eurofins | Craft Technologies

Measurement of Nutritional and Inflammatory Biomarkers in ViveBio Dry Matrix Spots (DMS)

FRANCISCO X. ARREDONDO, NEAL E. CRAFT AND MATTHEW K. FLESHMAN.

Eurofins Craft Technologies, Inc. Wilson, NC 27893.

BILL & MELINDA GATES foundation

OBJECTIVE

To conduct time and temperature stability experiments on Nutritional Biomarkers (NBs) and inflammation markers extracted from ViveBio PRISM™ Dry Matrix Spots (DMS).

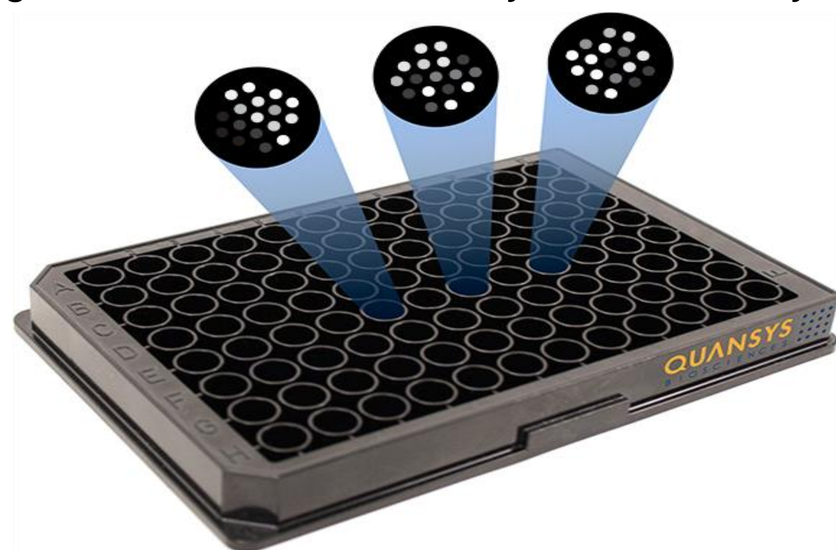
METHODS

Blood samples were created at three hematocrits (20, 30 and 45%) by combining purchased red blood cells (RBC) and sera. Blood samples of 35 µL were loaded onto ViveBio PRISM™ blood separators (Figure 1). The underlying pads retaining the serum were stored at 45°, 23°, 4°. -20° and -70°C. Samples were pulled for testing on days 0, 1, 3, 7, 14, 28, 84 and 168, and eluted in a maximum of 200 µL volume.

Figure 1 Prototype of ViveBio Prism Plasma Separator

Five NBs (α-acid Glycoprotein [AGP]), C-Reactive Protein (CRP), Ferritin, Retinol Binding Protein (RBP4) and Thyroglobulin (Tg) were measured using the Quansys Biosciences, Q-Plex chemiluminescent immunosorbent array, (Figure 2) This platform permits the use of small extracted volumes from the ViveBio PRISM™ dry serum matrix (1). Values measured in the liquid serum controls were used as reference values for the DMS.

