Assessment of Vitamin D status using Mitra® volumetric absorptive microsampling (VAMS) device

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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 wider 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.

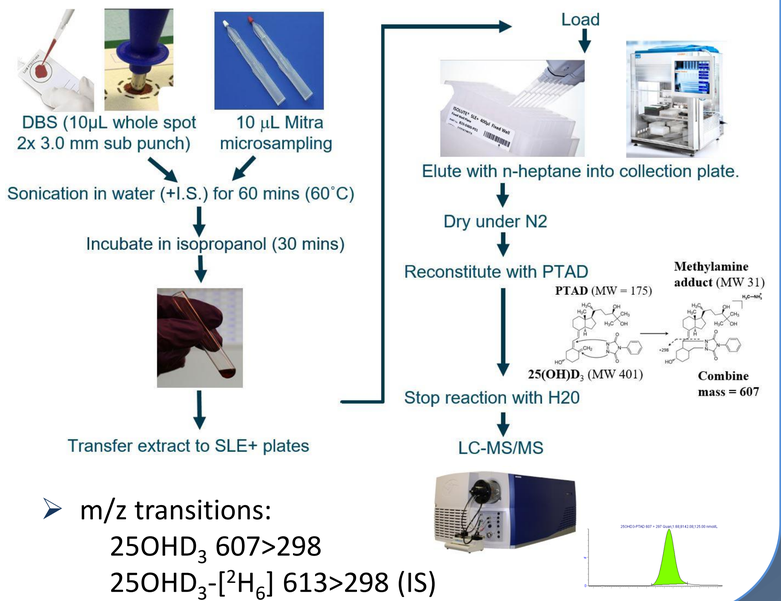
Aims and Objectives

- To describe the use of Mitra® volumetric absorptive microsampler (VAMS) (Torrance, CA, USA) for LC-MS/MS measurement of $25OHD_3$ 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 10006), then left to dry for 18 hours at room temperature prior to storage at -20°.
- Prior to analysis, DBS samples were extracted by 1) cutting the whole spot (wDBS), or by 2) making two 3 mm sub-punches (spDBS).

Sample extraction procedure



- m/z transitions:
  $25OHD_3$ 607>298
  $25OHD_3$-[$^2H_6$] 613>298 (IS)

Assay Characteristics

Intra-assay precision

	wDBS	SpDBS	VAMS
	Mean $25OHD_3$ nmol/L (%CV) (n = 10)		
1	7.5 (16.1)	7.6 (13.6)	7.3 (8.2)
2	23.4 (8.9)	23.2 (9.0)	20.5 (6.7)
3	43.6 (9.1)	45.0 (12.0)	40.3 (6.4)
4	64.2 (6.5)	71.1 (9.8)	59.5 (7.7)

Inter-assay precision

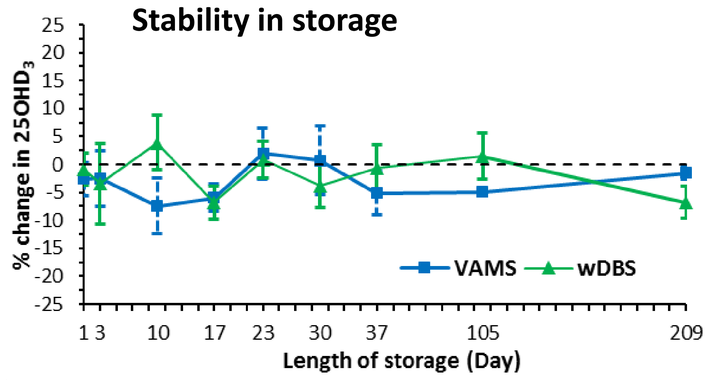
	wDBS	SpDBS	VAMS
	Mean $25OHD_3$ nmol/L (%CV) (n = 6)		
Low QC	10.6 (16.6)	12.2 (15.1)	10.9 (7.4)
Medium QC	57.7 (6.9)	62.7 (7.5)	64.8 (7.1)
High QC	82.0 (3.6)	93.3 (8.3)	91.3 (7.0)

- Linearity from 0-125 nmol/L (Typical r^2 value ≤ 0.98)
- Lower limit of quantification: wDBS 1.6 nmol/L, spDBS 2.5 nmol/L, Mitra® VAMS 1.5 nmol/L.

