A new microfluidic-based sampling device enabling the simple and precise collection of defined blood volume on commercially-available DBS cards

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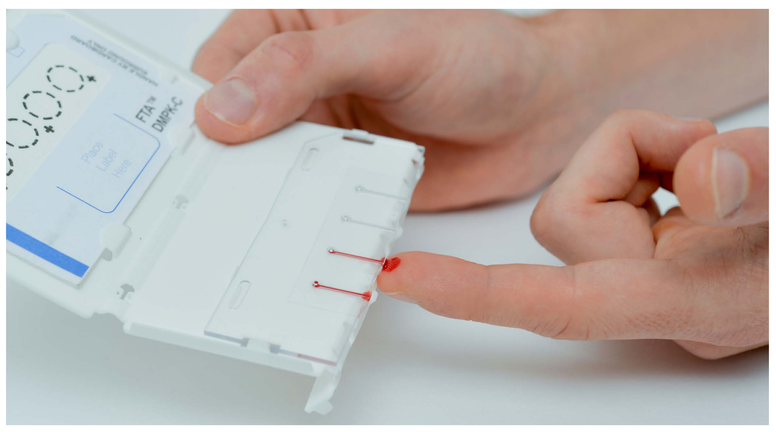
Introduction

- Hematocrit (Hct) is one of the most critical issues associated with the bioanalytical methods used for dried blood spot (DBS) sample analysis.
- Since Hct determines the viscosity of blood, it may affect the spreading of blood onto the filter paper.
- Hence accurate quantitative data can only be obtained in such cases if the whole DBS spot is extracted and contains a defined volume of blood.
- We present here a new microfluidic-based sampling device enabling the simple and precise collection of 5 or 10 µL of blood on commercially-available DBS cards.

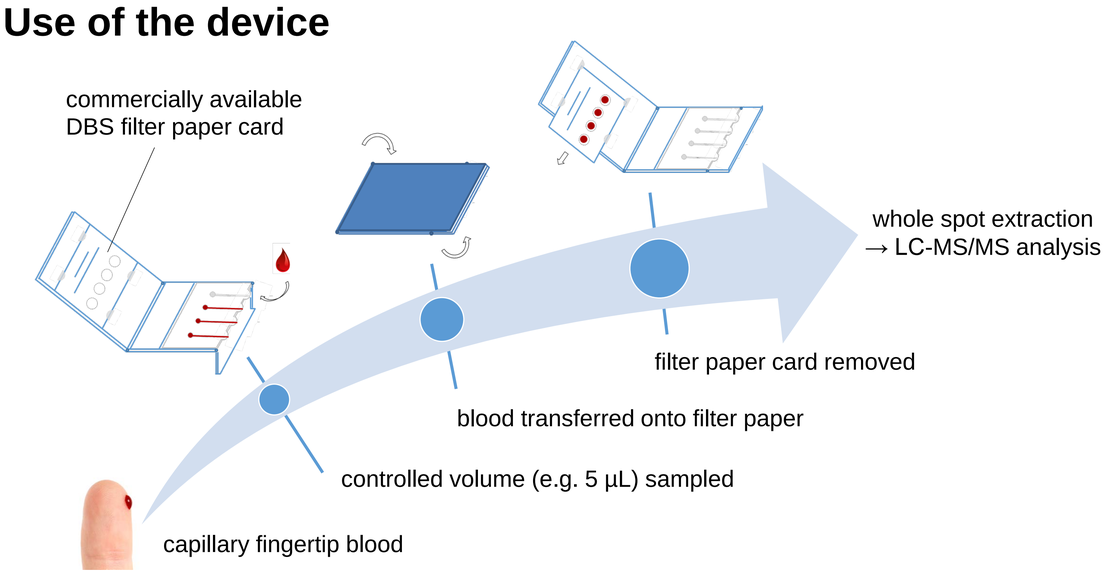
Instrumentation

- **Microfluidic device (DBS System - patent WO/2013/144743 A1)**



The dimensions of the 4 channels enable the collection of a predefined volume of blood. On this picture, 2 channels are filled with 10 µL of capillary blood.

