

A novel non-contact method to estimate the hematocrit of dried blood spots

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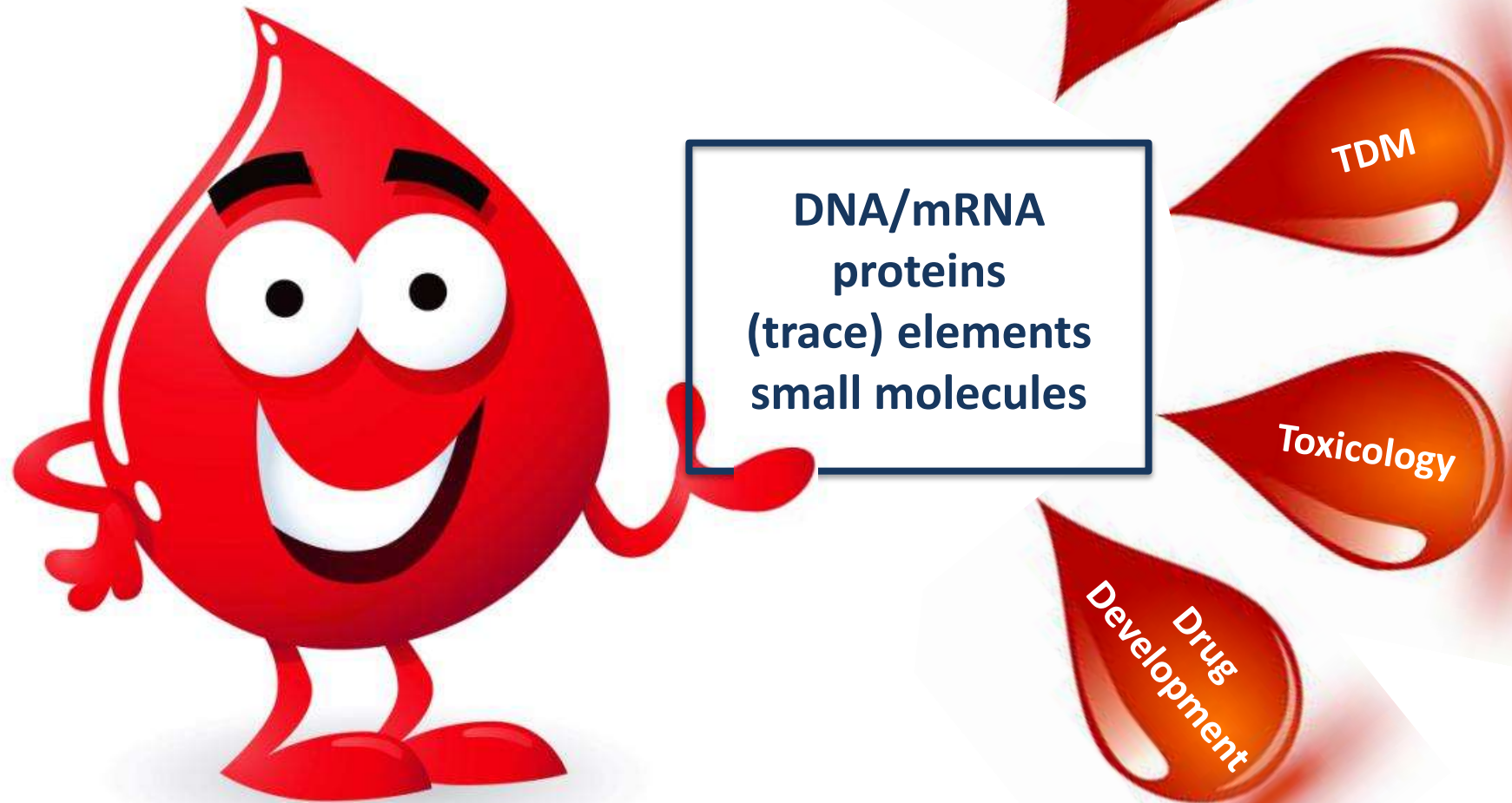
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What can be analyzed with DBS?

(Almost) everything:



General pros and cons of DBS sampling



- 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 ↓



- 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

General pros and cons of DBS sampling

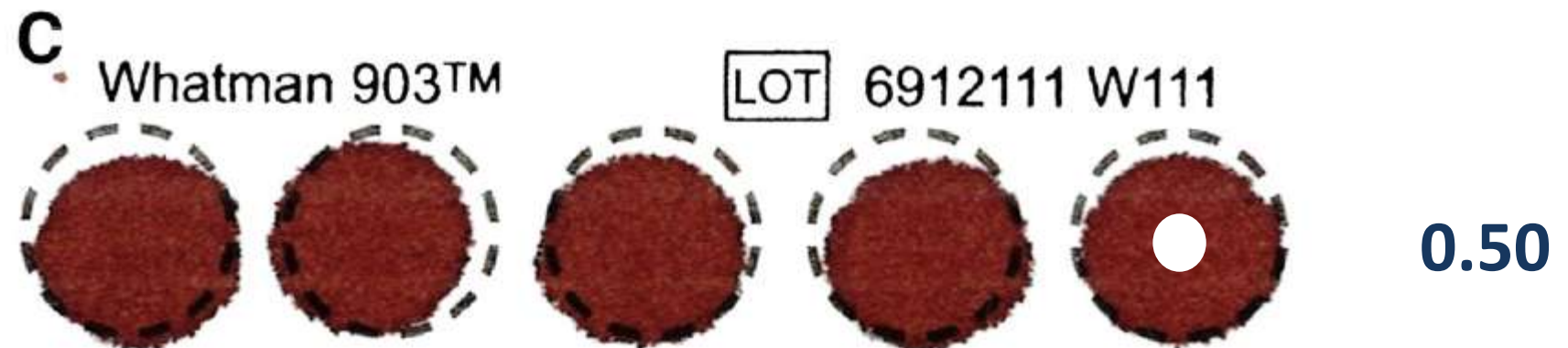
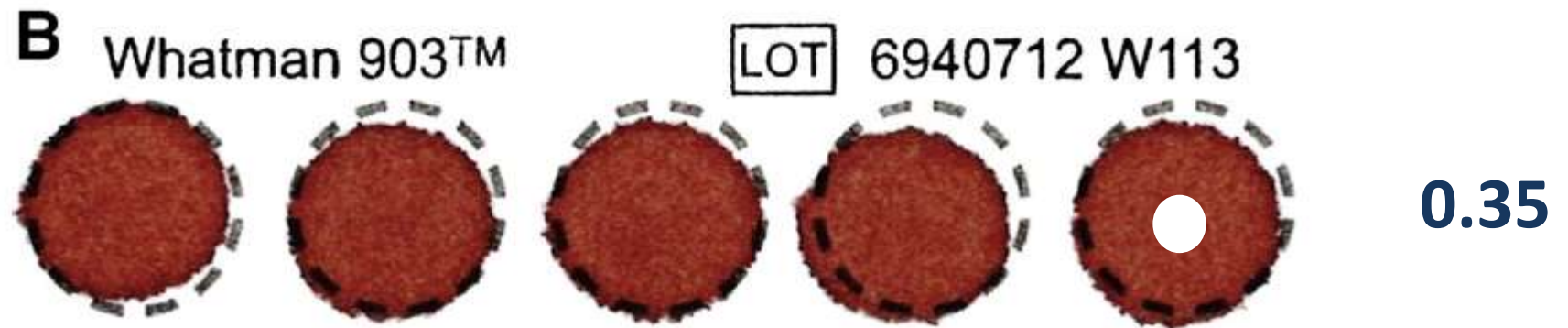
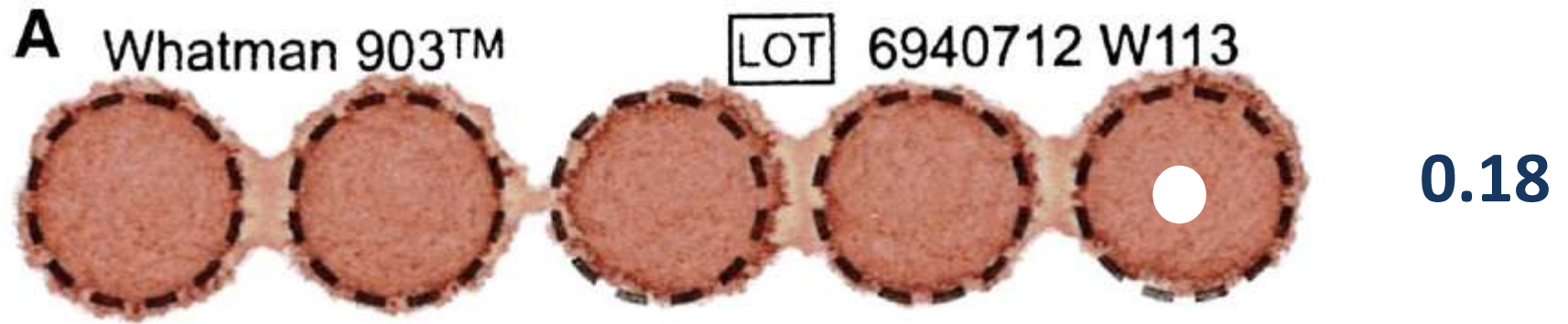


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The Hct effect in practice



The Hct effect: a two-fold problem

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 [])

Prediction of the Hematocrit of Dried Blood Spots via Potassium Measurement on a Routine Clinical Chemistry Analyzer

Sara Capiiau,[†] 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 \gg $[K^+]$ plasma and $> 99\%$ of cells are RBCs
- $[K^+]$ are tightly controlled \rightarrow no large interindividual variability
- K^+ is universal
- K^+ proved to be stable in DBS
- K^+ easily measurable in DBS

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Bring a 3-mm DBS punch in a small tube



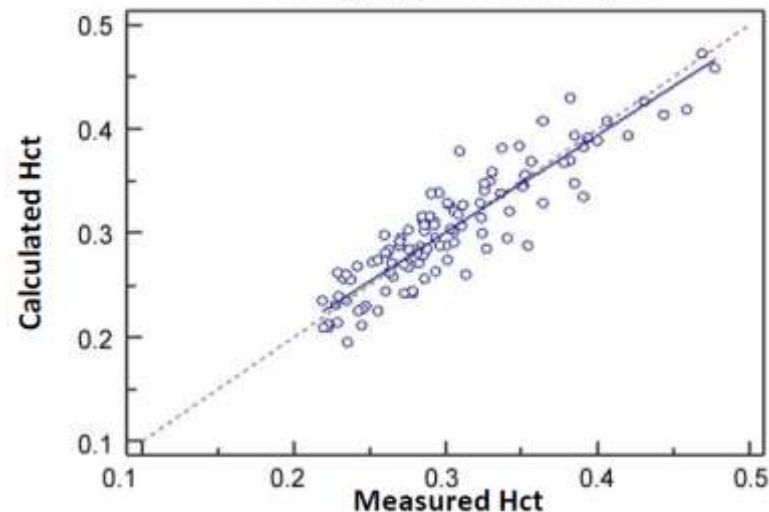
Extract 2X with 50 μ l 2.5 mM KCl in ultrapure H₂O



