Evaluation of the Mitra™ microsampling device against Dried Blood Spot cards for measurement of 25(OH)D_3 by LC/MS-MS

Holly Nicholls[1], Jonathan C.Y. Tang[1], John J. Dutton[1], Christopher J. Washbourne[1], Isabelle Piec[1], James B. Rudge[2], William D. Fraser[1]

[1]BioAnalytical Facility, Bob Champion Research Building, Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich Research Park, Norwich, Norfolk, United Kingdom NR4 7UQ.

[2]Neoteryx 421 Amapola Avenue, Torrance, CA 90501, USA

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Corresponding author:

Jonathan.tang@uea.ac.uk

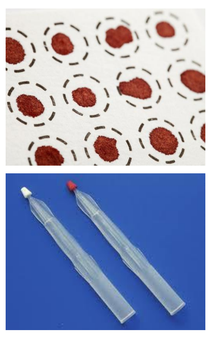
1. Introduction

- Vitamin D deficiency has become a major public health concern. In addition to poor outcome in bone health[1], it is now associated with hypertension[2], diabetes[3], mental health problems[4] and cancer[5].
- There are several advantages of dried blood spots (DBS). They are less invasive than venepuncture and a phlebotomist may not be required so patients can potentially take samples in their own homes then send them to hospital. Reduced need for phlebotomist appointments, as well as fewer shipping and storage requirements, could make the sampling process more cost-effective.
- However, some issues prevent the widespread use of DBS for analysis including the volumetric accuracy of spots and the uniformity of blood distribution, which are affected by haematocrit and therefore the location of any sub-punches becomes important.
- Mitra™ tips are a form of volumetric absorptive microsampler (VAMS) which take up a fixed volume of blood via an absorptive tip and could eliminate some of the problems encountered with dried blood spots.

2. Aims and Objectives

- To develop a LC/MS-MS assay for the quantitation of 25(OH)D_3 from whole blood using dried blood spots and Mitra™ tips as sampling methods.
- To compare the results produced using dried blood spots and Mitra™ tips and identify any advantages of the different sampling methods.
- Investigate effects of haematocrit and determine ways to correct for bias.

3. Method and Analysis

1 

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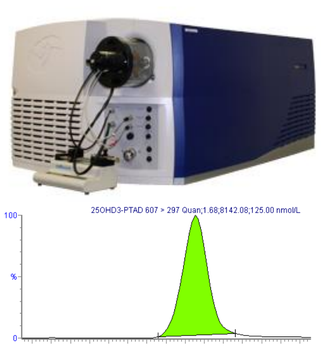
4 

1. Whole blood samples (10µL) were applied onto DBS cards and Mitra™ tips, and allowed to dry overnight at room temperature.
2. Whole blood spots, 2x3mm DBS discs and a Mitra™ tip were incubated with internal standard in IPA:H_2O for 30 mins at 35°C. The extract was pipetted into an supported liquid extraction (SLE+) plate and processed by the Biotage Extrahera automated plate processor.
3. The eluent was dried and reconstituted with 50 µl PTAD then incubated for 30 minutes. Water (50 µl) was added to stop the reaction.
4. The extract was injected into the LC/MS-MS. A typical chromatogram is shown above.

4. Micro-sampling vs Standard Plasma Method

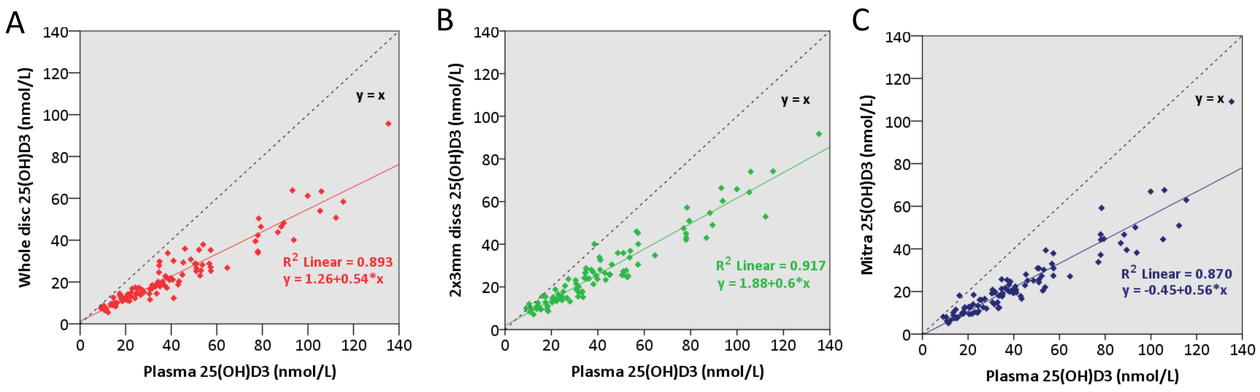
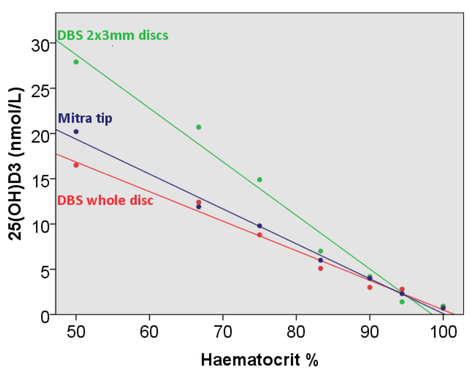


