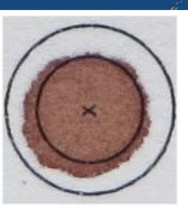
Prediction of the hematocrit of dried blood spots via potassium measurement on a routine clinical chemistry analyzer



Sara Capiau, Veronique V. Stove, Willy E. Lambert, Christophe P. Stove
Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, Ghent University, Belgium
christophe.stove@ugent.be

1. Introduction

Dried Blood Spot (DBS) sampling is increasingly used as a **minimally invasive tool** to acquire a representative blood sample in the context of therapeutic drug monitoring (TDM) and toxicology. However, the analysis of DBS is associated with **several issues**, such as contamination risk, blood volume spotted, blood spot homogeneity and hematocrit (Hct). Of these, the **Hct is undoubtedly the most widely discussed challenge**, as strongly deviating Hct values may significantly impact DBS-based quantitation. First of all, the Hct strongly influences the **spreading** of a blood drop on filter paper, with higher Hct values leading to smaller, more concentrated spots. Second, the Hct may influence parameters such as **recovery and matrix effect**. Third, when DBS results are to be **compared with** those obtained from **plasma**, the distribution of an analyte in red blood cells and plasma needs to be examined on a case-by-case basis. These Hct-associated issues make that, when compared to conventional plasma analysis, **DBS-based quantitation suffers from an additional unknown factor of uncertainty**. Although some advocate the use of volumetrically applied spots, this approach is difficult to sustain when aiming at patient self-sampling. **Hitherto, there is no approach that allows Hct prediction from non-volumetrically applied DBS.**

2. Optimized protocol for K^+-extraction from DBS

Bring a 3-mm DBS punch in a small tube → Add 50 µl 2.5 mM KCl in ultrapure H_2O → Spin down, Shake 5' (@1400 rpm) @ RT, Spin down → (1) Transfer 40 µl / (2) Transfer 50 µl → Measure $[K^+]$ via indirect potentiometry using the ISE (Ion Selective Electrode) module of the Cobas 8000 Clinical Chemistry Analyzer

3. Validation

Calibration curve parameters ($[K^+]$ in function of Hct)

- Data are homoscedastic (no need for weighting)
- Calibration curve is linear
- Slope & intercept: 3.15 [2.96 – 3.34] & -0.09 [-0.20 – -0.05].

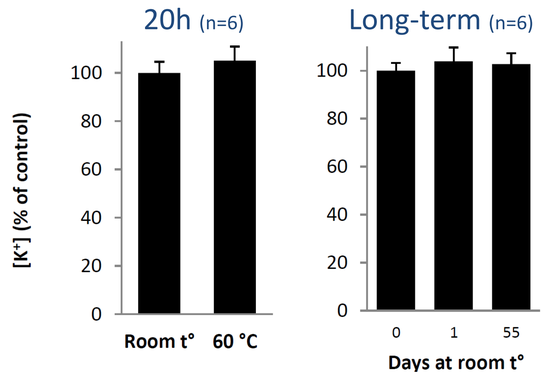
Precision & Accuracy

	Accuracy (% bias)	Intrabatch precision (% RSD)	Interbatch precision (% RSD)
A) QC LOW (Hct 0.24)	-1.07	8.49	11.69
QC MEDIUM (Hct 0.41)	0.09	3.30	5.05
QC HIGH (Hct 0.58)	-2.25	3.42	6.72
B) LLOQ (Hct 0.19)	4.20	9.25	9.25
ULOQ (Hct 0.63)	2.75	4.76	9.17
C) QC LOW (donor 2)	-2.05	8.75	10.57
QC MEDIUM (donor 2)	0.57	3.37	6.26
QC HIGH (donor 2)	-1.31	3.54	6.61

Overview of the data for accuracy and inter- and intrabatch (n = 8 duplicate measurements) precision. A and B respectively give the data obtained for QCs (3 Hct levels) and LOQs (LLOQ and ULOQ), prepared from blood from the same donor (donor 1) as the one in which the calibrators were prepared. C gives the data for QCs prepared from blood from another donor than the one in which the calibrators were prepared.

