FACULTY OF
PHARMACEUTICAL SCIENCES

DEPARTMENT OF BIOANALYSIS
LABORATORY OF TOXICOLOGY

HEMATOCRIT PREDICTION OF DRIED BLOOD SPOTS: CURRENT STATUS AND FUTURE OUTLOOK

Liesl Heughebaert, Lisa Delahaye, Christoph Lühr, Stijn Lambrecht and Christophe Stove

14th EBF symposium – 25/11/2021

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PRESENTATION OUTLINE

- Current status:
  - Dried blood spots and the hematocrit effect
  - Strategies to cope with the hematocrit effect

- Near-infrared-based hematocrit prediction of DBS: an in-depth evaluation
  - Extensive evaluation of a commercially available NIR set-up:
    - Performance of the calibration model
    - Method validation and stability
    - Robustness
    - Method comparison and application

- Future outlook: where are we heading?

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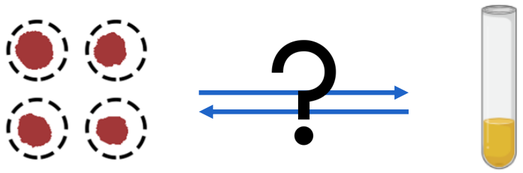
CURRENT STATUS: DRIED BLOOD SPOTS AND THE HEMATOCRIT EFFECT

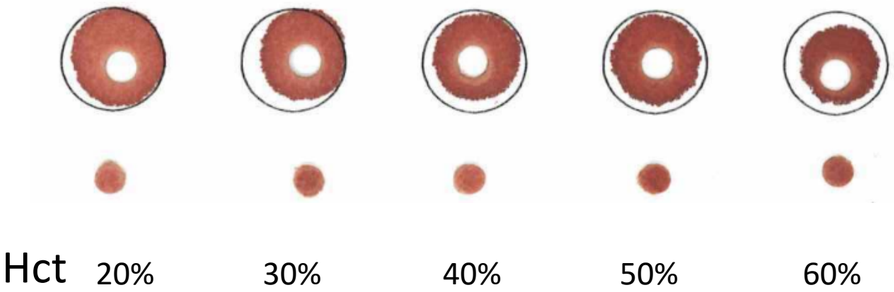
THE HEMATOCRIT EFFECT

Physiological aspect



Analytical aspect

- Area bias
- Recovery bias
- Matrix bias



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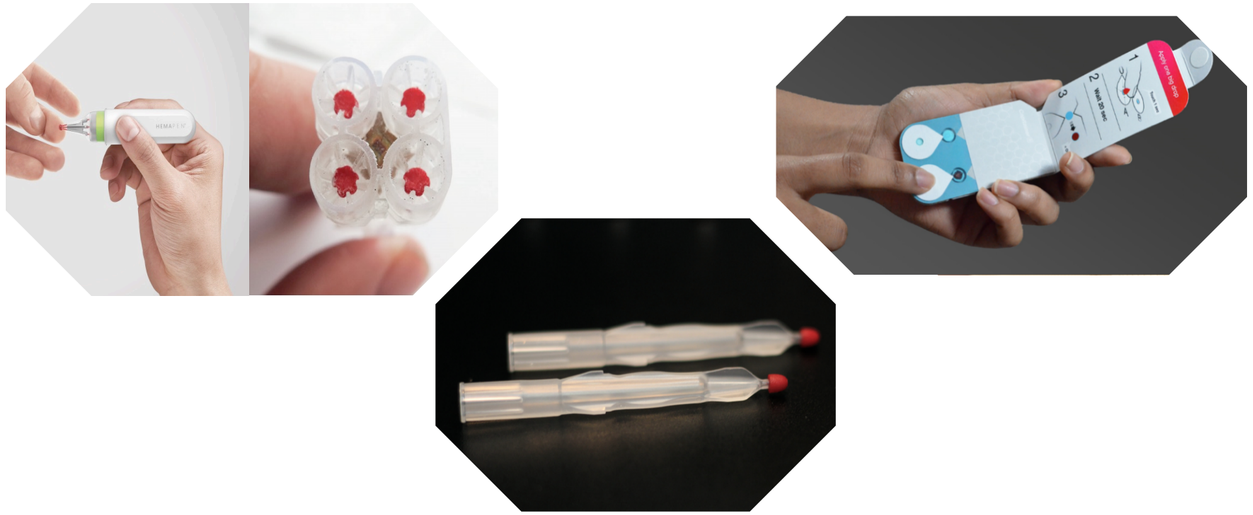
STRATEGIES TO COPE WITH THE HEMATOCRIT EFFECT

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**

+

- No more Hct-based area bias
- Patient centricity

−

- Hct-based effect on recovery/matrix
- Automated analysis is still in development