Assay recovery

Expected $25OHD_3$ nmol/L	Mean recovery (%)		
	wDBS	SpDBS	VAMS
8.5	102.4	110.2	98.4
21.0	90.0	108.6	104.0
37.5	93.3	97.1	101.7
62.5	89.2	96.7	100.7
87.5	90.3	104.6	95.4
106.3	91.8	102.5	99.6
Overall average (range)	92.8 (78.9-114.1)	103.3 (88.3-120)	100.0 (93.4-114.1)

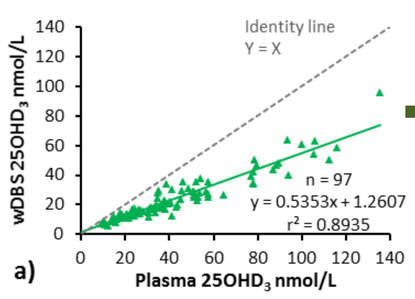
Stability in storage

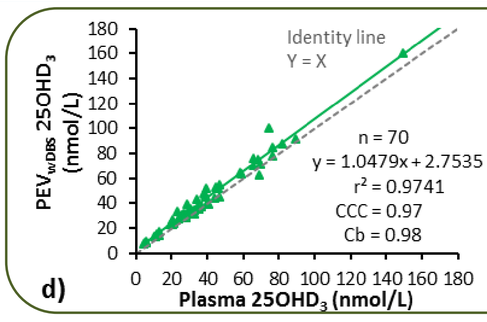


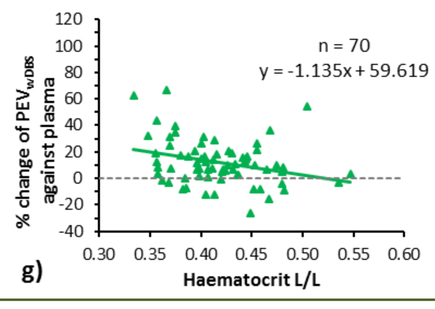
- A 209-day stability plot showing percentage change of $25OHD_3$ concentration from day one in samples collected by wDBS and VAMS stored at -20°C.

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

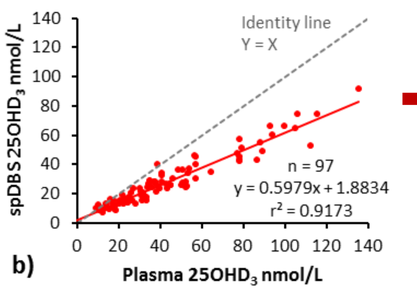
1) Whole spot (wDBS)

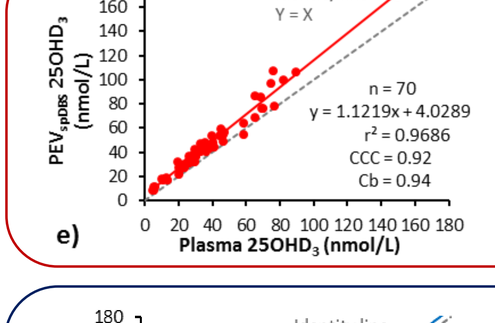


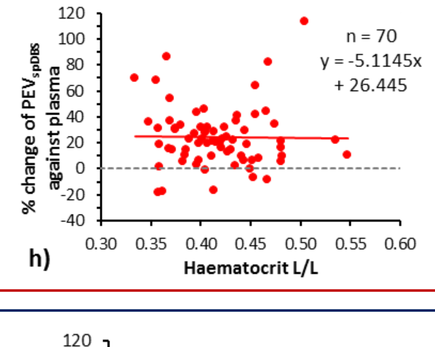




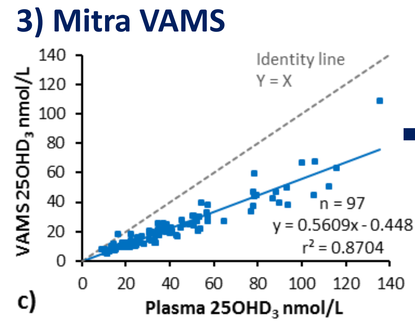
2) 2x 3mm sub-punches (spDBS)

