

# Collection and storage of volumetrically accurate and precise dried blood spots in a contained device: hemaPEN®

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## Introduction

A paradigm shift in blood sampling is necessary to achieve and sustain better standard of care and meet the growing desire for patient centric data provision to enable healthcare to cater to the individual's needs.

Dried Blood Spotting (DBS) technology offers safe and easy sampling of small volumes and eliminates the need for cold chain logistics. However, the broader adoption into therapeutic drug monitoring or clinical pharmacology is limited through lack of sampling volumetric accuracy and precision, and analytical bias due to variable blood hematocrit (HCT) and sample contamination from external environment.

Trajan Scientific and Medical is currently developing an innovative tool based on DBS technology, which could solve the current limitations and is poised to help shift the paradigm. The hemaPEN® (Figure 1) is a novel device for the collection and storage of volumetrically accurate and precise dried blood spots in a contained environment.



Figure 1: hemaPEN® device for the collection and storage of volumetrically accurate and precise dried blood spots in a contained environment.

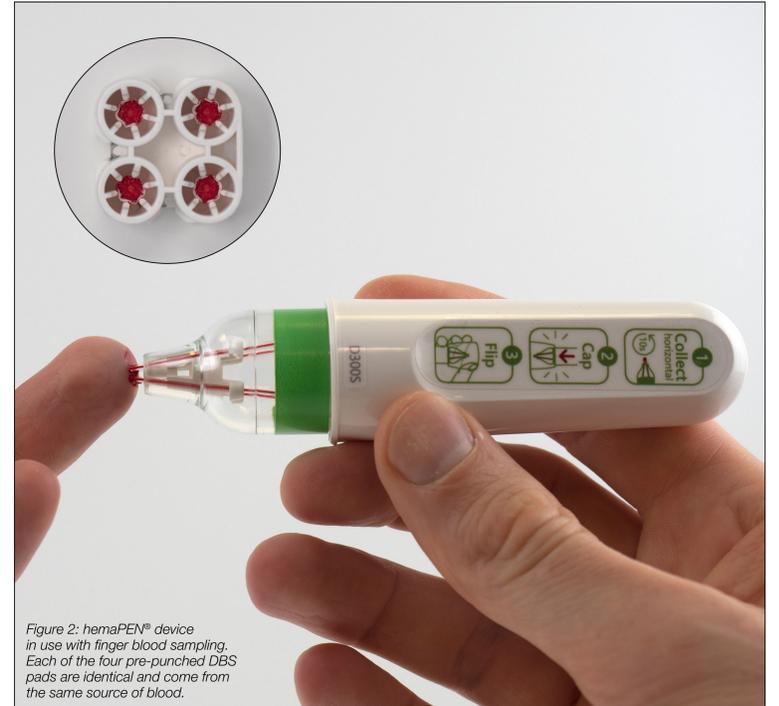


Figure 2: hemaPEN® device in use with finger blood sampling. Each of the four pre-punched DBS pads are identical and come from the same source of blood.

## Objectives

- To demonstrate the hemaPEN (Figure 2) delivers on the collection and storage of 4 x 2.74  $\mu$ L accurate and precise volumes of a blood sample irrespective of HCT.
- To demonstrate that samples collected by the hemaPEN dry inside the contained body of the device.

## Method

### Volumetric Accuracy and Precision

- Volume collected and transferred to DBS was calculated by weight of 4 x 3.5 mm discs, immediately after collection of the blood sample using the hemaPEN (n = 18).
- Blood samples at three HCT levels (34%, 45% and 59%), prepared from a single source of EDTA blood by adjusting plasma volume and measuring with the HemoCue® HB 201+, were used.
- Density of each hematocrit blood was calculated from gravimetric measurements of 25  $\mu$ L volume using calibrated positive displacement pipette (Drummond) and was compared to two set of data from literature<sup>1,2</sup> to verify the methodology employed.
- Volumes were calculated from the weight and the relevant blood densities defined by Burstain et al. 1994<sup>1</sup>.
- Accuracy was calculated by mean actual weight of wet DBS as a percentage of the theoretical weight (calculated from 4 x target volume (2.74  $\mu$ L) and literature blood density).
- Inter-device precision was calculated from the volume for each device independent of HCT.

### Sample Integrity

Drying efficiency was assessed by measuring the change in weight of 4 x 3.5 mm discs after being stored in the hemaPEN (n = 42) under different environmental conditions (23°C/41% RH, 21°C/100% RH and 40°C/100% RH) for two storage periods (60 and 120 minutes). hemaPENS were either stored in polyfoil packaging bags or directly in the environment.

## Results

The hemaPEN dispensed between 103.5% and 104.5% of the expected theoretical 2.74  $\mu$ L volume (as defined by the manufacturers' specification), independent of hematocrit onto each dried blood spot (Figure 3 and Figure 4). Inter-device volumetric precision was calculated at 0.7% coefficient of variance independent of HCT.

