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500ce, Agilent Technologies, Tokyo, Japan), equipped with a collision/reaction cell system, as previously described [9] and detailed in Table 1.

Both plasma and erythrocytes were diluted 1:25 with an alkaline solution containing 1% NH_4OH (Merck, Darmstadt, Germany), 2% g/L 1-butanol, 0.05% EDTA, 0.05% Triton X-100, and 20 μg/L of internal standards (Ge, Rh, Lu and Ir; CPI International, Amsterdam, Netherlands). 1-Butanol (anhydrous, 99.8%), EDTA (H_4-EDTA, 99.995%), and Triton X-100 were all obtained from Sigma–Aldrich (Schnelldorf, Germany). We also measured multi-elemental concentrations in a sample of Ficoll-Paque medium diluted 1:100 with the alkaline solution.

Standard solutions for the external calibration were prepared by serial dilution of stock standards with the alkaline solution. Standards of Mg, Fe, Cu and Zn were obtained from CPI International (Amsterdam, Netherlands); P, S, I and Cs from Inorganic Ventures (Virginia, USA); Ca (ULTRAgrade™) from ULTRA Scientific (North Kingstown, USA); and other elements (multi-element standard solution VI for ICP-MS Certipur®) from Merck (Darmstadt, Germany).

The analytical performance was checked using commercial SERO AS control materials (Billingstad, Norway): Seronorm™ Trace Elements whole blood L-1, ref. 210105, lot 1103128; Seronorm™ Trace Elements whole blood L-2, ref. 210205, lot 1103129; Seronorm™ Trace Elements Whole Blood L-1, ref. 201505, lot MR4206; and Seronorm™ Trace Elements Serum, lot MI0181. The preparation of blanks and control materials was identical to that of erythrocyte and plasma samples, i.e., with alkali dilution.

Polypropylene labware used in ICP-MS analysis were soaked in 1.6 M HNO_3 (Suprapur®, 65%, Merck, Darmstadt, Germany) for a minimum of 2 h, rinsed five times with deionised water, then dried prior to use.

Table 1
Typical operating parameters of ICP-MS (Agilent 7500ce).

Operating parameters	Operating conditions
Forward plasma power (W)	1360
Sampling depth (mm)	8
Plasma gas flow (L/min)	15
Carrier gas flow (L/min)	0.65
Make up gas (L/min)	0.30
Sample uptake	0.1 rpm (round per second)
Sampling cone	Ni, 1 mm orifier
Skimmer cone	Ni, 0.4 mm orifier
Cell gas mode	Standard (no gas), He (4.5–5.5 mL/min), and H_2 (4.5 mL/min)
Isotopes monitored	Standard mode: ^{7}Li, ^{127}I, ^{133}Cs, ^{137}Ba, ^{208}Pb and ^{238}U He mode: ^{24}Mg, ^{31}P, ^{34}S, ^{39}K, ^{55}Mn, ^{59}Co, ^{63}Cu, ^{66}Zn, ^{75}As, ^{85}Rb, ^{88}Sr, ^{95}Mo and ^{111}Cd H_2 mode: ^{43}Ca, ^{56}Fe and ^{78}Se
Internal standards	^{74}Ge for elements in the mass range from 7 to 90 u, ^{103}Rh for ^{95}Mo, ^{111}Cd and ^{127}I, ^{175}Lu for ^{133}Cs and ^{137}Ba, and ^{193}Ir for ^{208}Pb and ^{238}U