Figure 2 Illustration of Quansys Q-Plex Assay Plate



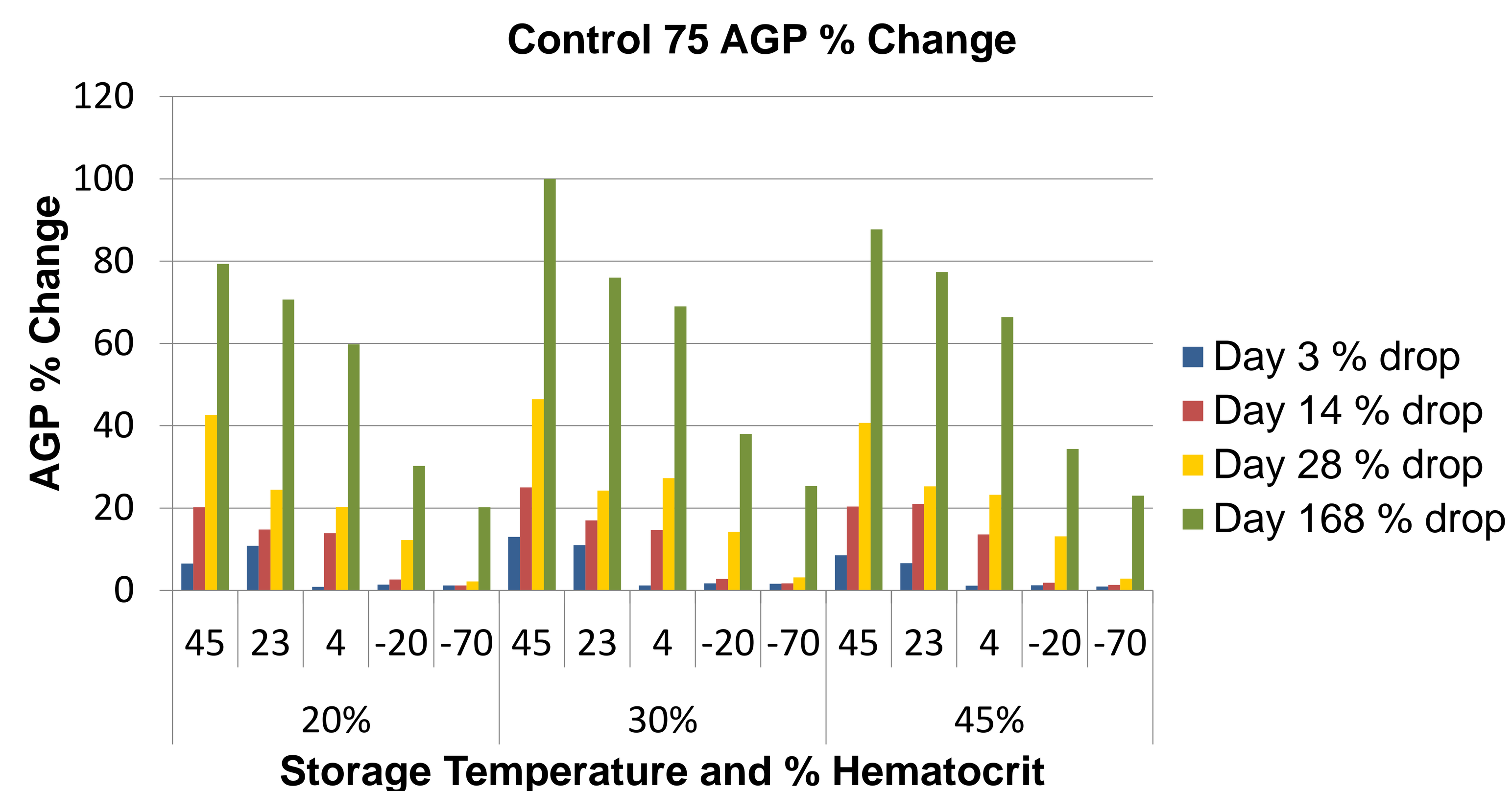
RBC folates and serum vitamin B12 were measured using a 96-well plate microbiological assay (ALPCO-Immundiagnostik AG, Bensheim, Germany). RBC folates were processed following established procedures, in a laboratory-generated 30% hematocrit blood sample (2, 3).