Methodologies to predict the hematocrit

analytical chemistry — Article — pubs.acs.org/ac

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

Sara Capiau,† Veronique V. Stov
†Laboratory of Toxicology, Department
‡Department of Laboratory Medicine, Gl

analytical chemistry — Article — Cite This: *Anal. Chem.* 2018, 90, 1795−1804 — pubs.acs.org/ac

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

ders,‡ and Christophe P. Stove*,†
s, Ghent University, Ottergemse-steenweg
ity of Amsterdam, Meibergdreef 9,

FOCUS SERIES: ALTERNATIVE SAMPLING STRATEGIES

Development and Validation of Hematocrit Level Measurement in Dried Blood Spots Using Near-Infrared Spectroscopy

Daan van de Velde, BSc, Jordy L. van der Graaf, BSc,* Mariam Boussaidi, BSc,* Ruud Huisman,* Dennis A. Hesselink, PhD,† Henk Russcher, PhD,‡ Annelies C. Kooij-Egas, BSc,§ Erik van Maarseveen, PhD,§ and Brenda C.M. de Winter, PhD**

+

- Automated analysis of DBS is available
- Non-destructive

−

- Destructive
- In-house generated configurations

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PRESENTATION OUTLINE

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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

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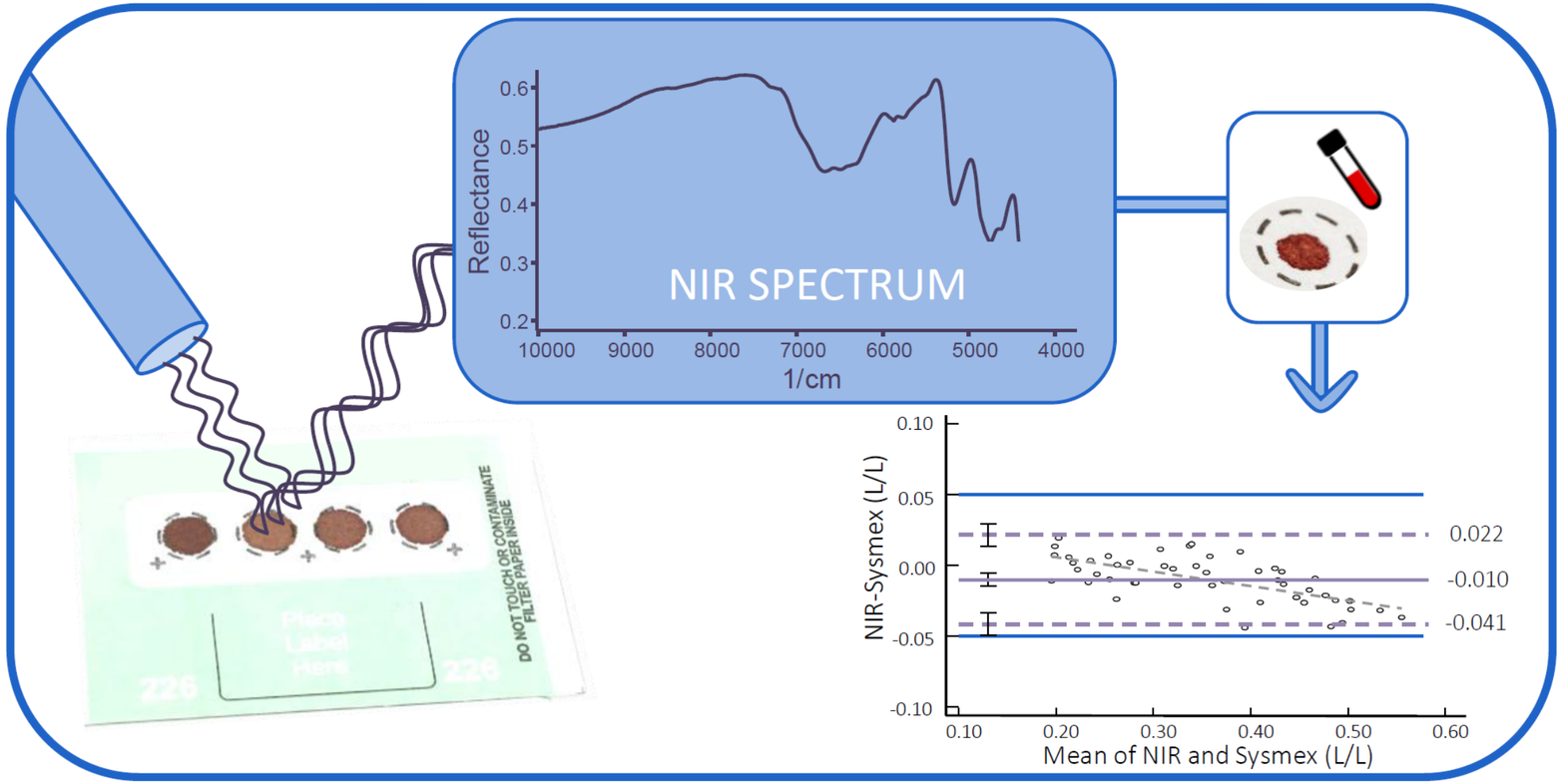
NIR-BASED PREDICTION OF THE HEMATOCRIT: OBJECTIVES