- **Use of the device**



Methods

- ***In vivo* sampling**. One volunteer drank coffee. After ca. 1 hour, 10 µL of capillary blood sampled (n = 5) on Whatman DMPK-C cards, with both the microfluidic device and with a manual pipette. Caffeine and paraxanthine were quantified by LC-MS/MS (Method : Bosilkovska M *et al*. *Bioanalysis* **2014**; 6:151-64).
- ***In vitro* assessment of accuracy, precision and of the hematrocrit effect**. Blood centrifuged at 4°C for 10 min at approximately 1500 g and plasma separated from the erythrocyte pellet. The latter gently mixed with collected plasma to obtain blood batches with measured Hct levels of 26%, 37%, 62% and 82%. The Hct for the blood used for calibration standards (Cs) and quality control samples (QCs) (reference) was 47%. Mavoglurant (AFQ056) spiked at different Hct levels and QC levels, 5 µL sampled on Whatman DMPK-A cards with the microfluidic device and a pipette (when used for comparison). Extraction from the DBS punches (6 mm diameter, whole spot) with methanol/water (1:1) (200 µl) containing the internal standard ([$^{13}CD_3$]Mavoglurant, 50 ng/mL). Mixing for 30 min. Supernatant transferred to a well-plate, and 50 µL injected into the chromatographic system. LC-MS/MS system: Thermo Vantage, APCI, positive mode, Rheos pumps. Mobile phases: 20 mM ammonium acetate in water (A) and methanol (B). 40% B for 1.0 min isocratically, increased to 90% B over the next 1.0 min, kept isocratically for the next 1.5 min, flow rate of 1.0 mL/min. Analytical column: Agilent Zorbax SB-C18, 30 x 4.6 mm, 3.5 µm, 60°C.

Results

In vivo sampling

- Comparison of mean concentrations and CV(%) (n = 5) obtained by microfluidic vs. manual pipette sampling.

		Caffeine		Paraxanthine	
Sampling	Spot area sampled	Mean conc. (µg/mL)	CV (%)	Mean conc. (µg/mL)	CV (%)
Microfluidic device	Whole spot	1.49	8.3	0.82	4.7
Pipette	Whole spot	1.51	6.8	0.85	7.8

In vitro assessment of accuracy and precision

- Comparison of mean concentrations and CV(%) (n = 10) obtained by microfluidic sampling at different Hct levels.

	Mavoglurant (AFQ056)					
	QC1 (2000 ng/mL)			QC5 (15.0 ng/mL)		
Hct (%)	Mean conc. (ng/mL)	Mean bias (%)	CV (%)	Mean conc. (ng/mL)	Mean bias (%)	CV (%)
26	2069	3.4	6.6	16.6	10.9	7.2
62	1930	-3.5	7.4	14.8	-1.6	7.6

In vitro assessment of the hematrocrit effect

- Comparison of mean concentrations and CV(%) (n = 3) obtained by microfluidic sampling at different Hct levels.

Hct (%)		Mavoglurant (AFQ056)		
		QC1	QC2	QC3
26	Mean conc. (ng/mL)	1915	90.7	13.0
	Mean bias (%)	-2.8	-6.6	-2.8
	CV (%)	2.3	8.0	15.9*
37	Mean conc. (ng/mL)	1805	91.6	13.7
	Mean bias (%)	-5.9	-5.2	0.0
	CV (%)	n.c.	5.5	8.8
47	Mean conc. (ng/mL)	1745	93.3	14.3
	Mean bias (%)	-6.7	-10.1	-2.3
	CV (%)	6.5	8.7	5.3
62	Mean conc. (ng/mL)	1835	83.5	11.9
	Mean bias (%)	10.1	0.6	-11.9
	CV (%)	4.0	6.3	10.1

Note: mean bias (%) calculated between the individual concentrations obtained with the microfluidic sampling when compared to the ones obtained with the pipette sampling. * Bias > 15%. n.c., not calculated, as n = 2 (one concentration excluded as outlier).

Conclusions

- Tested on prototype devices sampling 5 µL or 10 µL on resp. Whatman DMPK-A and DMPK-C cards, with similar results.
- This new microfluidic sampling is as accurate and precise as a manual pipette.
- Much simpler in use than a pipette, improved quality of spots (e.g. no multiple spotting possible).
- Tested without success at the non-physiological Hct level of 82%, due to the high blood viscosity.
- The blood sampling and analysis of Mavoglurant is not affected by Hct values.
- Adequate to circumvent the issue associated with the Hct effect in the range of 26 to 62%, along with using the whole spot for analysis.