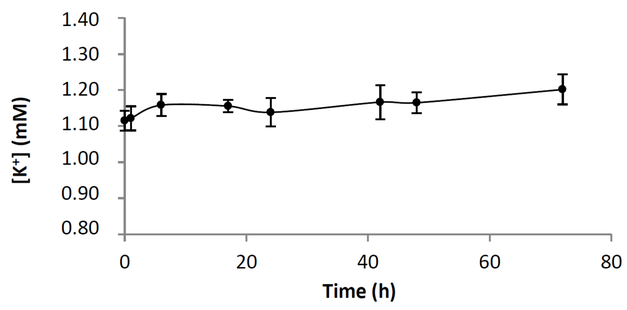
Stability

No effect of storage of DBS on $[K^+]$



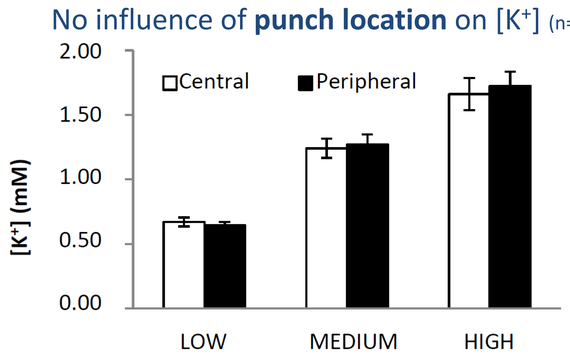
Extracts can be stored up to 3d at 4°C

No decrease in $[K^+]$ post-extraction (n=6)

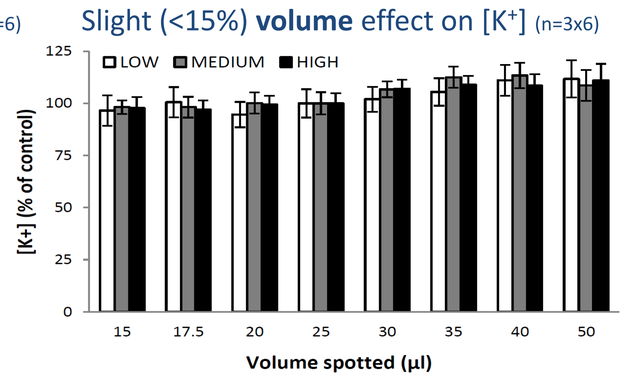


Effect of punching site and blood volume on $[K^+]$, tested at 3 Hct levels

No influence of **punch location** on $[K^+]$ (n=6)



Slight (<15%) **volume** effect on $[K^+]$ (n=3x6)

