Different strategies for coping with the hematocrit effect in dried blood micro-sampling

Sara Capiau, Pieter M.M. De Kesel and Christophe P. Stove
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What can be analyzed with DBS?

(Almost) everything:

DNA/mRNA, proteins, (trace) elements, small molecules
General pros and cons of DBS sampling

**Pros:**
- Ease of sampling/home sampling
- Minimally invasive
- Small blood volumes
- Representative matrix (blood)
- Stabilizing effect
- Reduced biohazard
- Convenient & cost-effective transport and storage
- Straightforward sample processing and analysis
- Automatable
- # Animals ↓

**Cons:**
- Correct sampling
- Contamination risk
- Sensitive analysis required
- Extensive validation required
  - Site of punching
  - Blood volume spotted
  - Hematocrit effect
- Correlation between venous and capillary blood levels

De Kesel et al., Bioanalysis, 2013
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- Correlation between venous and capillary blood levels

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De Kesel et al., Bioanalysis, 2014
The Hct effect in practice

A. Whatman 903™

B. Whatman 903™

C. Whatman 903™

The impact of blood hematocrit can be divided into two aspects:

**ANALYTICAL IMPACT**

- Viscosity of the blood (differential spreading of blood with high and low Hct)
- Extraction efficiency of the compounds (i.e. recovery)
- Matrix effects

**PHYSIOLOGICAL IMPACT**

- Blood-to-plasma ratio of compounds (cfr. comparison blood-plasma [ ])

Whole DBS analysis

Pre-cut Dried Blood Spots

Perforated Dried Blood Spots
Li et al., Bioanalysis, 2011

Dried Matrix On Paper Disks
Meesters et al., Bioanalysis, 2012

All require volumetric application of DBS!
DBS: Direct application vs. volumetric application

Volumetric application vs. Non-volumetric application

Whole DBS analysis vs. Partial punch analysis

Lab or Hospital Sampling @ home
New Microfluidic-Based Sampling Procedure for Overcoming the Hematocrit Problem Associated with Dried Blood Spot Analysis

Luc Alexis Leuthold,† Olivier Heudi,*† Julien Déglon,‡ Marc Raccuglia,† Marc Augsburger,§ Franck Picard,† Olivier Kretz,† and Aurélien Thomas§
Microfluidic-based volumetric sampling (1)

http://dbs-system.ch/technology/
Microfluidic-based volumetric sampling (2)

A. Unknown fluid volume
B. Dissolving the thin film at the inlet
C. Defined blood volume
D. Break through at the inlet
E. Pinch-off region
F. Membrane dissolved
G. Excess fluid (waste)

G. Lenk, Karolinska Inst, SWEDEN
Microfluidic-based volumetric sampling (3)

HemaPEN™ (Trajan Scientific and Medical)

hemaPEN instantly collects a precise volume of blood
Volumetric Absorptive Microsampling

VAMS (Mitra®, Phenomenex)

Volumetric Absorptive Microsampling: A Dried Sample Collection Technique for Quantitative Bioanalysis

Philip Denniff and Neil Spooner*

Volumetric sampling (10.2 ul), irrespective of hematocrit
Does volumetric absorptive microsampling eliminate the hematocrit bias for caffeine and paraxanthine in dried blood samples? A comparative study

Pieter M.M. De Kesel, Willy E. Lambert, Christophe P. Stove

Study Set-up:

- Use left-over EDTA-anticoagulated blood from patients (≠ hospital departments)
- Compare liquid blood – VAMS – DBS
- Analytes: caffeine & paraxanthine

De Kesel et al., Analytica Chimica Acta, 2015
Volumetric Absorptive Microsampling

De Kesel et al., Analytica Chimica Acta, 2015
Volumetric Absorptive Microsampling

De Kesel et al., Analytica Chimica Acta, 2015
Volumetric Absorptive Microsampling

De Kesel et al., Analytica Chimica Acta, 2015
Volumetric Absorptive Microsampling

Evaluation of impact of hematocrit on recovery:

- VAMS: Similar extraction procedure as for DBS
- ↓ recovery at higher hematocrits

Absolute recovery and matrix effect data (n = 3) for caffeine and paraxanthine at two concentration levels in VAMS samples prepared using whole blood with varying Hct values.