Figure 1: (A) Whole disc DBS vs 100 µl plasma (B) 2x3mm discs vs 100 µl plasma (C) Mitra™ tips vs 100 µl plasma

- Patient samples (n= 97) with the haematocrit range 0.26-0.53 (26-53%) were tested using each method.
- There was a linear relationship between the whole blood and plasma 25(OH)D_3 concentrations.
- 25(OH)D_3 whole blood results from DBS and Mitra™ showed strong correlation with plasma 25(OH)D_3 (r^2 between 0.870-0.917), but whole blood values were on average 42.0% lower than plasma concentrations.
- This is due to the haematocrit portion of a whole blood sample.

5. Haematocrit Investigation



- To investigate the effect of the haematocrit level on the 25(OH)D_3 measurements, non-lysed red cells were added in stages to a whole blood sample until saturation.
- There was a proportional decrease in 25(OH)D_3 concentration as the blood haematocrit level increased, suggesting the majority of 25(OH)D_3 is present in plasma and only trace amounts are present in the cellular compartment.

6. Haematocrit Adjustment

- A further 60 samples were analysed using dried blood spots and Mitra™ tips and the linear equations from the initial comparison (section 4) were used to calculate plasma equivalent 25(OH)D_3 concentrations.

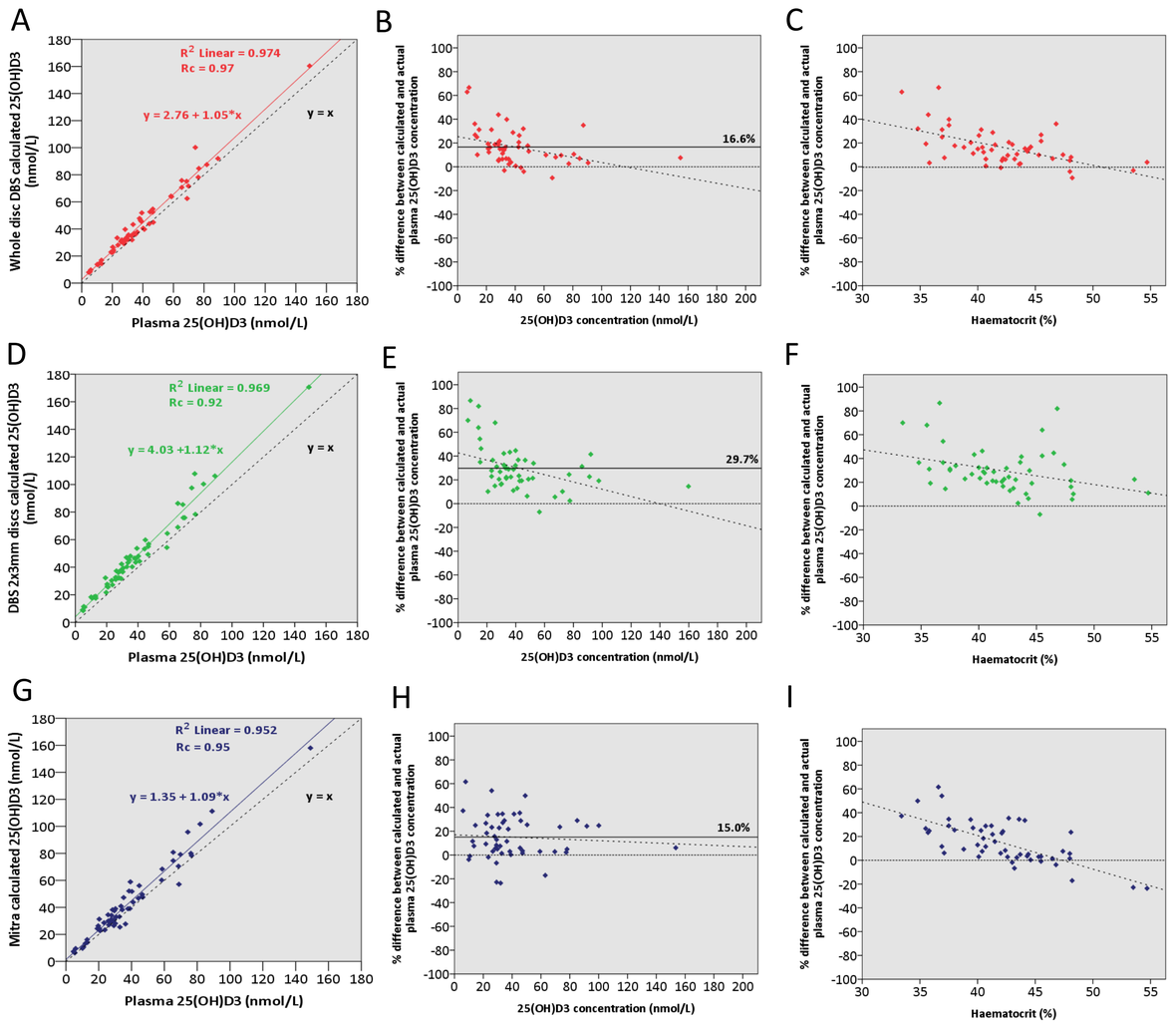


Figure 2: (A) Whole disc DBS calculated value vs plasma (D) 2x3mm discs calculated value vs plasma (G) Mitra™ tips calculated value vs plasma (B,E,H) Bland-Altman plots for each method with average % bias (C,F,I) % difference between calculated and actual results vs haematocrit for each method

