Introduction

- Since the introduction of Guthrie cards in the 70s, filter paper method for collection of dried blood spots (DBS) has gained popularity as an alternative form of sampling technique to venepuncture.
- DBS sampling is less invasive than venepuncture; whilst the low sample volume requirement (typically 10-50 μL) is ideally suited for use in paediatric practice and in the elderly population.
- The reduced storage and shipping requirements allow patients to send their samples via the post, which could streamline the process of transporting samples to the laboratory and improve efficiency.
- However, analysts face many challenges with paper-based methods: concerns over volumetric inaccuracy, variability in spot sizes, analyte stability and reproducibility of measurements in sub-punches, have prohibited the widespread use of DBS sampling techniques. Volumetric microsampling devices are able to accurately collect a fixed amount of samples, and because sub-punching is not required, it overcomes many drawbacks associated with filter paper collection method.

Aims and Objectives

- To describe the use of Mitra® volumetric absorptive microsampling (VAMS) (Torrance, CA, USA) for LC-MS/MS measurement of 25(OH)D and interpretation of vitamin D status.
- To compare assay performance of Mitra VAMS against paper-based dried blood spot techniques.

Method of analysis

- Whole blood K3EDTA samples from 157 patients were selected at random, following routine analysis.
- 10 μL of blood was pipetted into Whatman® 903 protein saver cards or sampled using Mitra® VAMS (10 μL fixed volume: product number 10096), then left to dry for 18 hours at room temperature prior to storage at -20°C.
- Prior to analysis, DBS samples were extracted by 1) cutting the whole spot (wDBS), or by 2) making two 3 mm sub-punches (spDBS).

Comparison of dried blood-to-plasma equivalency values (PEV)

1) Whole spot (wDBS)

- Fig a-c) Raw 25OHD3 values produced from DBS and VAMS (n=97) were correlated with plasma concentration, but showed an average negative bias of -39.3%.

2) 2x 3mm sub-punches (spDBS)

- Fig d-e) Transforming raw DBS values into a clinically-relevant PEV (n=70). Analysis of concordance correlation coefficient (CCC) and correctional bias (CS) showed good agreement with plasma concentrations.

3) Mitra VAMS

- Fig f-g) Bland-Altman plots showed the assay bias was negatively associated with the increase in Hct levels.
- Mitra VAMS showed the least deviation across the Hct range.

Use of dried blood-to-plasma equivalency value for interpretation of vitamin D status

- Following interpretation guidelines from the IOM, we classified the vitamin D status in our patient cohort (n = 70).
- Using PEVs resulted in a small underestimation of individuals with deficiency status.
- Between the three microsampling techniques, results produced from VAMS were most representative of plasma.

Conclusion

- VAMS demonstrated benefits over the conventional paper-based method: the consistency in sampling volume, ease of use without the need for sub punches, and preservation of sample constituency.
- Our study provides validation of microsampling methodologies for measurement of 25(OH)D and interpretation of vitamin D status.
- VAMS produced more precise measurements of 25(OH)D and the most accurate reflection of vitamin D status compared to wDBS and spDBS.
- Although the recovery of the analyte remains Hct-dependent, the use of an empirically-derived model to transform DBS values into clinically-relevant equivalency improves the interpretability of results.