1. Performance of the initial calibration model

2. Method validation

3. Robustness

4. Method comparison and application



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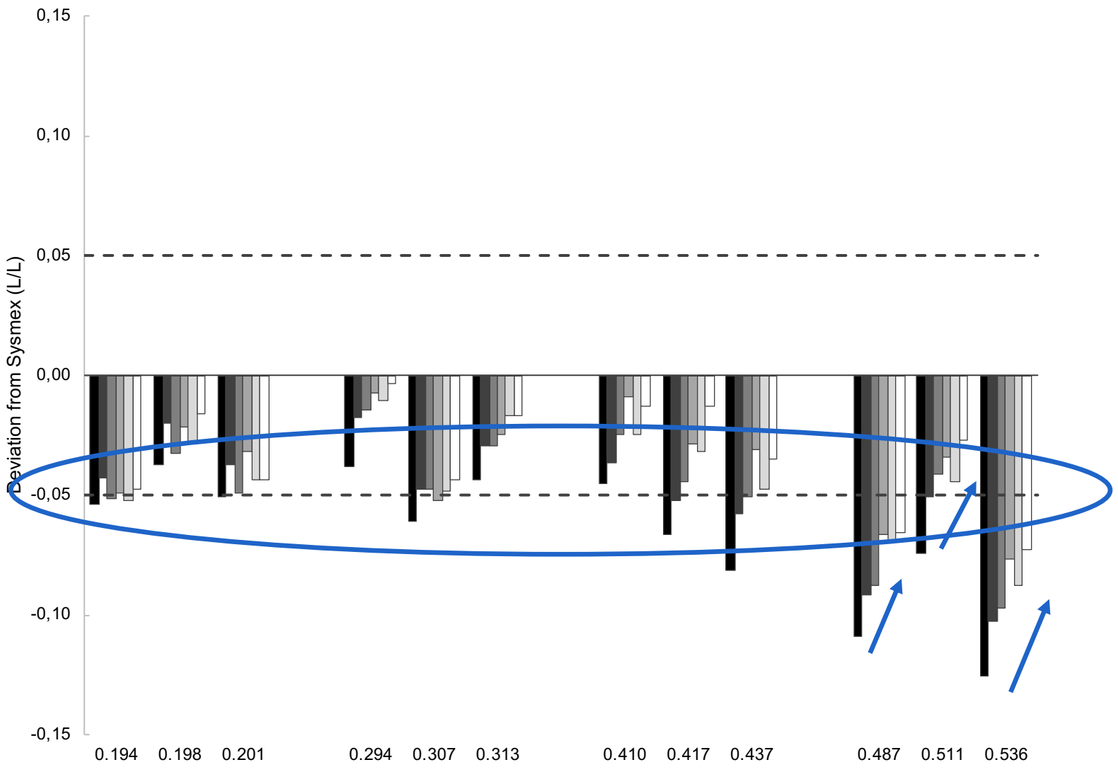
NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT



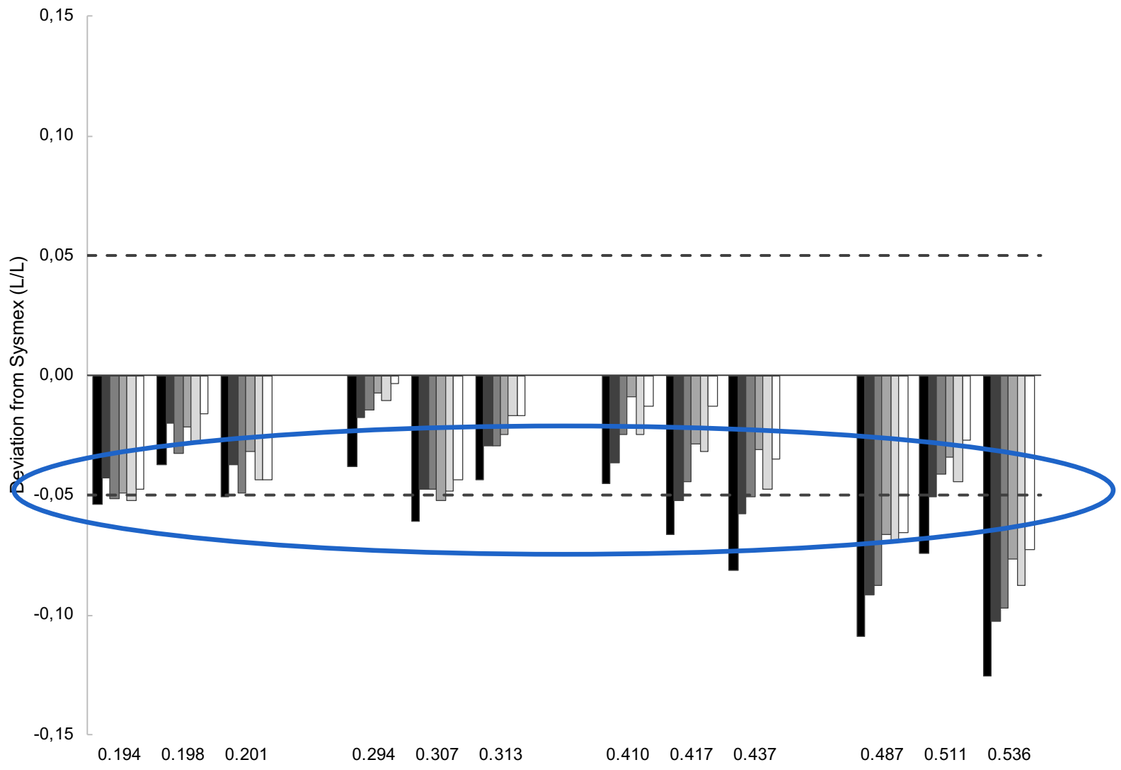
Original calibration model

Upon evaluation of an initial calibration model, two issues were seen:

1. An unnacceptable negative bias

2. A time-dependent bias

NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT



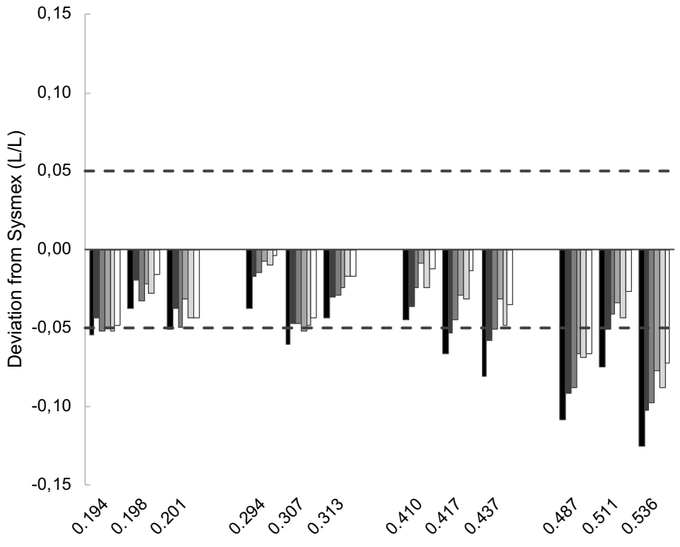
Original calibration model

Upon evaluation of an initial calibration model, two issues were seen:

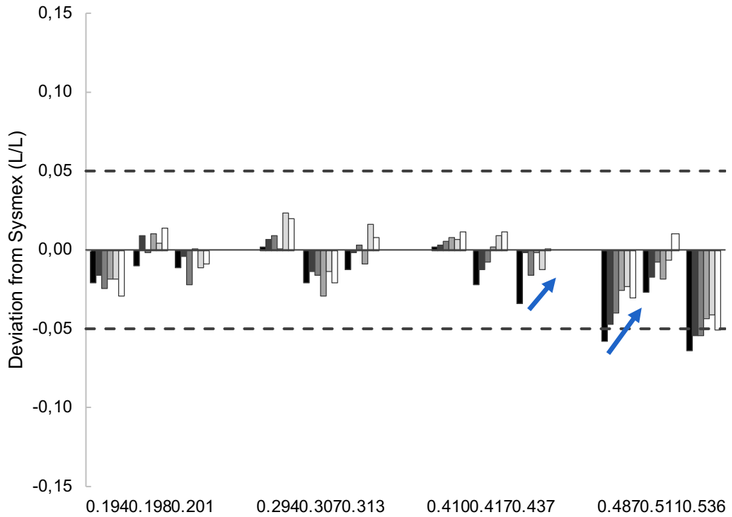
1. An unnacceptable negative bias: tackled by adding the spectra of 150 (duplicate) DBS

2. A time-dependent bias

NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT



Original calibration model



Update with data from 150 patient samples

Upon evaluation of an initial calibration model, two issues were seen:

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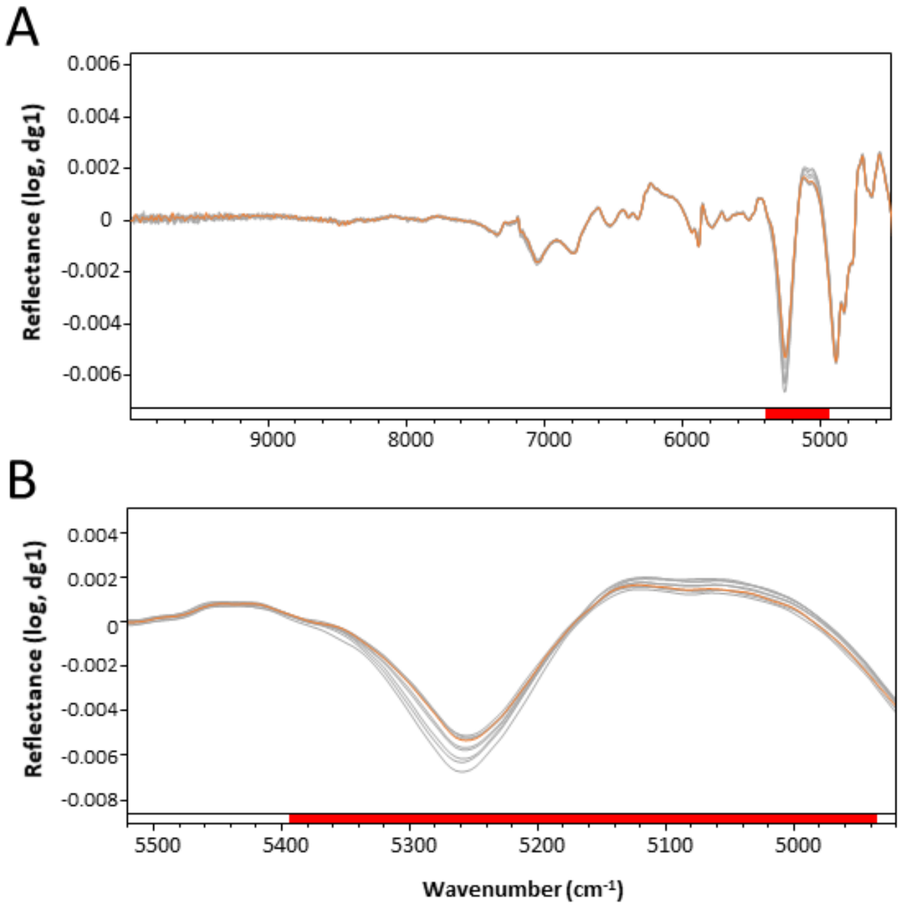
2. A time-dependent bias

NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT



Upon evaluation of an initial calibration model, two issues were seen:

1. An unnacceptable negative bias: tackled by adding the spectra of 150 (duplicate) DBS

2. A time-dependent bias: tackled by excluding the wavenumber range 5380-4968 cm^{-1}

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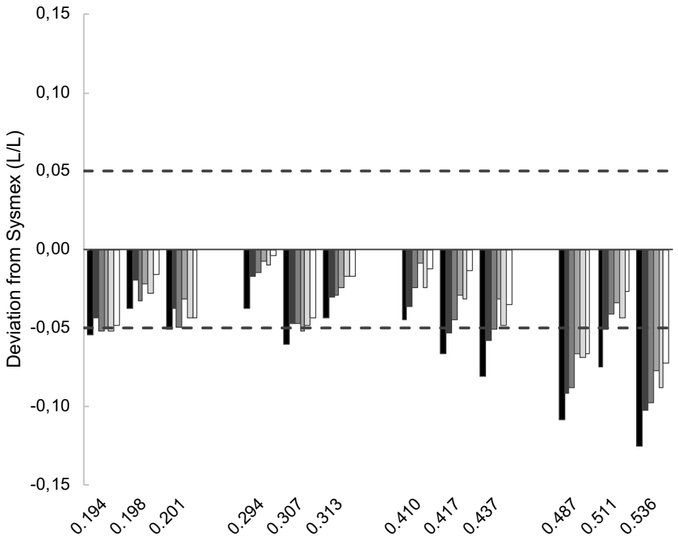
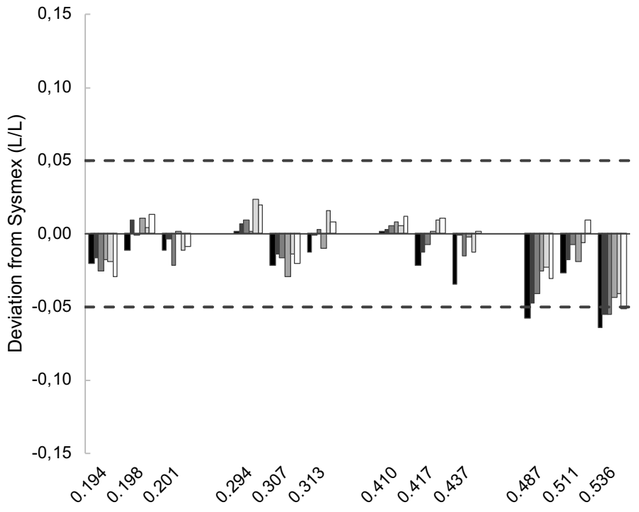
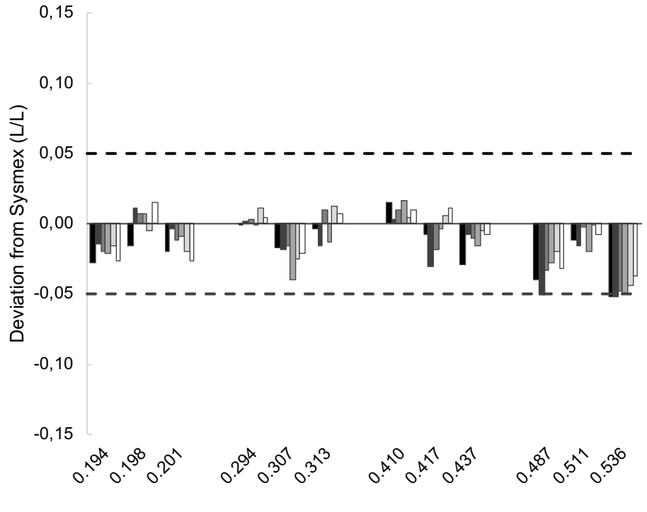
NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT



Original calibration model



Update with data from 150 patient samples



Exclusion of wavenumber range 5380-4968 cm^{-1}

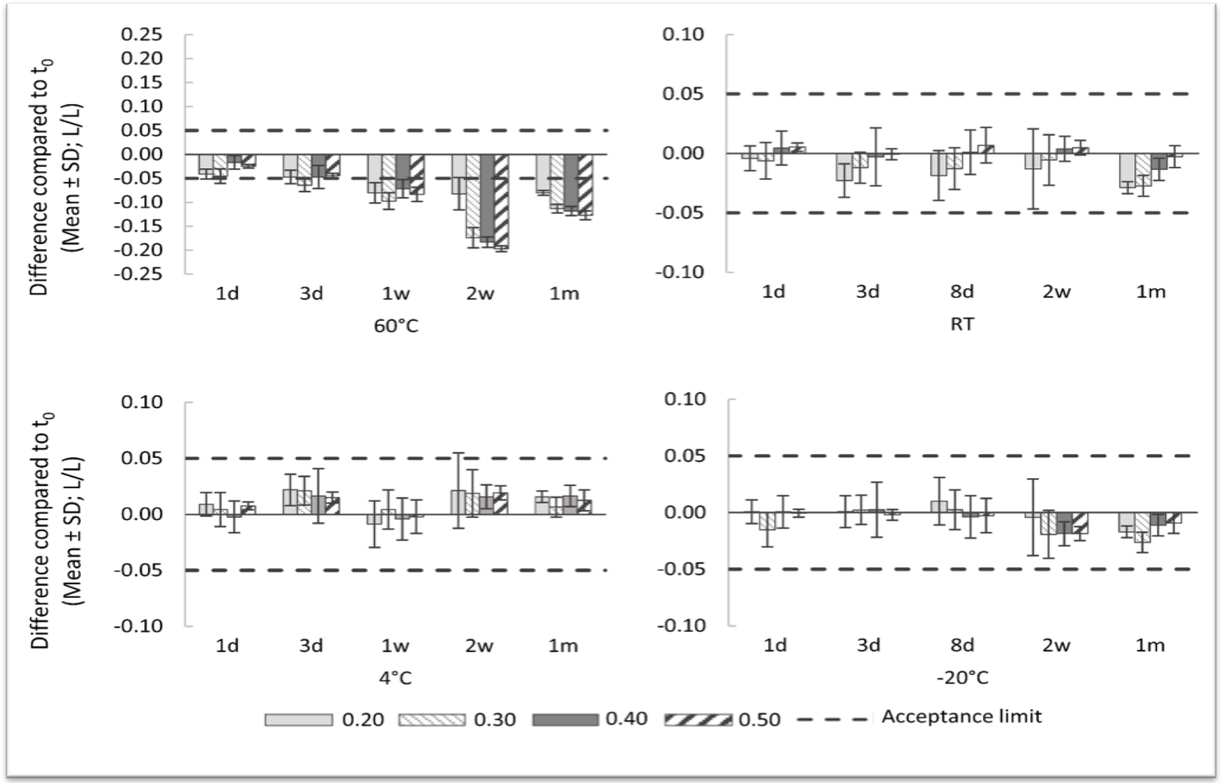
NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT

Method validation

- Accuracy: maximum bias of 0.012 L/L
- Precision: maximum total imprecision of 4.5 %

Hct range	Number of samples	Intra-day precision (CV, %)	Total precision (CV, %)	Bias %	Bias L/L
<0.20	3	4.5	4.5	4.2	0.008
0.20-0.25	7	4.4	4.4	5.4	0.012
0.25-0.30	7	3.5	3.5	1.4	0.004
0.30-0.35	7	3.5	3.5	1.8	0.006
0.35-0.40	7	4.2	4.2	0.8	0.003
0.40-0.45	7	1.9	2.6	0.7	0.003
0.45-0.50	7	2.8	2.8	-2.8	-0.013
>0.50	4	3.0	3.0	-0.8	-0.005

Stability



NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT

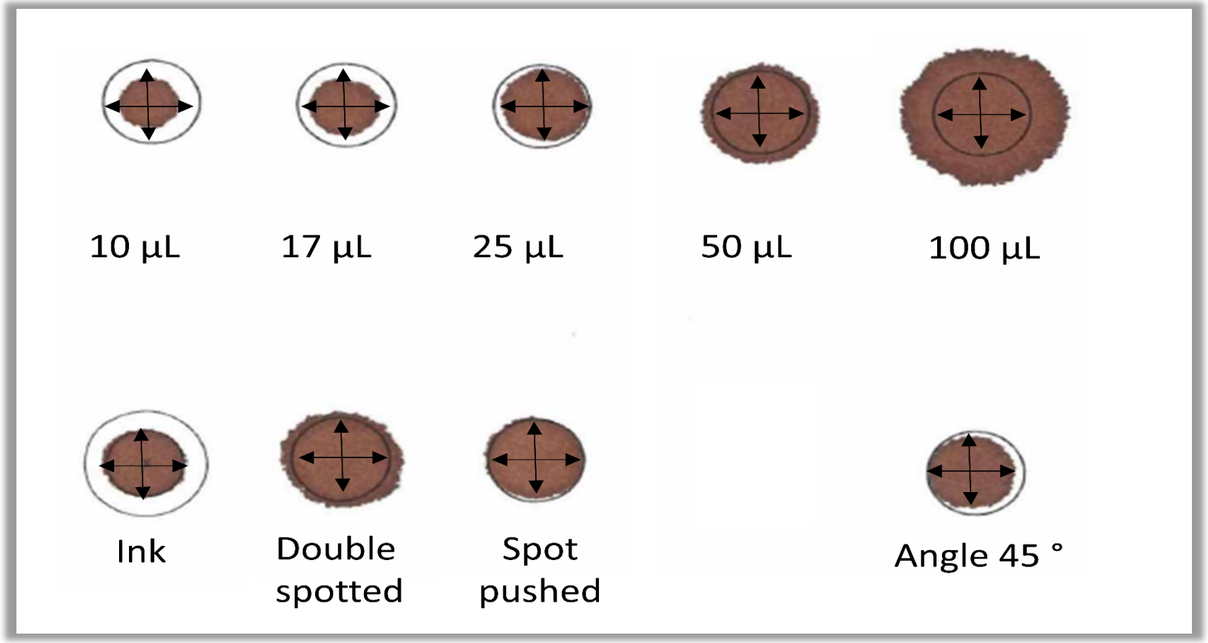
‘A good result can only be collected from a correctly collected sample.’

No inter-operator variability.

No relevant effect of measurement location or type of filter paper.

No relevant effect of the volume spotted except for 10 µL spots.

NIR-based Hct prediction proved to be very robust.

