FACULTY OF PHARMACEUTICAL SCIENCES

DEPARTMENT OF BIOANALYSIS
LABORATORY OF TOXICOLOGY

In-depth evaluation of automated non-contact reflectance-based hematocrit prediction of dried blood spots

Laura Boffel[#], Liesl Heughebaert[#], Stijn Lambrecht, Marc Luginbühl, Christophe Stove // EBF 9th YSS // 12/05/2023

[#]equally contributed

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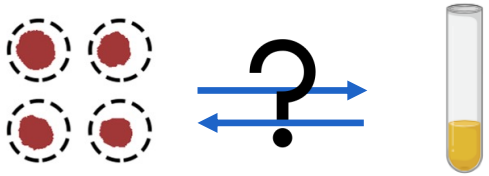
fwo Research Foundation Flanders
Opening new horizons

Dried blood spots and the hematocrit effect

The hematocrit effect

Physiological aspect



Analytical aspect

- Area bias
- Recovery bias
- Matrix bias

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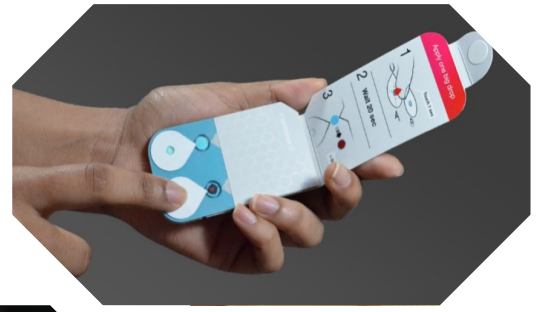
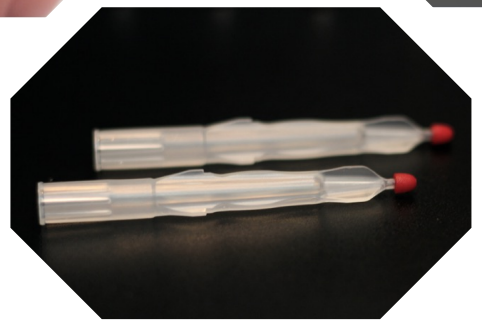
Strategies to cope with the hematocrit effect

Alternative microsampling devices





FOCUS SERIES: ALTERNATIVE SAMPLING STRATEGIES

Alternative Sampling Devices to Collect Dried Blood Microsamples: State-of-the-Art

Lisa Delahaye, PharmD, Herman Veenhof, PhD,† Birgit C. P. Koch, PhD,‡ Jan-Willem C. Alffenaar, PhD,§¶|| Rafael Linden, PhD,** and Christophe Stove, PhD**

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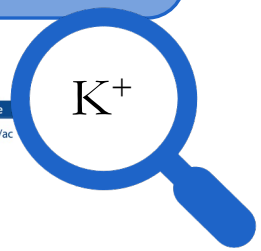
Methodologies to predict the hematocrit



analytical chemistry

Article

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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

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Clinica Chimica Acta 523 (2021) 239–246

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Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Near-infrared-based hematocrit prediction of dried blood spots: An in-depth evaluation

Lisa Delahaye [a,1], Liesl Heughebaert [a,1], Christoph Lühr [b], Stijn Lambrecht [c], Christophe P. Stove [a,*]

[a] Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent, Belgium
[b] BÜCHI Labortechnik GmbH, Essen, Germany
[c] Laboratory of Clinical Chemistry and Hematology, Ghent University Hospital, Ghent, Belgium

analytical chemistry

Article

Cite This: Anal. Chem. 2018, 90, 1795–1804

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UV-Vis

Correction for the Hematocrit Bias in Dried Blood Spot Analysis Using a Nondestructive, Single-Wavelength Reflectance-Based Hematocrit Prediction Method

Sara Capiau,† Leah S. Wilk,‡ Pieter M. M. De Kesel,† Maurice C. G. Aalders,‡ and Christophe P. Stove*,†

†Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemse-steenweg 460, Ghent 9000, Belgium

‡Department of Biomedical Engineering and Physics, Academic Medical Center, University of Amsterdam, Meibergdreef 9, Amsterdam 1105 AZ, The Netherlands