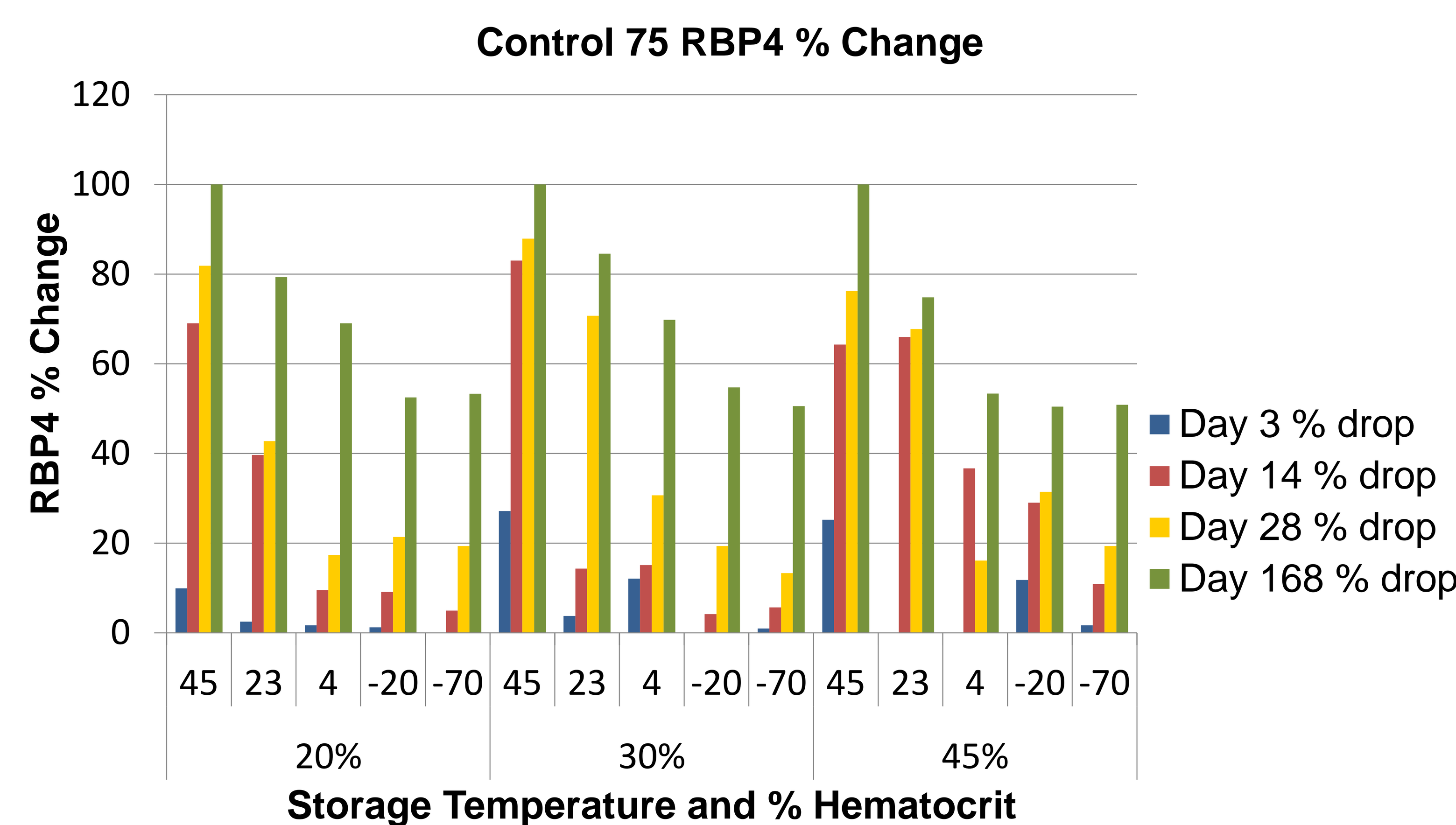
RESULTS

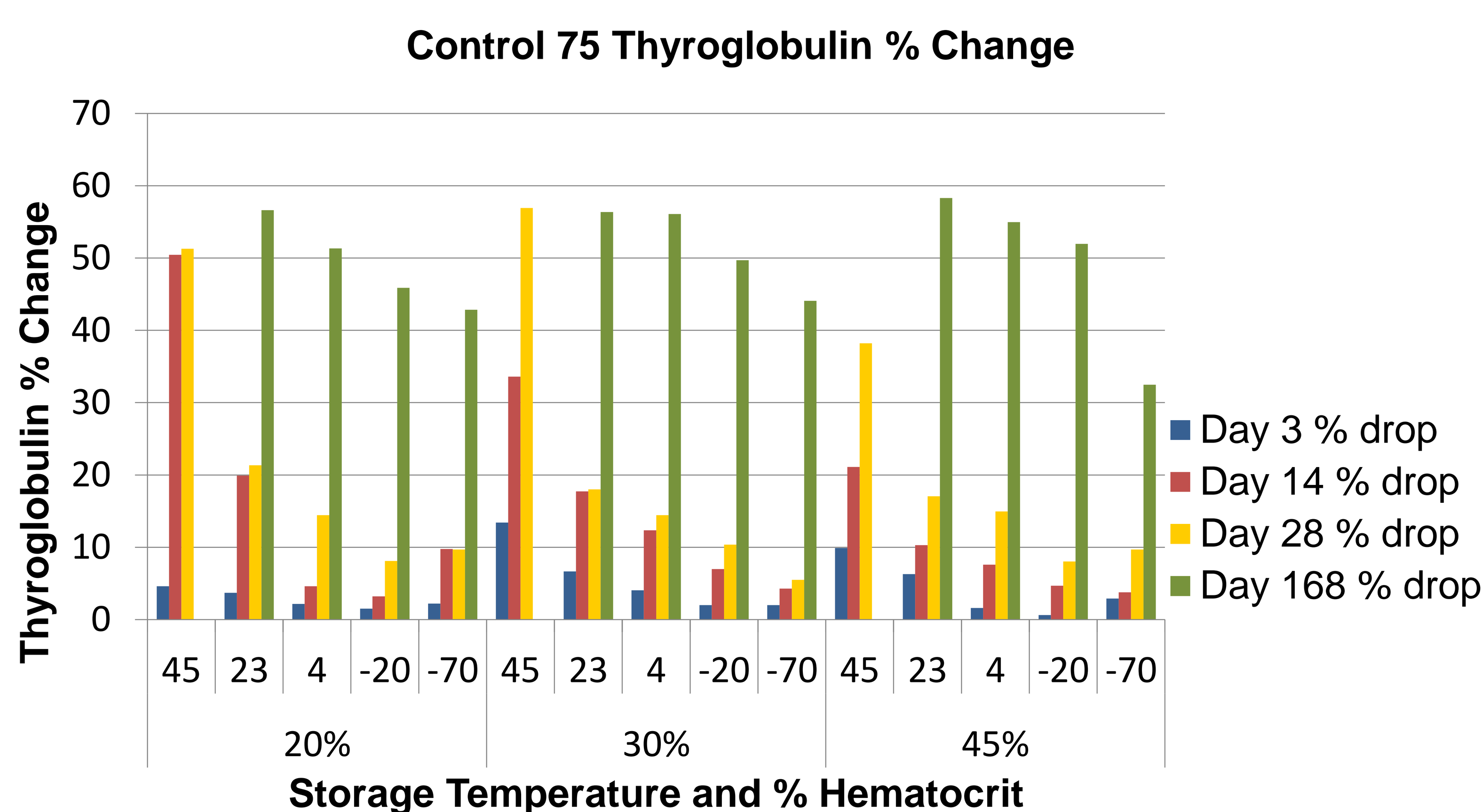
Overall the obtained values for AGP, CRP, Ferritin, RBP, and Thyroglobulin (Tg) followed the trend of diminishing values with increasing storage temperature and length of storage.

For illustrative purposes, percentage retained from time 0 is shown for days 3, 14, 28 and 168 for AGP, RBP4 and Tg.