The DBS pads were 80% to 100% dry within one hour and 100% dry within 2 hours under all evaluated environmental conditions and at each HCT level (Figure 5).

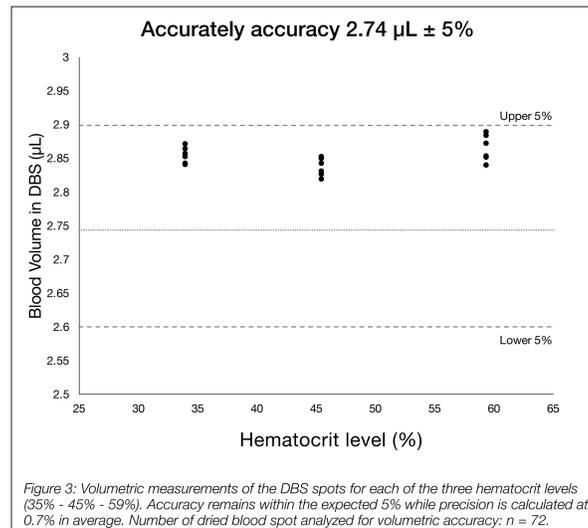


Figure 3: Volumetric measurements of the DBS spots for each of the three hematocrit levels (35% - 45% - 59%). Accuracy remains within the expected 5% while precision is calculated at 0.7% in average. Number of dried blood spot analyzed for volumetric accuracy: n = 72.

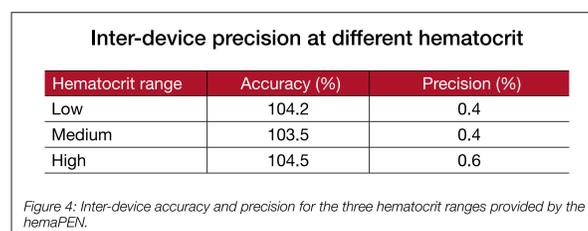


Figure 4: Inter-device accuracy and precision for the three hematocrit ranges provided by the hemaPEN.

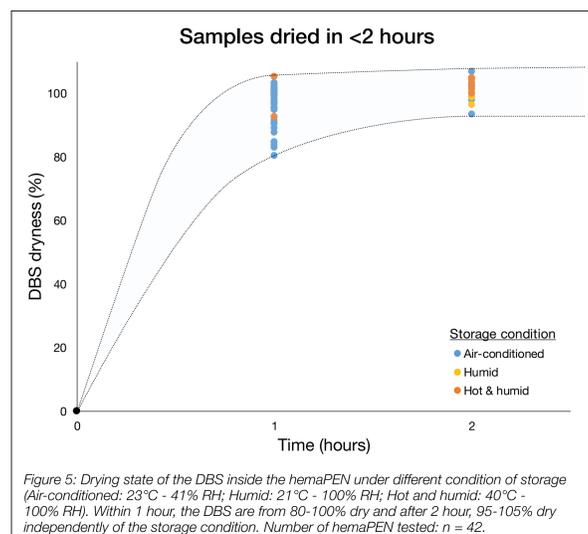


Figure 5: Drying state of the DBS inside the hemaPEN under different condition of storage (Air-conditioned: 23°C - 41% RH; Humid: 21°C - 100% RH; Hot and humid: 40°C - 100% RH). Within 1 hour, the DBS are from 80-100% dry and after 2 hour, 95-105% dry independently of the storage condition. Number of hemaPEN tested: n = 42.

## Conclusion

Blood volume collection and storage on pre-punched 3.5 mm DBS papers using the hemaPEN technology, is accurate to 2.74  $\mu$ L  $\pm$  5% and demonstrates inter-device precision 0.7%.

The European Bioanalysis Forum Liquid Microsampling Forum<sup>3</sup> has previously reported that the volumetric accuracy and precision at volumes less than 4  $\mu$ L, using manual pipetting (2  $\mu$ L 80-115% accuracy and 15% CV) and end-to-end capillary (2  $\mu$ L 90-125% and 20% CV), make it difficult to meet criteria for regulated bioanalysis. What is an acceptable volumetric accuracy and precision for microsampling is still debated, however, the tighter accuracy and precision reported could make the hemaPEN an ideal tool for bioanalysis. Additional work to verify these results with different operators and different manufacturer's batches of capillaries is recommended.

The four identical DBS samples within the hemaPEN are protected from the environment within the body of the hemaPEN as shown by the ability of the sample to dry within one hour irrespective of the external temperature and relative humidity. This confers a high level of sample integrity during sample collection, storage and transport. The entire sample then enters the analytical workflow eliminating the volumetric aspect of hematocrit bias inherent with the conventional DBS cards<sup>4</sup>.

The hemaPEN has the potential to remove historical barriers associated with DBS technology adoption in therapeutic drug monitoring and clinical pharmacology, and this microsampling device has the potential to be a disruptive tool to change blood sampling and bioanalysis paradigms.

## References

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