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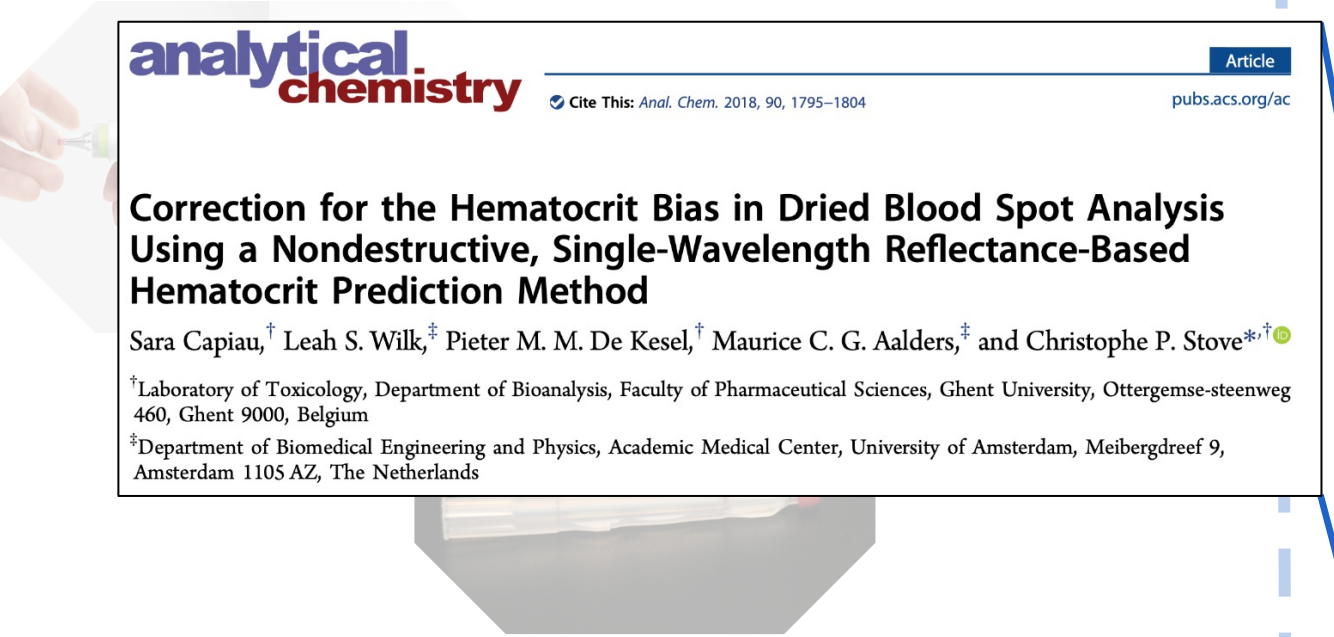

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UV-Vis-based hematocrit prediction module incorporated into the automated CAMAG® DBS-MS 500 HCT system

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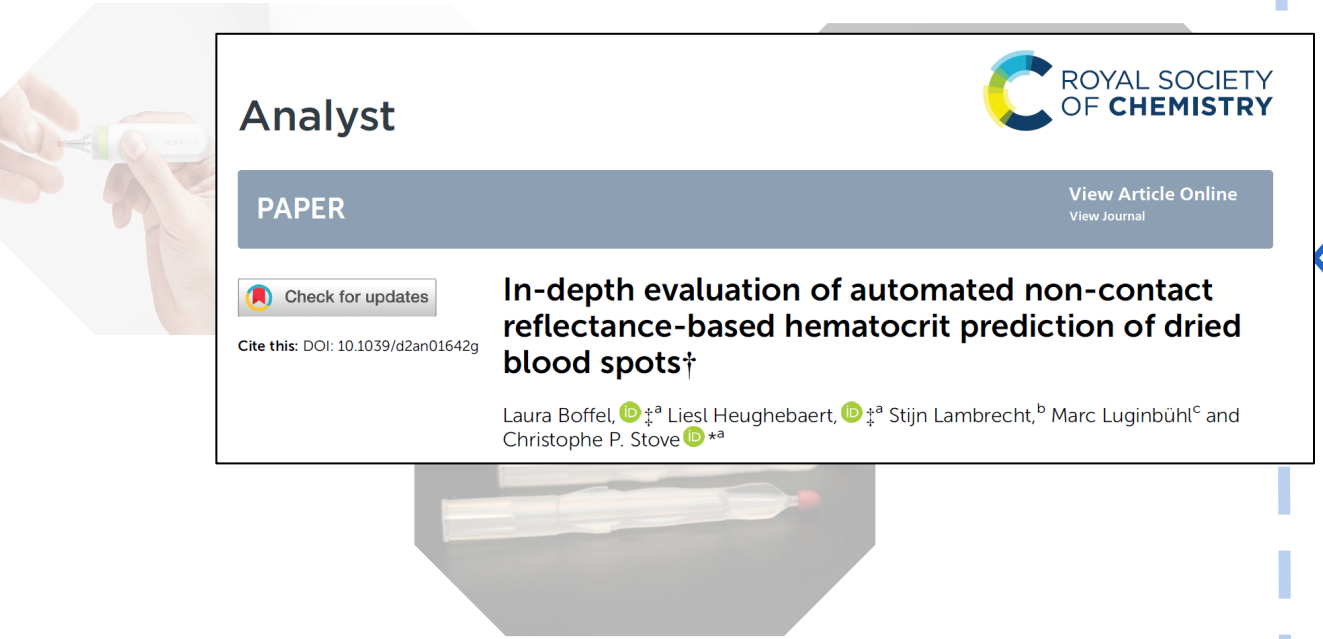