RESULTS

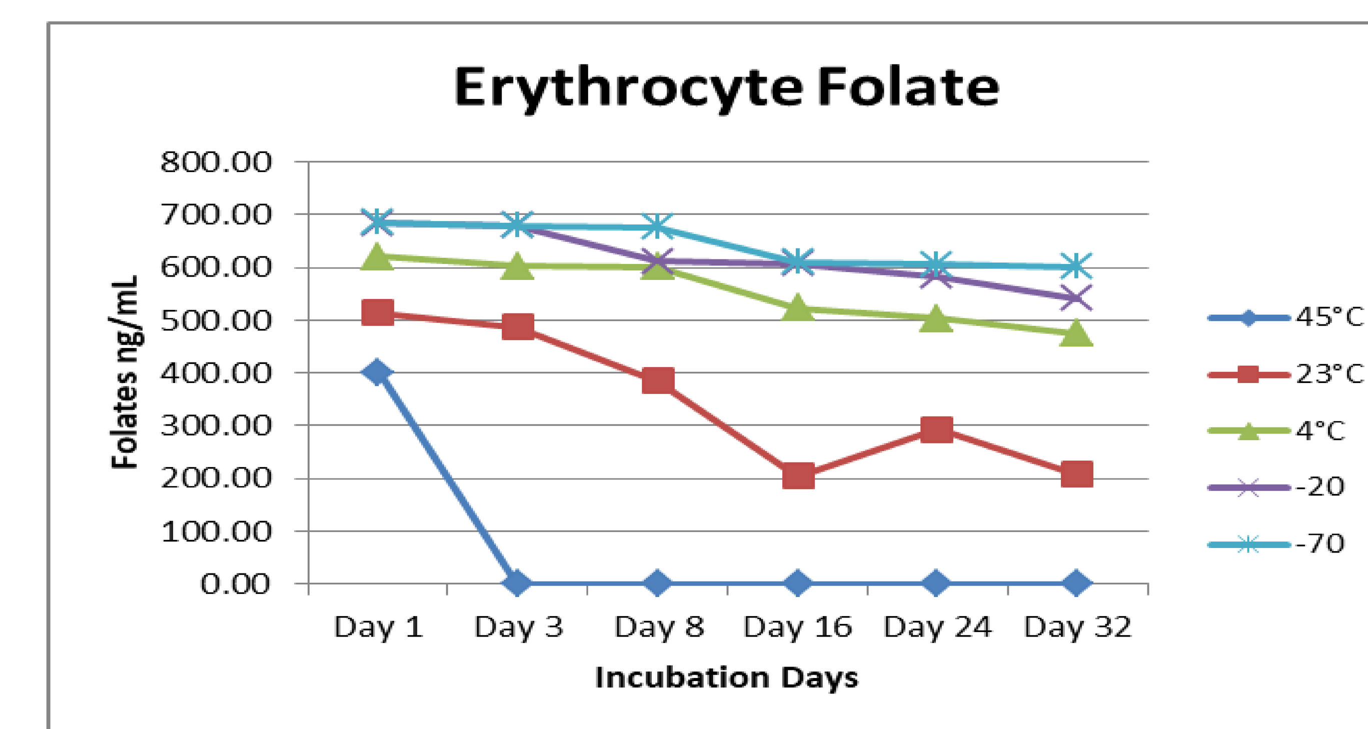






For the microbiological turbidimetric vitamin B12 assay a minimum of 3 dry matrix pads where used, it was found that, at every storage temperature, the stability of the analyte is poor and B12 could not be measured for more than 3 days.(Data not shown)

RBC folates by microbiological turbidimetric assay was measured in RBCs from a normal individual not taking folic acid supplements. The assay requires the presence of ɣ-glutamyl hydrolase (GGH). The source of the enzyme in these assays was sodium heparin plasma, immediately stored at -70°C. The RBC folates value was obtained according to Piyathilake, et al (3).



CONCLUSIONS

We have demonstrated that the studied biomarkers of nutrition and inflammation can be eluted from the ViveBio PRISM™ DMS, and analyzed in low volumes employing the Quansys Q-Plex chemiluminescent immunosorbent array.

The ViveBio PRISM™ separates the erythrocytes from the plasma in a vertical flow. The removal of hemoglobin from the plasma on the collection pad prevents the methemoglobin oxidation of most of the studied markers. The collection of nearly plasma-free RBCs permits the estimation of RBC folates which reflect the long-term folate status.

Unfortunately, the rapid decline of several of the NBs, even at temperatures below ambient, indicates that further work must be done to identify methods to stabilize the vitamins and proteins. There is some evidence that the incorporation of antioxidants and binding proteins could improve the stability.

REFERENCES

1. Quansys Bio. Logan Utah. 2017.
2. Wright AJA, Finglas PM and Southon S. Erythrocyte Folate analysis: Saponin Added during Lysis of Whole blood can increase apparent folate concentrations depending on hemolysate pH. Clinical Chemistry. 2000; 46-12; 1978-1986.
3. Piyathilake, CJ, Robinson CB and Cornwell P. A Practical Approach to Red Blood cell Folate Analysis. Analytical Chemistry Insights. 2007; 2; 107-110.

ACKNOWLEDGEMENTS

This work was funded by The Bill and Melinda Gates Foundation, OPP1135483.