Screening of Dried Matrix Spot Collection Devices to be Used for Nutritional Biomarker Analysis.

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Background

Dry Matrix Spots (DMS) are dried spots of body fluids, including blood, serum, urine, saliva and breast milk. DMS have been employed as a convenient sample matrix to collect in non-clinical and remote settings. DMS can be collected by staff with minimal training and, for a limited time, they can be stored and transported without a cold-chain. Historically, nutritional biomarkers (NB) were measured in dried blood spots (DBS). Aging of the blood, exposure to elevated humidity and temperature can alter the concentrations of most analytes. A collection device that would separate RBCs from serum without the need of electricity or centrifugation would be a huge advancement over whole blood spot collection.

Objective

To evaluate blood collection devices and identify ones best suited to obtain DMS for measurement of NBs. To determine what methods would be most useful to measure NBs from collected DMS.

Methods

We obtained samples of 9 commercial blood collection devices. Criteria were established to identify successful characteristics of collection devices and analytical methods. Ideally, devices should: separate RBCs from serum without gradient or chromatographic effects, provide adequate and quantifiable volume, be reproducible, provide moderate stability with minimal refrigeration, and offer simple collection. NBs were chosen from those identified by BOND working groups *(Raitan, AJCN 2011)*. Vitamin D was added due to the high percentage of the world's population with insufficient vitamin D intake. The NBs to be measured included: serum α-Acid Glycoprotein (AGP), C-Reactive Protein (CRP), Ferritin (Fer), Homocysteine (HCY), Methylmalonic Acid (MMA), Retinol Binding Protein (RBP), Thyroglobulin (Tg), Zinc (Zn), vitamin B12 and D along with RBC Folates. The analytical methods to evaluate the NBs must be sensitive (≥ppb), affordable, reproducible (±15%), accurate (±15%) and rugged.

Commercial Blood Collection Devices

903 Protein Saver Card
www.gelifesciences.com
Volume: 70-80 µL/circle

Capillary whole blood, imprinted with four half-inch circles. Well characterized but does not separate RBC's from serum.

FTA DMPK Cards
www.gelifesciences.com
Volume: 30-50 µL

DMPK-C is not impregnated with chemicals; suited for protein- based assays. DMPK-A and B are chemically treated that upon Blood contact the cells lyses and the proteins denature. Does not separtate RBC's from serum.

Asanté DBS Strips
www.sediabio.com
16-54 µL

Available in 3 widths (3, 5, & 10 mm) the 3 mm strip will collect a quantity of blood equivalent to a 6 mm DBS-disc. Does not separate RBC's from serum.

HemaSpot-HF
www.spotonsciences.com
65-105 µL

Capillary whole blood, sample applied to the center of the form and wicks out evenly to 8 blades. Does not separate RBC's from serum.

HemaSpot-SE
www.spotonsciences.com
65-105 µL

Capillary whole blood, sample applied to the center of the disc and RBC's separate from plasma laterally Zn contamination and chromatographic affects.

Advanced DX100 Card
www.adx100.com
50-100 µL

Patented in card serum from whole blood separation. Zn contamination and chromatographic affects.

Novilytic Noviplex Card
www.shimadzu.com/AN
50-70 µL

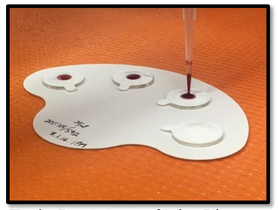
Laminated membrane stack; cells are removed by adsorption-filtration; plasma is drawn to the collection disc at the bottom. Serum volume (2 µL) too low for multiple analyses..

Results

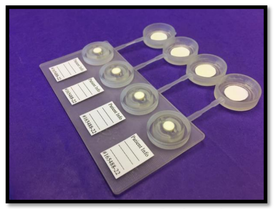
None of commercial products met the selection criteria. Many do not separate RBCs from serum, exhibit chromatographic effects, or provide too little serum for analytical testing. Linear and spiral devices had trace metal contamination and exhibited chromatographic effects as illustrated below.

	Albumin µg
DPS-1	180
DPS-2	186
DPS-3	194
DPS-4	205
DPS-5	255
DPS-6	233

CTI has identified a device (ViveBio, Alpharetta, GA) that allows the separation of RBCs in a vertical fashion with the concurrent separation and collection of a hemoglobin-free serum sample into a highly absorbent collection pad. The transfer efficiency is high (~60%) providing ~10-12µL of serum from 30-40 µL of whole blood to perform analytical tests.



Early Prototype of ViveBio Separation Card



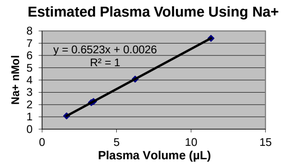
Current Version of ViveBio Blood Separation Tray

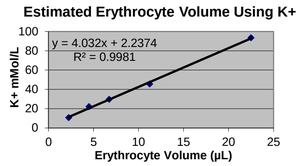
Choices of Analytical Methods

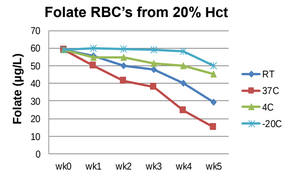
- Clinical Analyzers – inadequate volume available
- HPLC – limited sensitivity or selectivity
- ELISA – good for most protein NBs
- Microbiological – good for folate and B12, more expensive and laborious than ELISA
- LC-MS/MS – good for some NBs, expensive and highly technical
- ICP – good for minerals, including Zn

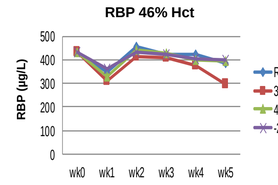
The analytical methods that have been selected to make the NB measurements are microbiological for Folates and B12; QuanSys Q-Plex for AGP, CRP, Fer, RBP, TfR, Tg; LC-MS-MS for MMA, HCY, vitamin D.

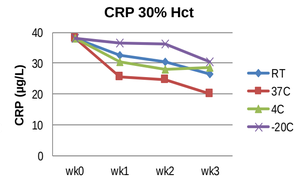
Representative Data Using ViveBio Device











Conclusions

- Essentially all commercial blood collection devices were eliminated by the selection criteria.
- Linear and spiral devices had trace metal contamination and exhibited chromatographic effects.
- Vertical separation of RBCs from serum appears to be best suited for DMS.
- The ViveBio pre-production blood collection device achieves vertical separation of RBCs from serum and meets nearly all selection criteria.

Advantages of this device over traditional DBS include: availability of RBCs for quantification of RBC folate, better serum volume estimation, direct serum measures of AGP, CRP, Fer, HCY, MMA, RBP, Tg, Zn, vitamins B12 and D.

CTI Nutrition Lab is currently measuring the stability of the 11 NBs at 5 temperatures.