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Methodologies to predict the hematocrit

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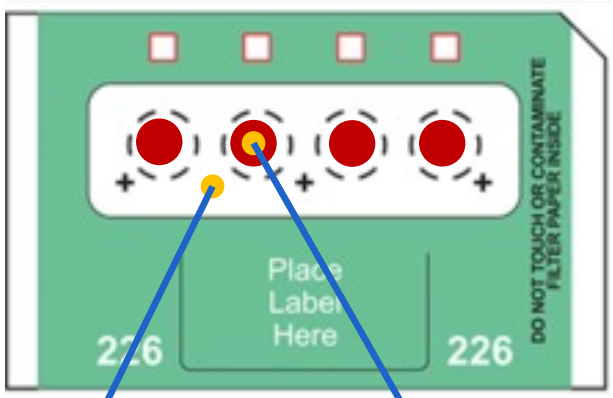
in-depth evaluation

analytical chemistry

Correction for the Hem
Using a Nondestructive
Hematocrit Prediction M

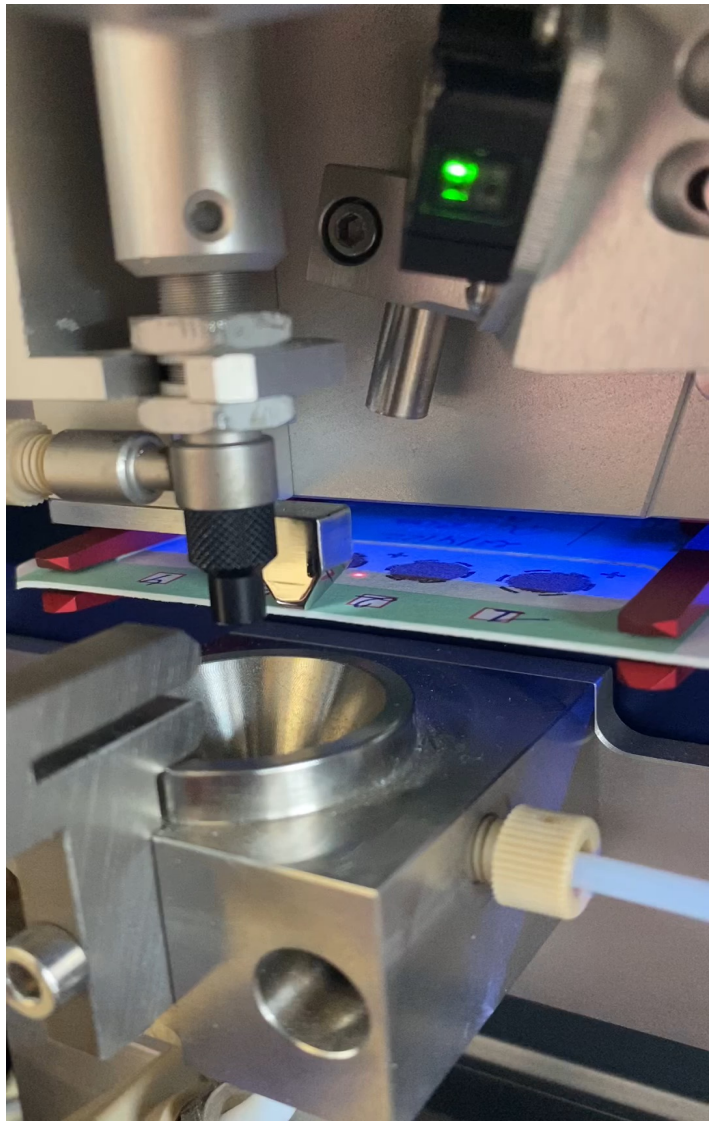
UV-Vis-based hematocrit prediction