- Lin's concordance correlation coefficient (Rc) shows there was a good agreement between the derived plasma values and the reference plasma values for each method.
- The Bland-Altman graphs showed there was a positive bias for all three methods. The bias was greatest with DBS 2x3mm discs.
- The positive bias was highest for the samples with low haematocrit levels for both whole disc DBS and Mitra™ but the zero bias line was intercepted at 50.7% and 47.4% haematocrit, respectively. However there was a positive bias across the entire haematocrit range with DBS 2x3mm discs.

7. Assay Characteristics

Assay precision

		DBS whole disc		DBS sub-punches 2x3mm		Mitra™	
	sample #	[25(OH)D3] (nmol/L)	CV (%)	[25(OH)D3] (nmol/L)	CV (%)	[25(OH)D3] (nmol/L)	CV (%)
intra-assay	1	7.5	16.1	7.6	13.6	7.3	13.6
	2	23.4	8.9	23.2	9.0	20.5	6.7
	3	43.6	9.2	45.0	12.0	40.3	6.4
	4	64.2	6.5	71.1	9.8	59.5	7.7
inter-assay	QC1	10.6	16.6	12.2	15.1	10.9	7.4
	QC2	57.7	6.9	62.7	7.5	64.8	7.1
	QC3	82.0	3.6	93.3	8.3	91.3	7.0

*intra-assay calculated over 10 runs, inter-assay calculated over 6 runs

Stability over 50 days

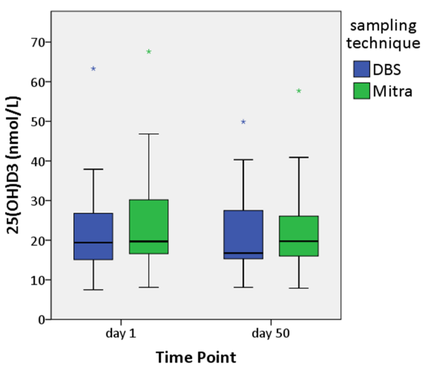


Figure 3: Box plot displaying 25(OH)D_3 concentrations at day 1 and day 50.

Assay recovery

	Recovery across three runs (%)		
[25(OH)D3] (nmol/L)	DBS whole disc	DBS sub-punches 2x3mm	Mitra™
8.5	102.4	110.2	120.4
21	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
106.25	91.8	102.5	99.6
average recovery across range	92.8 ± 3.1	103.3 ± 3.9	103.6 ± 7.8

- Linearity from 0-125 nmol/L
- Typical r^2 value ≤ 0.98
- Lower limit of quantification:
  - DBS whole disc 1.6 nmol/L
  - 2x3mm discs 2.5 nmol/L
  - Mitra™ 1.5 nmol/L

8. Conclusions

- The 25(OH)D_3 concentration in whole blood is affected by the presence of haematocrit with the sampling techniques used.
- Applying our correctional formula produced calculated results which correlated well and were in good agreement with the standard plasma assay, using both DBS and Mitra™, although there was a small positive bias for each method.
- Mitra™ performed similarly to the whole disc DBS, when an exact volume of blood was pipetted onto the cards.
- Mitra™ consistently produced CV values under 15% for the precision experiments.
- Mitra™ tips are easy to use and could replace DBS for this assay.

References

1. A.T. Francis R, Fraser W, Gittoes N, Javaid K, Macdonald H, Patel S, Selby P, Tanna N, Bowring C, Vitamin D and Bone Health: A Practical Clinical Guideline for Patient Management, National osteoporosis Society, 1.1 (2013) 2. Wang, T. J. 2016. 'Vitamin D and Cardiovascular Disease', *Annu Rev Med*, 67: 261-72. 3. Shen, L., Q. S. Zhuang, and H. F. Ji. 2016. 'Assessment of vitamin D levels in type 1 and type 2 diabetes patients: Results from metaanalysis', *Mol Nutr Food Res*, 60: 1059-67. 4. McGrath, J. J., T. H. Burne, F. Feron, A. Mackay-Sim, and D. W. Eyles. 2010. 'Developmental vitamin D deficiency and risk of schizophrenia: a 10-year update', *Schizophr Bull*, 36: 1073-8. 5. Trump, D. L., K. K. Deeb, and C. S. Johnson. 2010. "Vitamin D: considerations in the continued development as an agent for cancer prevention and therapy." In *Cancer J*, 1-9.