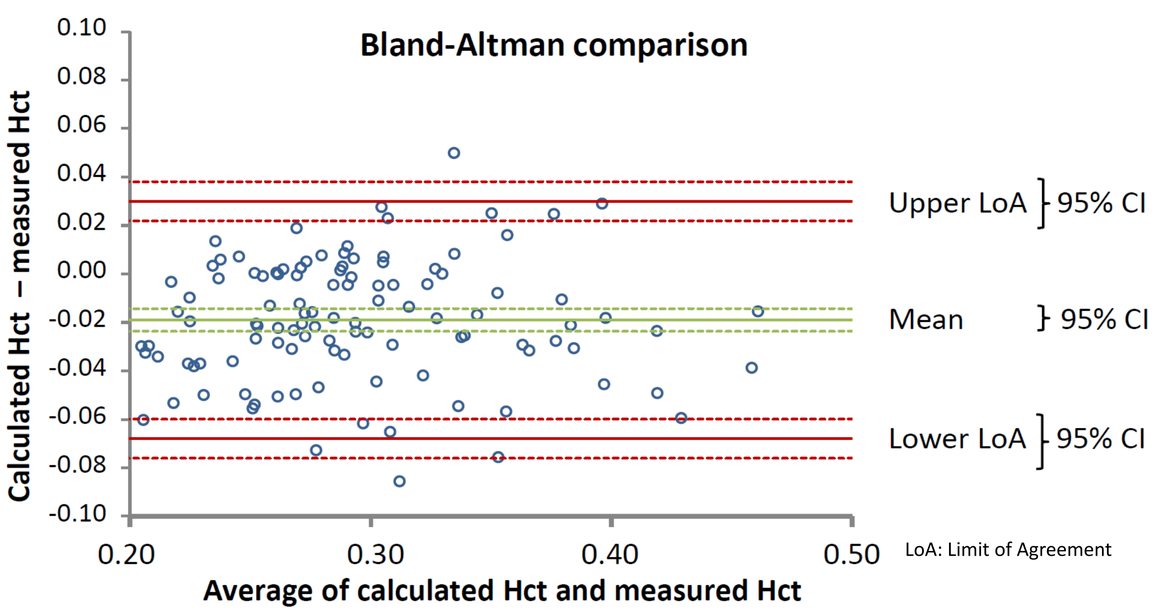


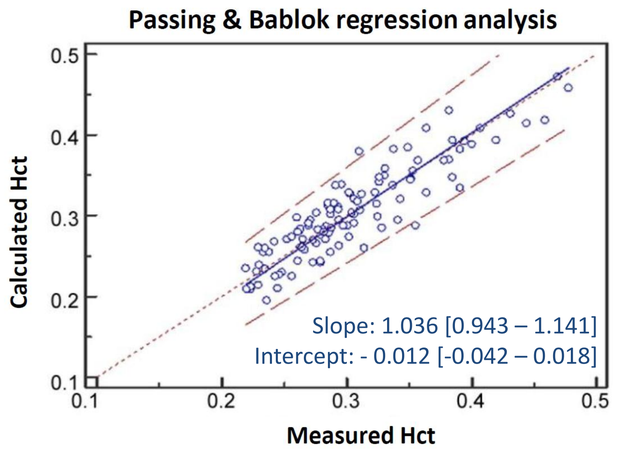
4. Method Application

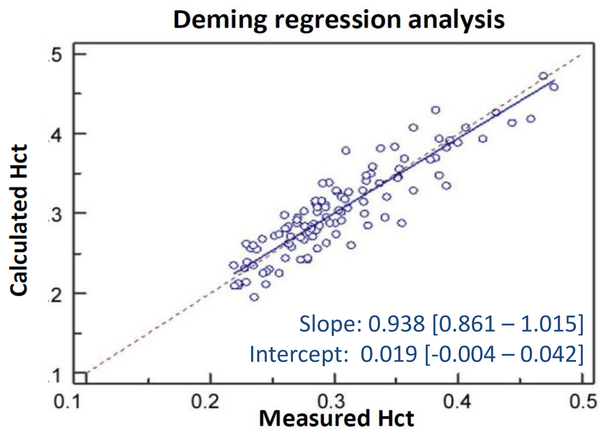
The method was applied on blood samples from patients (n = 111):

- Li-heparin blood was used to generate 25-µl DBS
- EDTA blood was used for routine Hct determination (Sysmex XE-5000)

⇒ **$[K^+]$ in 3-mm DBS was used to derive the 'calculated Hct'.**
⇒ **Bland-Altman comparison between 'calculated' & 'measured' Hct**
⇒ **Passing & Bablok and Deming regression analysis (after bias correction).**

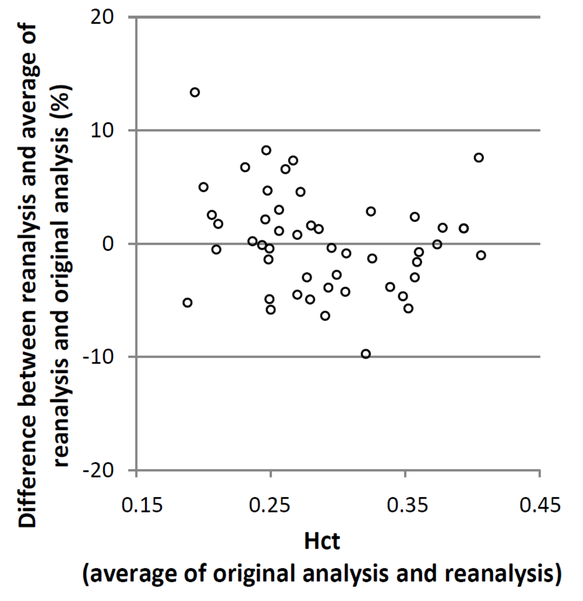






Incurred sample reanalysis

- subset of the patient samples (n=49)
- Criterium: 2/3 of repeated analyses should lie within ± 20% of the mean of the repeated and the original analysis
  ⇒ **FULFILLED!**



5. Conclusion and Future Perspectives

- **We developed and validated a procedure to predict the approximate Hct (LoA's of ±0.049, after bias correction) of DBS, based upon $[K^+]$ measurement.**
- **Successful application of the procedure on 3-mm punches of DBS, derived from patient samples, demonstrated its practical applicability.**
- **Being able to predict the Hct of any given DBS may render it possible in future to cope with -and possibly even to adjust for- the "hematocrit effect" in any given DBS-based analytical method.**
- **In future, this study should be extended to true capillary DBS.**