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



Deming regression analysis



Potassium based Hct estimation



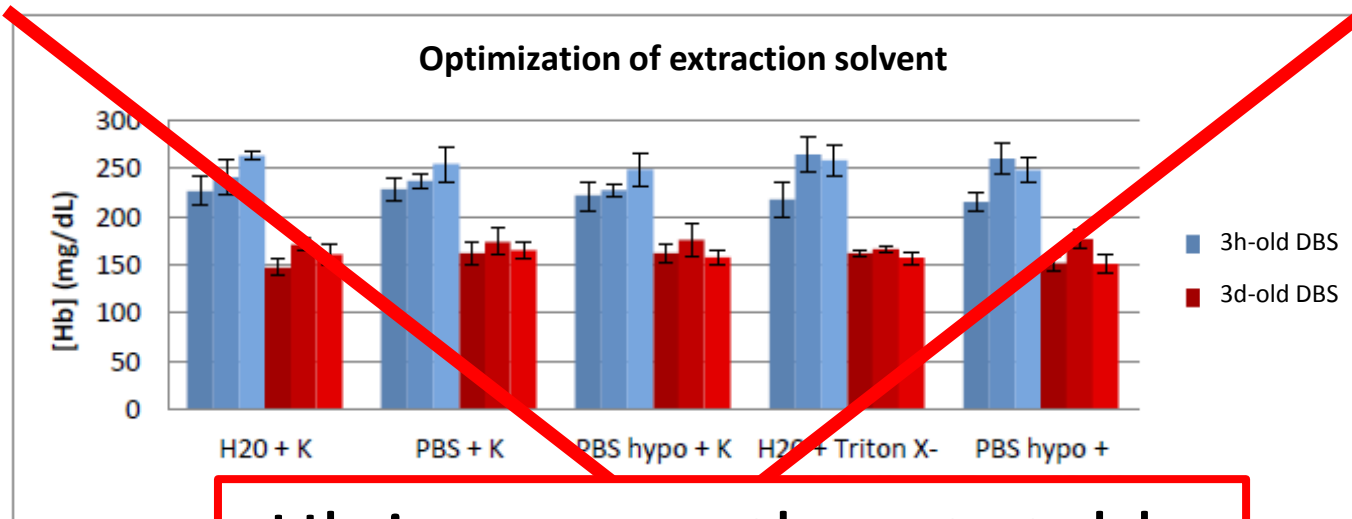
- Straightforward sample prep.
- Routine analyzer
- Cheap
- Stable
- 3-mm punch

- Adopted by other groups



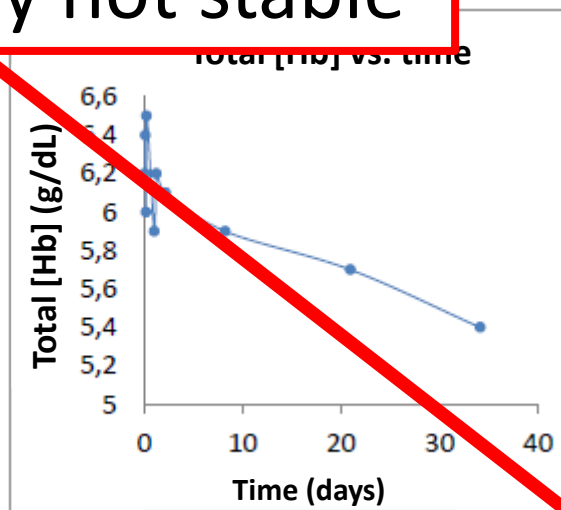
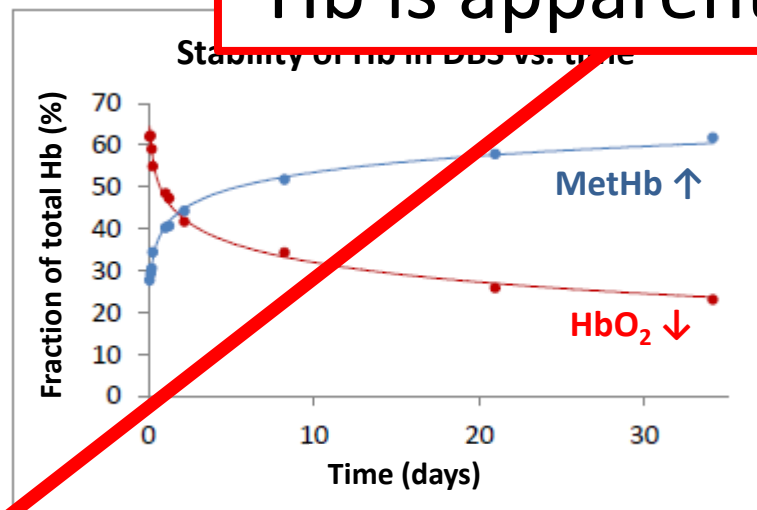
- Requires sample prep.
- Destructive
- Time-consuming

Why not use hemoglobin?