Reflectance$_{589nm}$ background

Reflectance$_{589nm}$ DBS

Normalized reflectance $= \frac{BG}{DBS}$



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Study objectives

1. Evaluation of measurement conditions

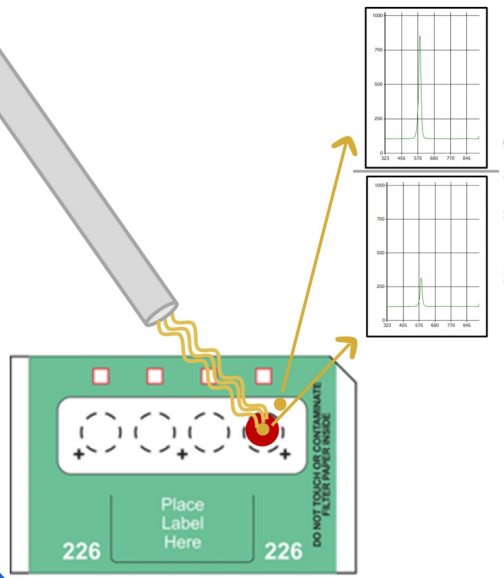
2. Set-up of calibration model

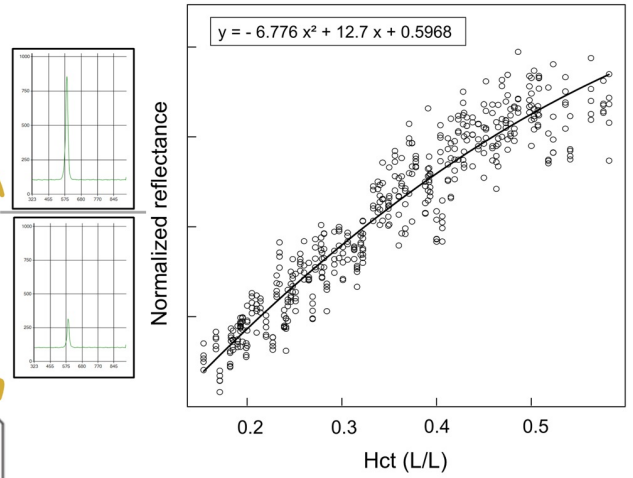
3. Method validation

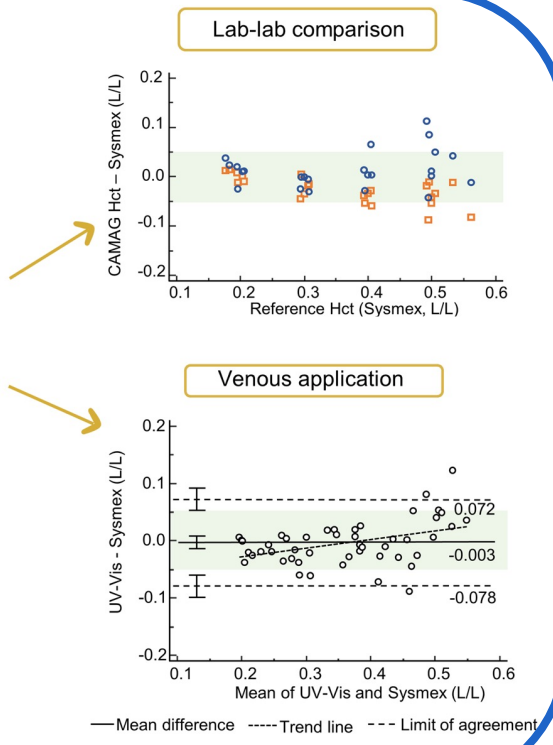