<table>
<thead>
<tr>
<th>Hct</th>
<th>Caffeine</th>
<th>Paraxanthine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low QC</td>
<td>High QC</td>
</tr>
<tr>
<td>0.21</td>
<td>101.45 ± 2.26</td>
<td>101.79 ± 0.67</td>
</tr>
<tr>
<td>0.42</td>
<td>101.30 ± 1.28</td>
<td>100.53 ± 2.67</td>
</tr>
<tr>
<td>0.48</td>
<td>93.86 ± 0.89</td>
<td>91.08 ± 2.03</td>
</tr>
<tr>
<td>0.62</td>
<td>92.01 ± 4.80</td>
<td>92.91 ± 4.74</td>
</tr>
</tbody>
</table>

The calibration line had been set up at a relative high Ht;  
⇒ because of the impact of the Ht, recovery of the vast majority of the samples will be slightly higher than that of the calib.’s

De Kesel et al., Analytica Chimica Acta, 2015
Evaluation of impact of hematocrit on recovery:

→ extraction at 60 °C

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<tr>
<td></td>
<td>Low QC</td>
<td>High QC</td>
</tr>
<tr>
<td>0.21</td>
<td>101.21 ± 3.40</td>
<td>98.45 ± 1.37</td>
</tr>
<tr>
<td></td>
<td>Absolute recovery</td>
<td>101.46 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>(mean ± SD, %)</td>
<td>106.77 ± 3.11</td>
</tr>
<tr>
<td>0.62</td>
<td>104.68 ± 3.77</td>
<td>100.70 ± 2.05</td>
</tr>
</tbody>
</table>
The impact of blood hematocrit can be divided into two aspects:

**ANALYTICAL IMPACT**

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- Matrix effects

**PHYSIOLOGICAL IMPACT**

- Blood-to-plasma ratio of compounds (cfr. comparison blood-plasma [ ])

⇒ Also to be evaluated in volumetric DBS applications
Investigation of Different Approaches to Incorporating Internal Standard in DBS Quantitative Bioanalytical Workflows and Their Effect on Nullifying Hematocrit-Based Assay Bias

Paul Abu-Rabie,*†,‡ Philip Denniff,† Neil Spooner,† Babur Z. Chowdhry,‡ and Frank S. Pullen‡

* Bioanalytical Science and Toxicokinetics, Drug Metabolism and Pharmacokinetics, Platform Technologies and Sciences, GlaxoSmithKline Research and Development, Ware, SG12 0DG, United Kingdom
‡ Faculty of Engineering and Science, University of Greenwich, Medway Campus, Chatham Maritime, Kent ME4 4TB, United Kingdom

Workflow for Nullifying Dried Blood Spot Hematocrit based Area and Recovery bias

1. Accurate volume blood dispense
2. Internal standard addition prior to extraction
3. Whole spot extraction/elution
**New materials**

**Qyntest® (Qynion):**

Non-cellulose multilayered material that would allow even spreading of blood, irrespective of hematocrit

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*Figure 4. Effect of hematocrit value of blood on the spot size on different DBS cards. In total 35 spots were measured, Ht20, 45 and 70 n = 9, Ht30 and 60 n = 4. Ht: Hematocrit.*

*Mengerink et al, Bioanalysis, 2015*
Hemaspot™ (Spot-On-Sciences):
Minimizing the Hct effect

Prepare calibrators in blood with an appropriate Hct

=> Define target population
The hematocrit issue: a chicken-and-egg-situation

Perform DBS analysis in a validated Hct interval

⇒ Only samples within this Hct range yield valid results

However, how does one know if the Hct of a given DBS lies within this interval?

⇒ Need for Hct prediction
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,† and Christophe P. Stove*†

†Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium
‡Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium

- 
  [K⁺] intracellularly >> [K⁺] plasma and > 99% of cells are RBCs
- [K⁺] are tightly controlled → no large interindividual variability
- K⁺ is universal
- K⁺ proved to be stable in DBS
- K⁺ easily measurable in DBS
Prediction of the Hematocrit of Dried Blood Spots via Potassium Measurement on a Routine Clinical Chemistry Analyzer

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‡Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium

Bring a 3-mm DBS punch in a small tube

Extract 2X with 50 µl 2.5 mM KCl in ultrapure H₂O

Measure [K+] in 90 µl via indirect potentiometry using the ISE (Ion Selective Electrode) module of the Cobas 8000 Clinical Chemistry Analyzer

Deming regression analysis
Potassium-based algorithm allows correction for the hematocrit bias in quantitative analysis of caffeine and its major metabolite in dried blood spots

Pieter M. M. De Kesel • Sara Capiau • Veronique V. Stove • Willy E. Lambert • Christophe P. Stove
A K⁺-based algorithm to correct for Hct effect was used. The study included healthy volunteers (n = 61) and hospital patients (n = 117). Venous whole blood samples were collected for [caffeine] and [paraxanthine] measurements. Venous DBS samples were also collected for [K⁺] measurements. These measurements were used to determine the Hct effect and predict Hct levels.
K⁺-based algorithm to correct for Hct effect

[caffeine] > LLOQ
✓ n = 100
✓ Hct range 0.18 – 0.47

Reference set (n = 50)

DEDUCE NEW CORRECTION ALGORITHM:

Corrected [DBS] = [DBS]*((-0.4141*[K⁺]) + 1.5052)
K⁺-based algorithm to correct for Hct effect

[caffeine] > LLOQ
✓ n = 100
✓ Hct range 0.18 – 0.47

Test set (n = 50)

APPLY NEW CORRECTION ALGORITHM:

Corrected [DBS] = [DBS]*((-0.4141*[K⁺]) + 1.5052)
K⁺-based algorithm to correct for Hct effect

[caffeine] > LLOQ
✓ n = 100
✓ Hct range 0.18 – 0.47

Test set (n = 50)
K⁺-based algorithm to correct for Hct effect

[paraxanthine] > LLOQ
✓ n = 103
✓ Hct range 0.17 – 0.47

Test set (n = 103)

Apply new correction algorithm:

Corrected [DBS] = [DBS]*((-0.4141*[K⁺]) + 1.5052)
**K⁺-based algorithm to correct for Hct effect**

[paraxanthine] > LLOQ

- n = 103
- Hct range 0.17 – 0.47

Test set (n = 103)
Estimating the Hct of DBS: K+

- Straightforward sample prep.
- Routine analyzer
- Cheap
- Stable
- 3-mm punch
- Adopted by other groups

- Requires sample prep.
- Destructive
- Time-consuming
Estimating the Hct of DBS: Hemoglobin

Optimization of extraction solvent

Hb is apparently not stable

Cobas 8000

Co-oximeter

Stability of Hb in DBS vs. time

Total [Hb] vs. time

Fraction of total Hb [%]

MetHb ↑

HbO₂ ↓

Time (days)
Estimating the Hct of DBS: Hemoglobin

- Additional Hb derivatives formed upon drying!

- Different Hb derivatives not all detected using routine measurements

HYPOTHESIS: \( \text{Sum HbO}_2, \text{metHb and HC} = \text{constant} = \text{measure for Hct?} \)

Estimating the Hct of DBS: Hemoglobin

- Bremmer et al.:

Method that estimates the age of bloodspatters on crime scenes using the relative abundance of these Hb-derivatives

Non-contact diffuse reflectance spectroscopy

Could we use this technique to measure the different Hb derivatives in DBS (and hence the Hb sum)?

YES! => non-contact Hct estimation before DBS analysis
Method completely validated (Hct estimation independent of age DBS!)

EDTA patient samples (n = 288)
- Whole blood: ‘true’ Hct using Sysmex XE-5000
- Venous DBS: calculated HCT using non-contact method

=> 234 out of 288 samples within 15% of true Hct
=> 270 out of 288 samples within 20% of true Hct
Use of Hct prediction for Hct correction

- Caffeine reference set: correlation between estimated Hct and extent of Hct effect
- Set up of Hct correction algorithm
- Application to test set
Use of Hct prediction for Hct correction

- Caffeine reference set: correlation between estimated Hct and extent of Hct effect
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- Application to test set

Caffeine test set (n = 48)
Estimating the Hct of DBS: Hemoglobin

Hemoglobin-based hematocrit prediction:
✓ Non-destructive
✓ No punching
✓ No sample prep.
✓ Very fast
✓ Does not interrupt workflow drastically
✓ Cheap
✓ Highly reproducible
✓ Applicable for hematocrit correction

MERE SCANNING SUFFICES TO KNOW THE HCT OF A DBS!
The use of a membrane filtration device to form dried plasma spots for the quantitative determination of guanfacine in whole blood

Yuanyuan Li¹, Jack Henion¹*, Richard Abbott² and Phillip Wang³
Figure 1. Construction of the autoDPS card. (A–E) AutoDPS card in exploded view: (A) adhesive overlay, (B) red blood cell filtration disks, (C) autoDPS label composed of cardstock, (D) plasma collection substrate containing hydrophilic plasma collection wells separated from one another by hydrophobic wax barriers, (E) cardstock for support. (F) Top and (G) bottom of the autoDPS card showing that the wax barrier penetrates the thickness of the plasma collection material. (H) Fully assembled autoDPS card and (I) an autoDPS card whose upper red blood cell filter is being removed.
Simple, Miniaturized Blood Plasma Extraction Method

Jin-Hee Kim,† Timothy Woenker,‡ Jiri Adamec,§ and Fred E. Regnier*,†,||
Dried Plasma Spots

Hemaspot SE™ (Spot-On-Sciences):

- Application surface
- Blood separation membrane
- Mesh support
- Desiccant

Moisture-tight cartridge
Conclusion

• New promising developments
  ➔ convenient sampling
  ➔ ‘no’ hematocrit issue
  ➔ proper IS addition

• More experience required
• Each approach has its limitations
• Automation compatible

=> Will result in more home sampling applications
=> Drug development?

=> Remaining issue: capillary [ ] ≡ venous [ ]?