Cobas 8000

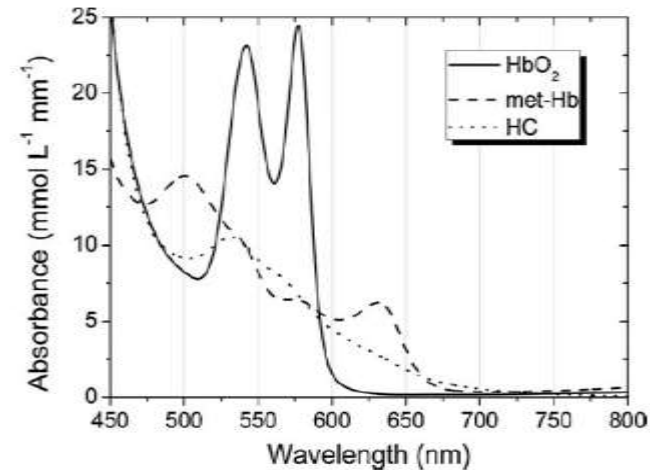
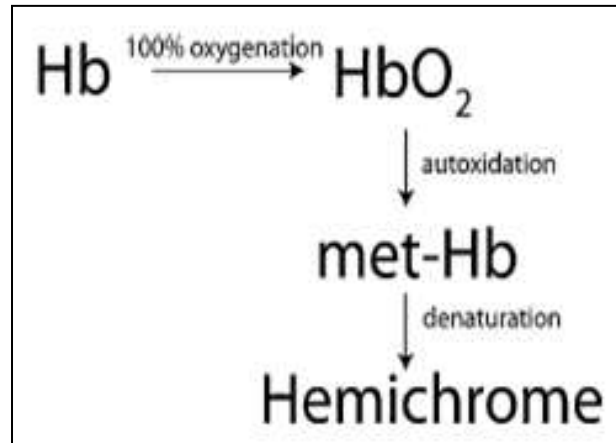
Hb is apparently not stable



Co-oximeter

New strategy

- Additional Hb derivatives formed upon drying!



- Different Hb derivatives not all detected using routine measurements



HYPOTHESIS: Sum HbO₂, metHb and HC = constant = measure for Hct?

New strategy

- 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



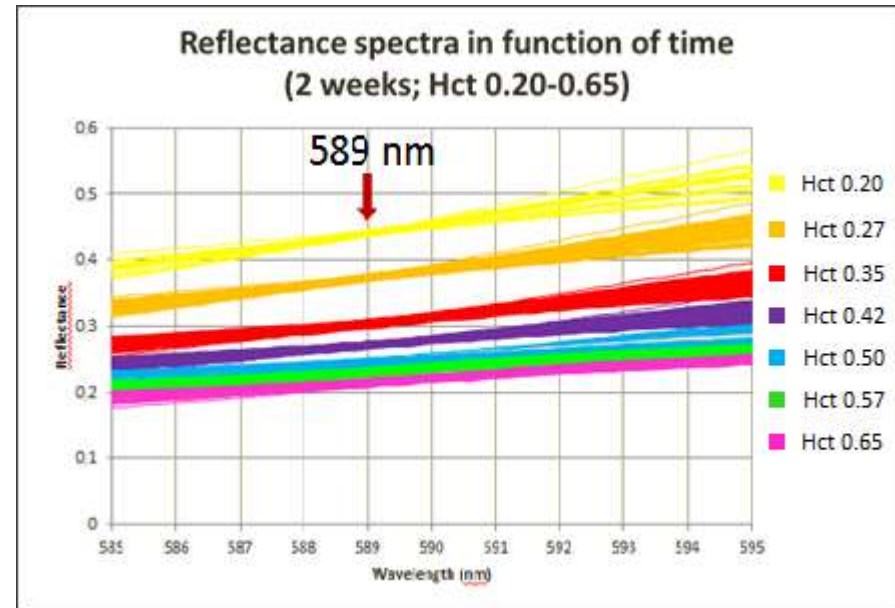
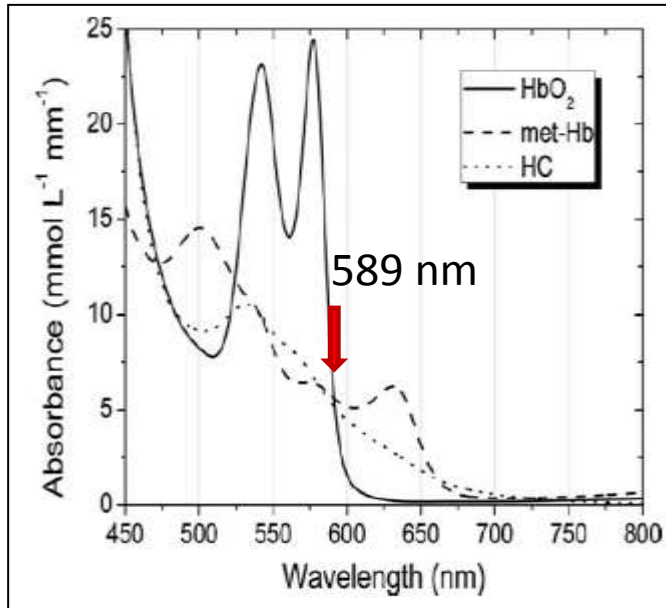
Could we use this technique to measure the different Hb derivatives in DBS (and hence the Hb sum)?