4. Robustness

5. Method comparison and lab-lab comparison

6. Venous application







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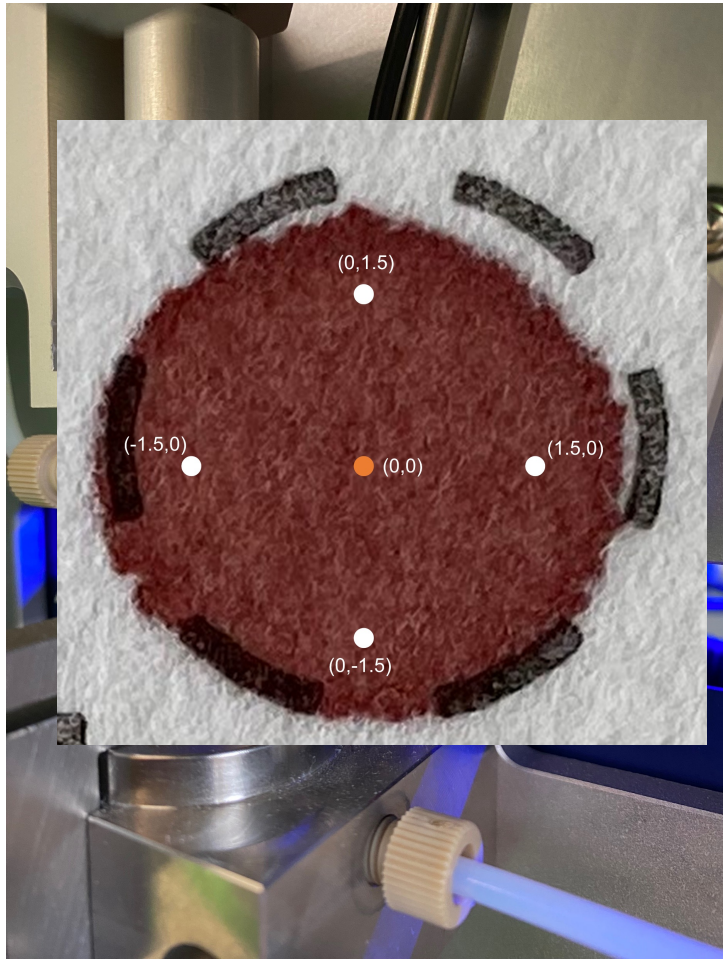
Results

Measurement conditions to optimize

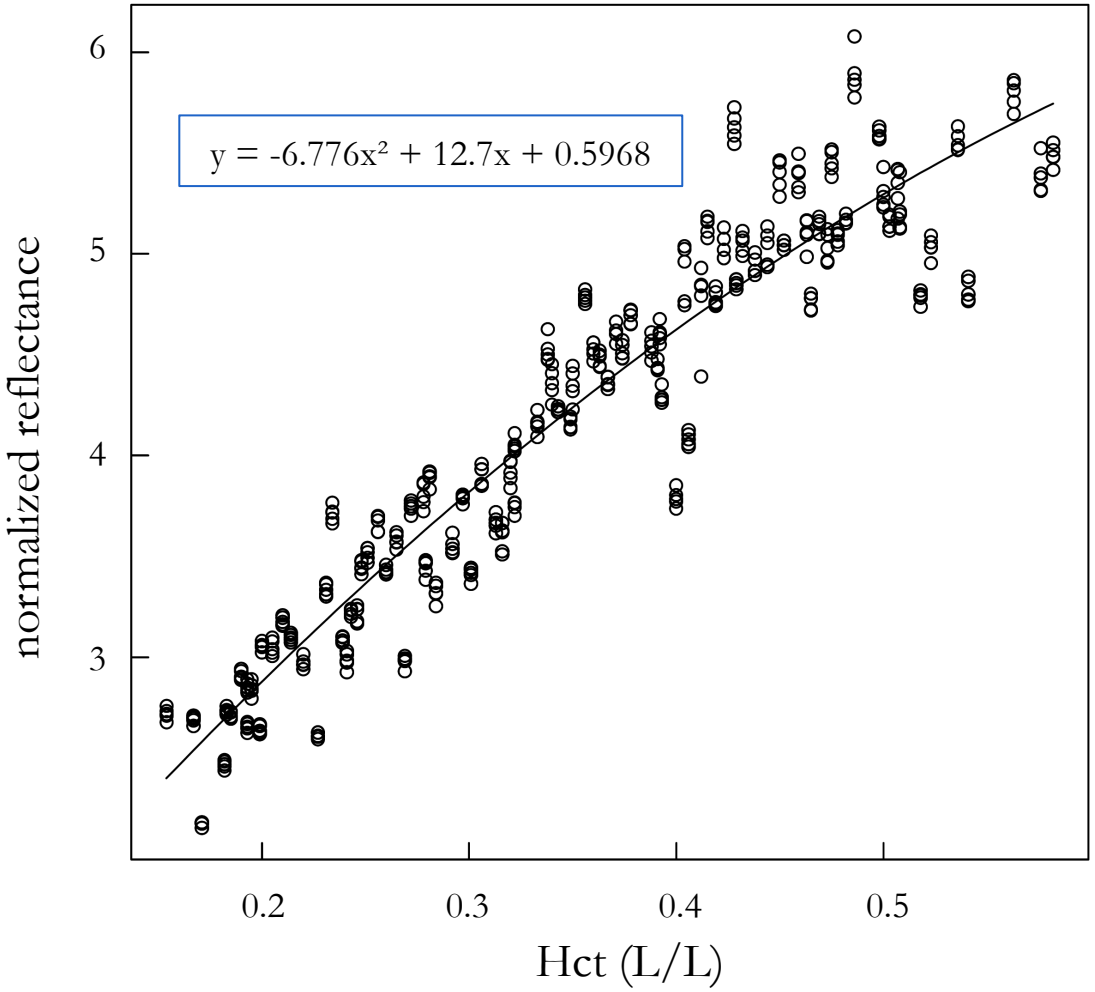
1. Probe-to-card distance 6.4 mm
2. Integration time 4500 µs
3. Use of multiple 'scans' per DBS 5 scans