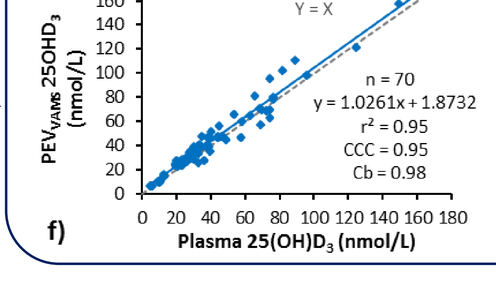


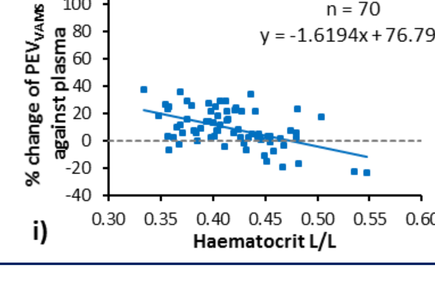




3) Mitra VAMS



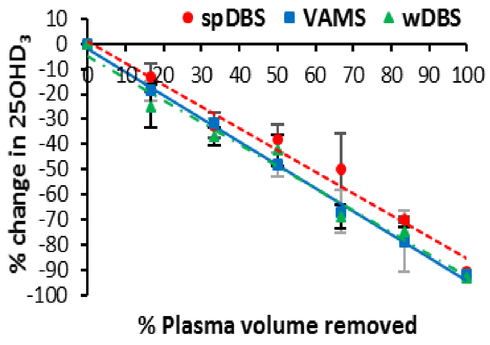




- **Fig a)-c)** Raw $25OHD_3$ values produced from DBS and VAMS (n=97) were correlated with plasma concentration, but showed an average negative bias of -39.3%.
- **Fig d)-e)** Transforming raw DBS values into a clinically-relevant PEVs (n=70). Analysis of concordance correlation coefficient (CCC) and correctional bias (Cb) showed good agreement with plasma concentrations.
- **Fig d)-e)** 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.

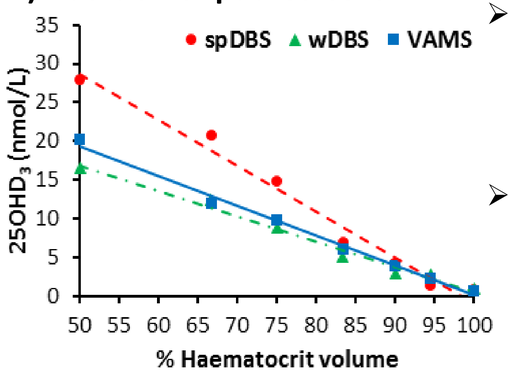
Effects of Haematocrit (Hct) displacement on $25OHD_3$ concentrations

1) Removal of plasma volume



- The plasma layer was taken off in steps until completely removed.
- Decrease in [$25OHD_3$] were proportional to the decrease in plasma volume.
- This shows $25OHD_3$ in blood is present primarily in the fluid compartment, the intracellular space contained <1.7% of total $25OHD_3$.

2) Addition of packed cells



- When plasma-free packed cells were added to a whole blood sample until full saturation, [$25OHD_3$] decreases as Hct level increased.
- Despite the constant plasma volume in the sample, the increasing level of Hct prevented the uptake of $25OHD_3$ into the microsampling devices.

- Findings from the above studies indicated the concentration of $25OHD_3$ in whole blood is dependent upon the level of Hct present, and that measurements using microsampling devices must be corrected for the level of Hct.

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

Vitamin D status definitions	Plasma (no. of cases, % in cohort)	PEV_{wDBS} (n, Δ%)	PEV_{spDBS} (n, Δ%)	PEV_{VAMS} (n, Δ%)
<30 nmol/L, Deficiency	27 (38.6%)	20 (↓10%)	15 (↓17.1%)	24 (↓4.3%)
30-50 nmol/L, Insufficiency	24 (34.3%)	29 (↑7.1%)	31 (↑10%)	26 (↑2.9%)
>50 nmol/L, sufficiency	19 (27.1%)	21 (↑2.9%)	24 (↑7.1%)	20 (↑1.4%)

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

Conclusions

- 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 $25OHD_3$ and interpretation of vitamin D status.
- VAMS produced more precise measurements of $25OHD_3$, 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.