YES! => non-contact Hct estimation before DBS analysis

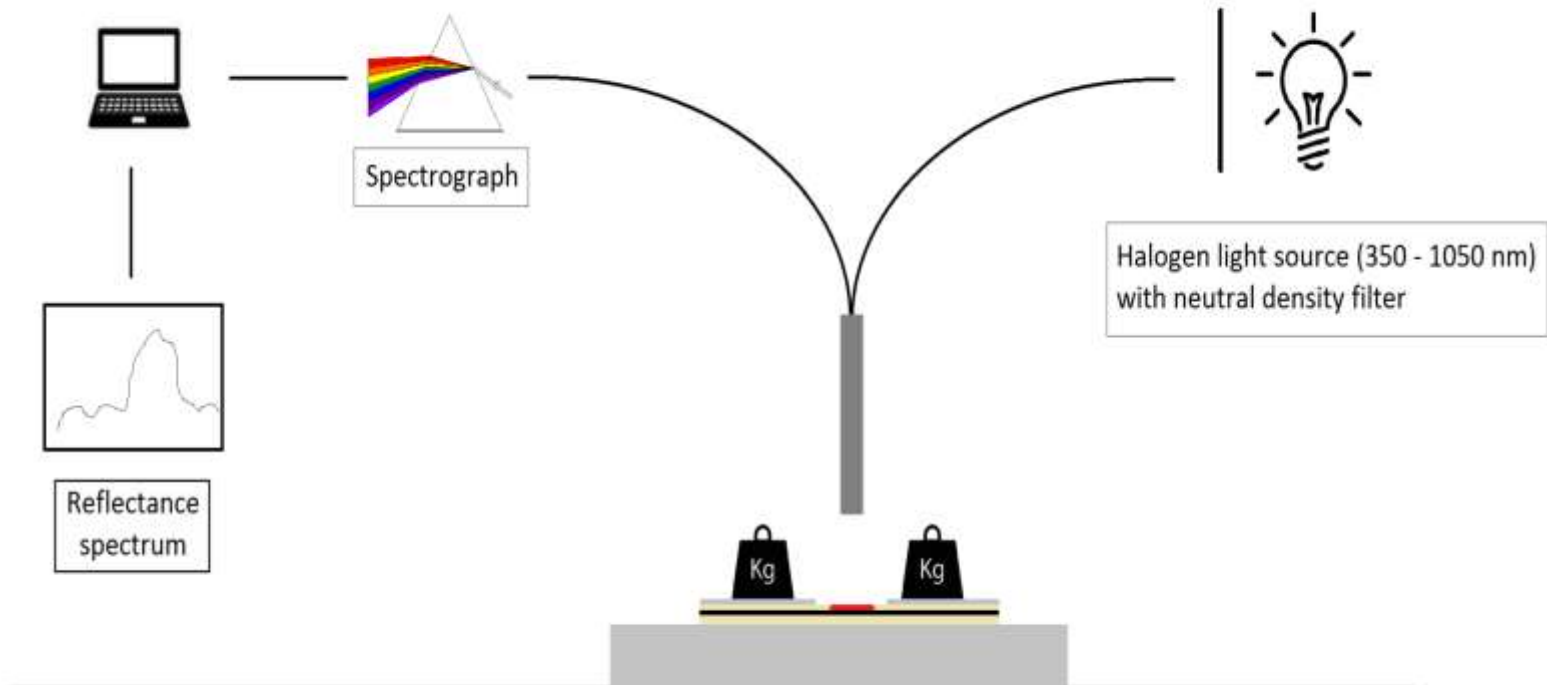
New strategy

Simplification:



Reflectance at 589 nm
= measure of 'total Hb' and Hct
= stable in function of time

Used set-up



Validation

Accuracy & Precision

3 x 2 set-up

Calibration curves set up in blood of person 1

QC's of person 1 + 5 other volunteers (Hct: 0.25, 0.42 & 0.65)

Calibration curves set up in blood of person 2

QC's of person 2 + 5 other volunteers (Hct: 0.25, 0.42 & 0.65)