Results

95 patient samples

0.154 – 0.582 L/L



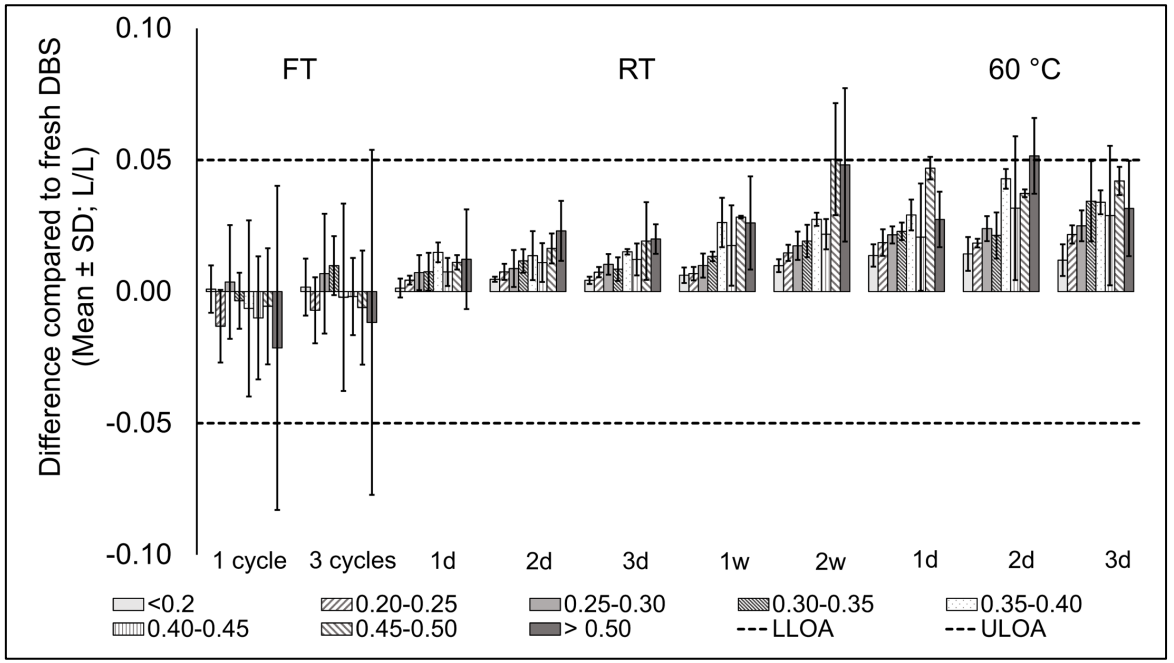
Results

Method validation

Hct range	Intra-day precision	Total precision	Bias	
	CV (%)	CV (%)	%	L/L
< 0.20	1.2%	1.7%	10.8%	0.019
0.20-0.25	1.6%	1.9%	7.9%	0.018
0.25-0.30	1.6%	1.9%	0.4%	0.000
0.30-0.35	1.5%	2.0%	0.1%	0.000
0.35-0.40	1.4%	1.7%	-0.9%	-0.003
0.40-0.45	1.4%	1.9%	0.1%	0.000
0.45-0.50	1.1%	2.0%	5.3%	0.025
> 0.50	2.2%	2.7%	-0.6%	-0.004
Total (42)	1.5%	2.0%	3.0%	0.007