Accuracy

Intraday precision

Interday precision

} $\leq 15 \%$

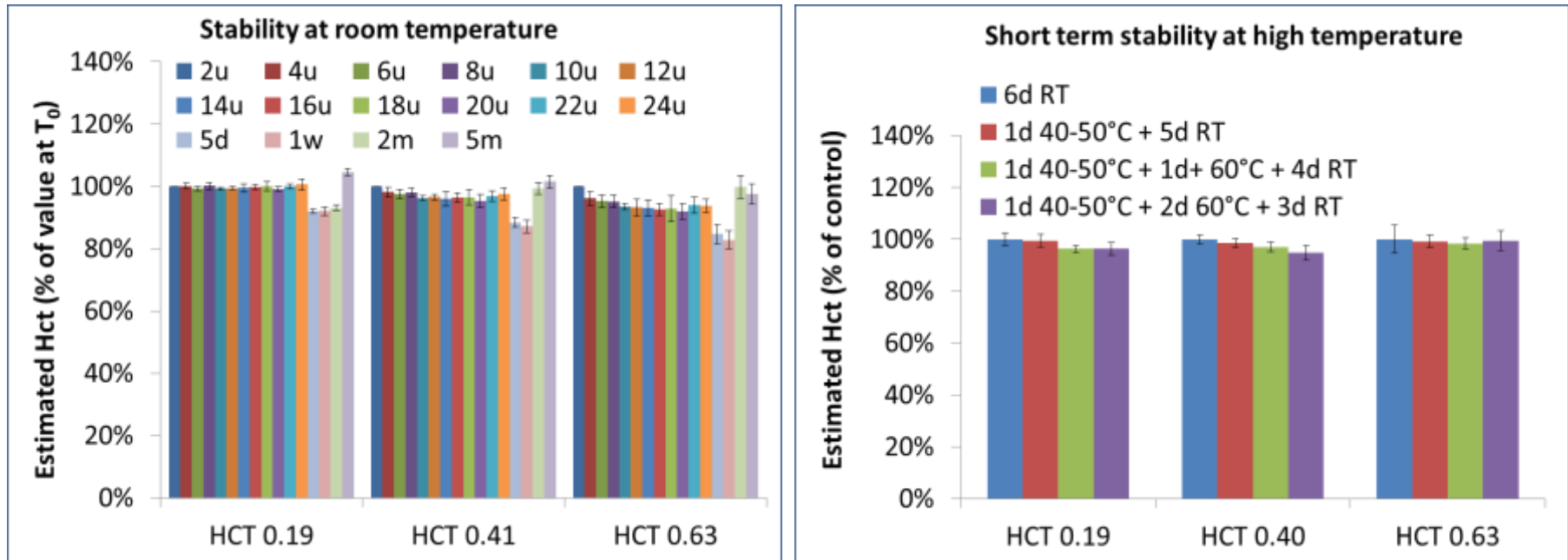


LLOQ = 0.20

ULOQ = 0.67

Validation

Stability

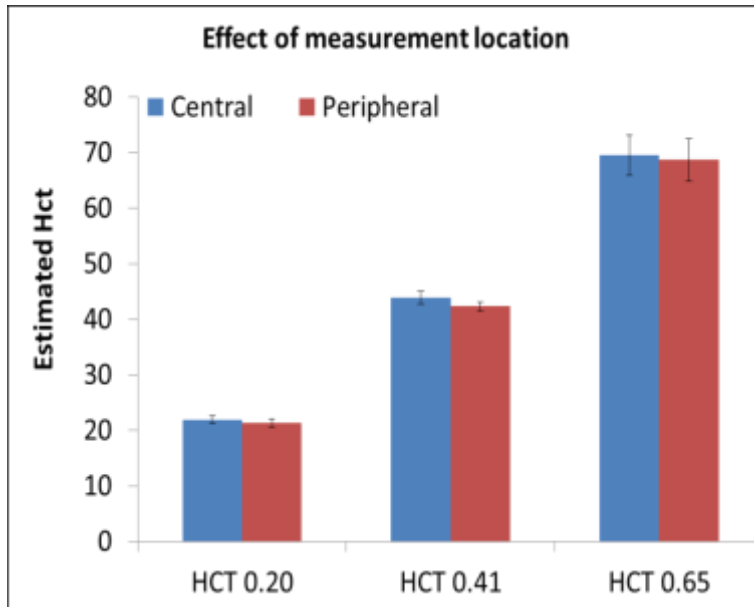


Estimated Hct stable @ RT \geq 5 months

Estimated Hct stable under extreme transport conditions

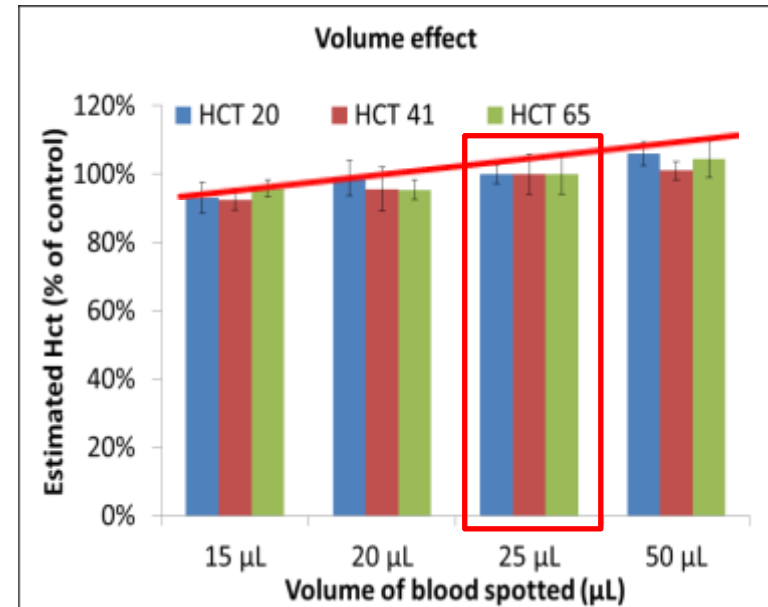
Validation

Volcano effect



No volcano effect
(no outer edge)