- Accuracy: maximum bias of 0.025 L/L
- Precision: maximum total imprecision of 2.7%

Stability



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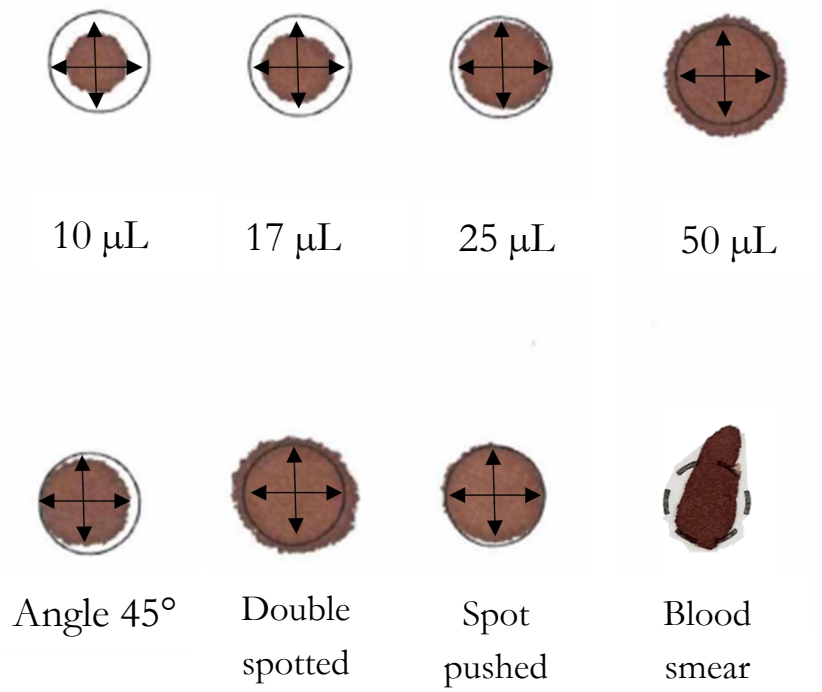
Results

'A good result can only be collected from a correctly collected sample.'

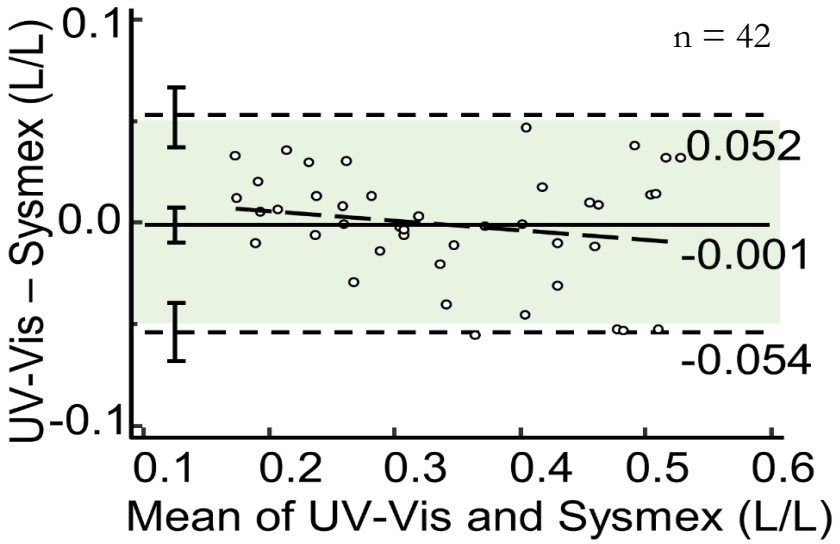


- Limited impact of the spotted volumes
- No impact of non-standard sampling strategies, except pushing the DBS
- No impact of filter paper type
- UV-Vis-based hematocrit prediction proved to be robust