Volume effect

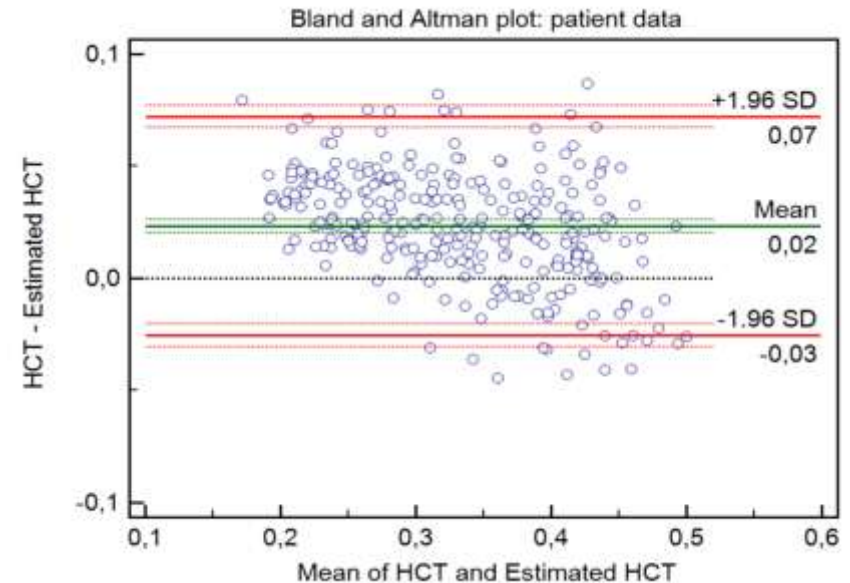
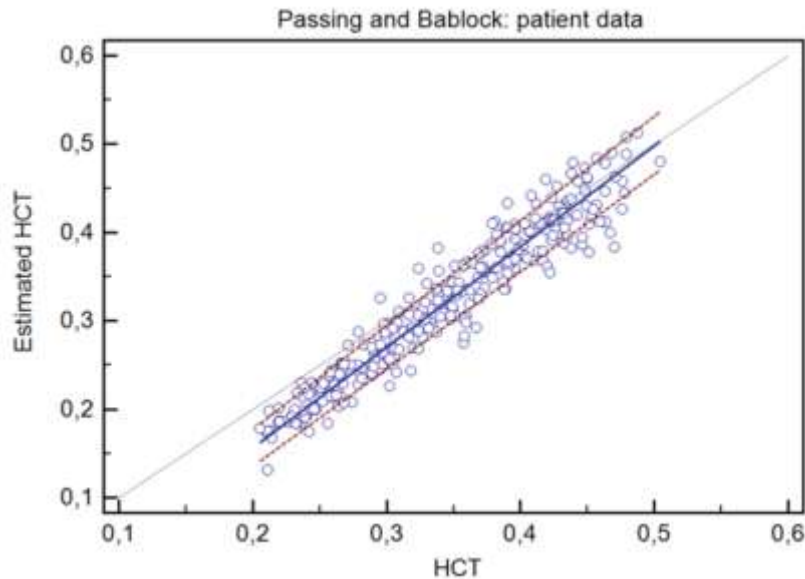


Slight volume effect
Within acceptable limits

Application

EDTA patient samples (n = 288)

- Whole blood: 'true' Hct using Sysmex XE-5000
- Venous DBS: calculated HCT using non-contact method



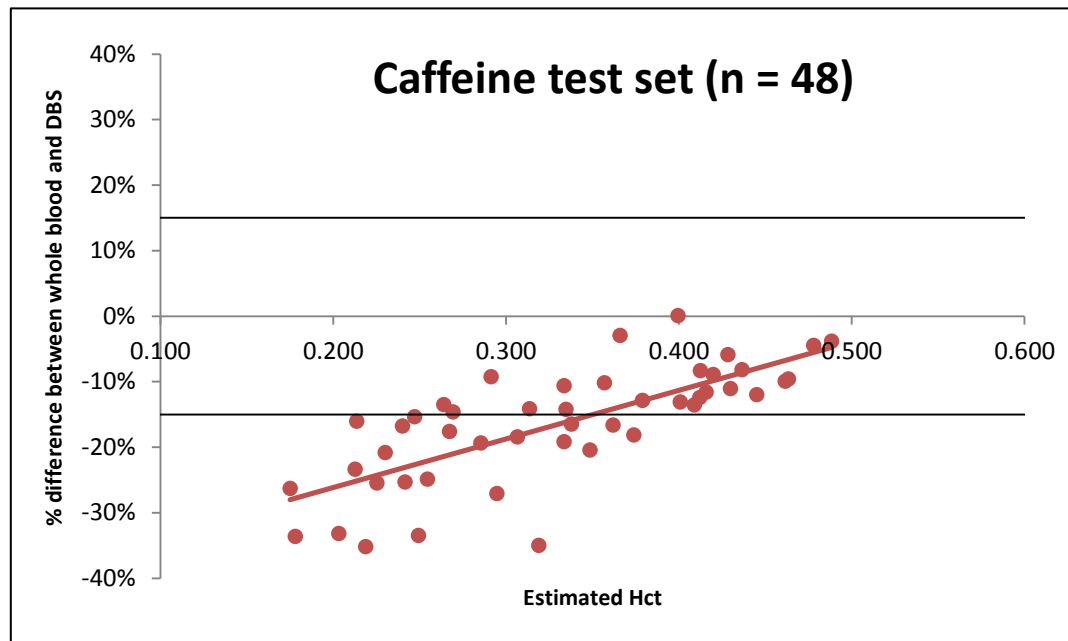
=> 234 out of 288 samples within 15% of true Hct