Results

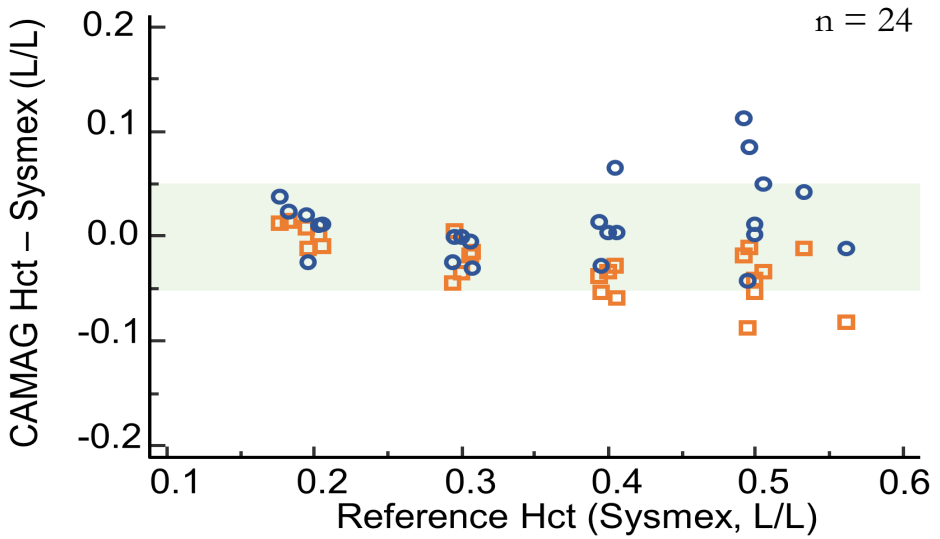
Method comparison

Conventional Hct measurement (via a hematology analyzer) *vs.* UV-Vis-based Hct prediction



Lab-lab comparison

Evaluation of the validated calibration model as 'generic' model across different CAMAG DBS-MS 500 Hct systems



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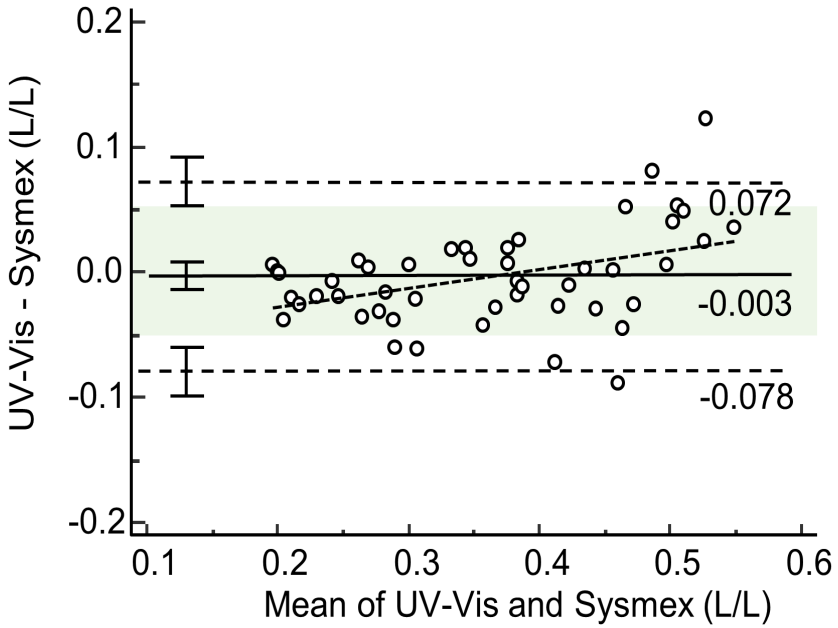
Results

Venous application

Application of the methodology on venous DBS collected in the framework of TDM of tacrolimus (n = 48)



Conclusion

- We successfully set up and validated a calibration model using authentic patient samples
- We demonstrated that the validated calibration model is 'generic', provided that the performance of the system and the model are tested before routine implementation
- The hematocrit can still reliably be predicted after 2 weeks of storage at room temperature
- UV-Vis-based hematocrit prediction proved to be robust against multiple DBS-related variables
- We demonstrated applicability on an independent venous DBS sample set collected in the context of TDM of tacrolimus

FUTURE

- Application of the method on capillary samples, covering a hematocrit range as large as possible
- Implementation of the validated calibration model into system software to increase the method's ease-of-use

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