=> 270 out of 288 samples within 20% of true Hct

Estimating the Hct of DBS: Hemoglobin

Use of Hct prediction for Hct correction

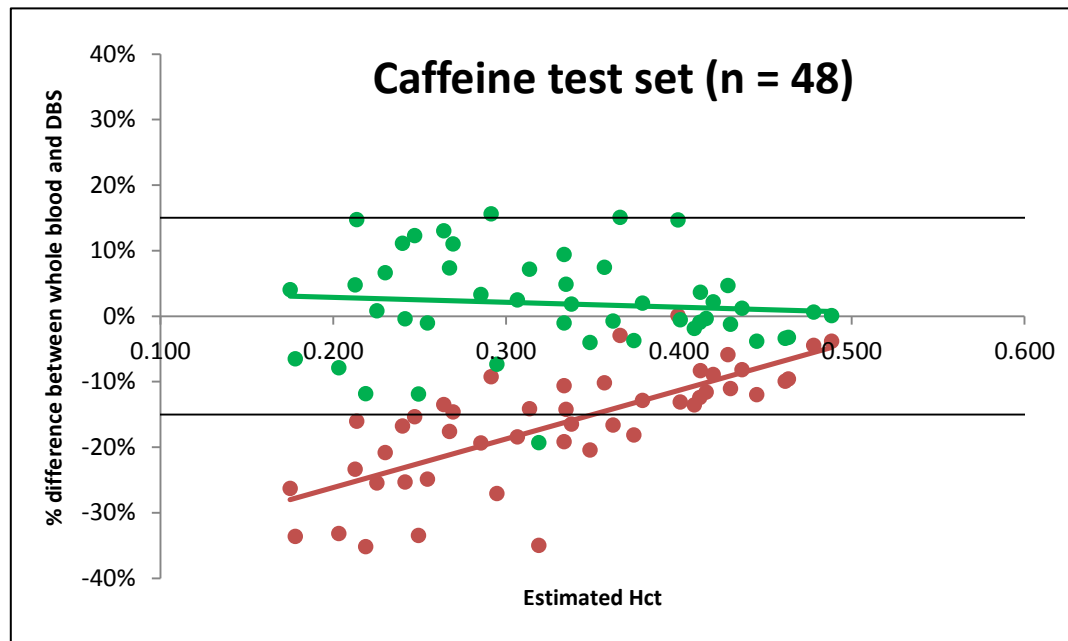
- Caffeine reference set: correlation between estimated Hct and extent of Hct effect
- Set up of Hct correction algorithm
- Application to test set



Estimating the Hct of DBS: Hemoglobin

Use of Hct prediction for Hct correction

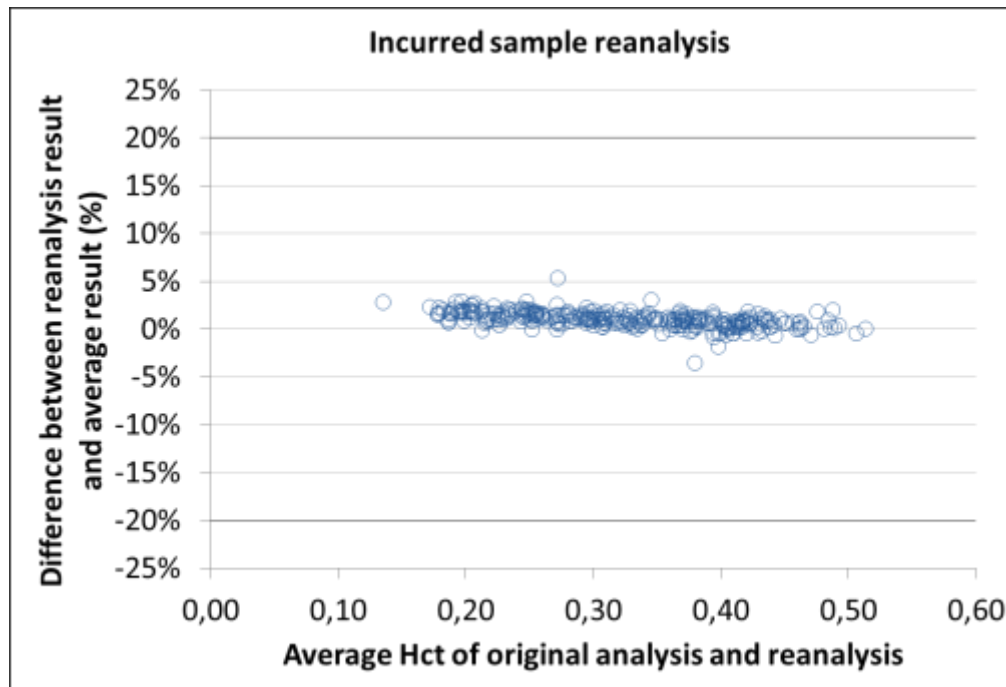
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Application

Incurring sample reanalysis:

- All patient samples reanalyzed after 3 days
- Criterium: 2/3 of repeated analyses should lie within $\pm 20\%$ of the mean of the repeated and the original analysis

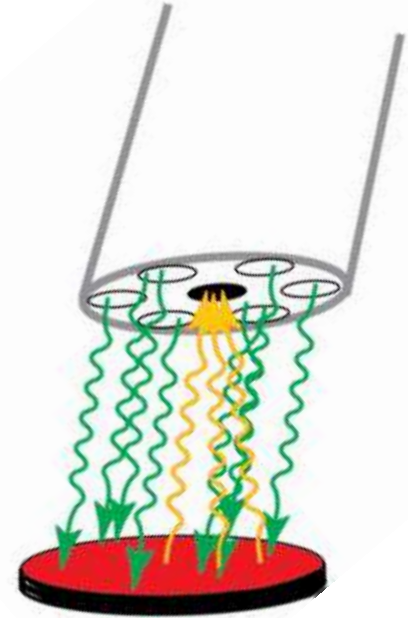


**CRITERIUM
FULFILLED!**

Conclusion

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!

Thank you!