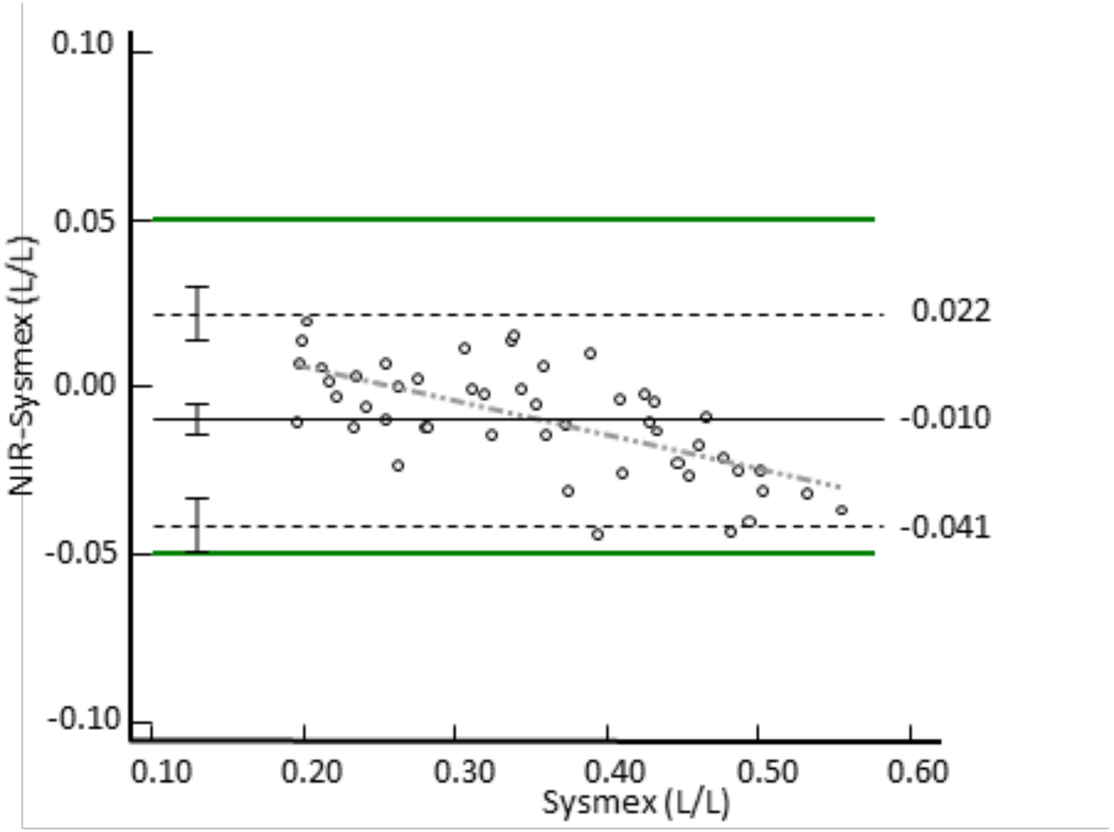


NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT

Method comparison

- Conventional Hct measurement (via a hematology analyzer) vs. NIR-based Hct prediction

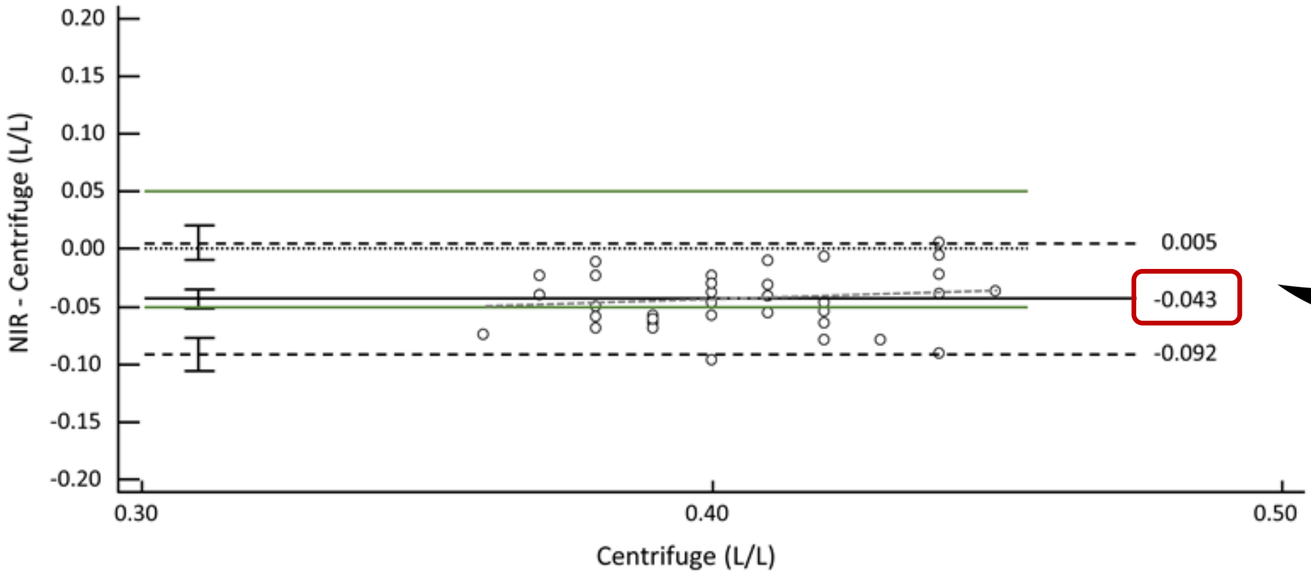
→ Difference between NIR and reference method within ±0.05 L/L



NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT

Method application

- Application of the method on capillary DBS (n=36)
  - NIR-based Hct vs Hct measured with a Hct centrifuge



Underestimation?

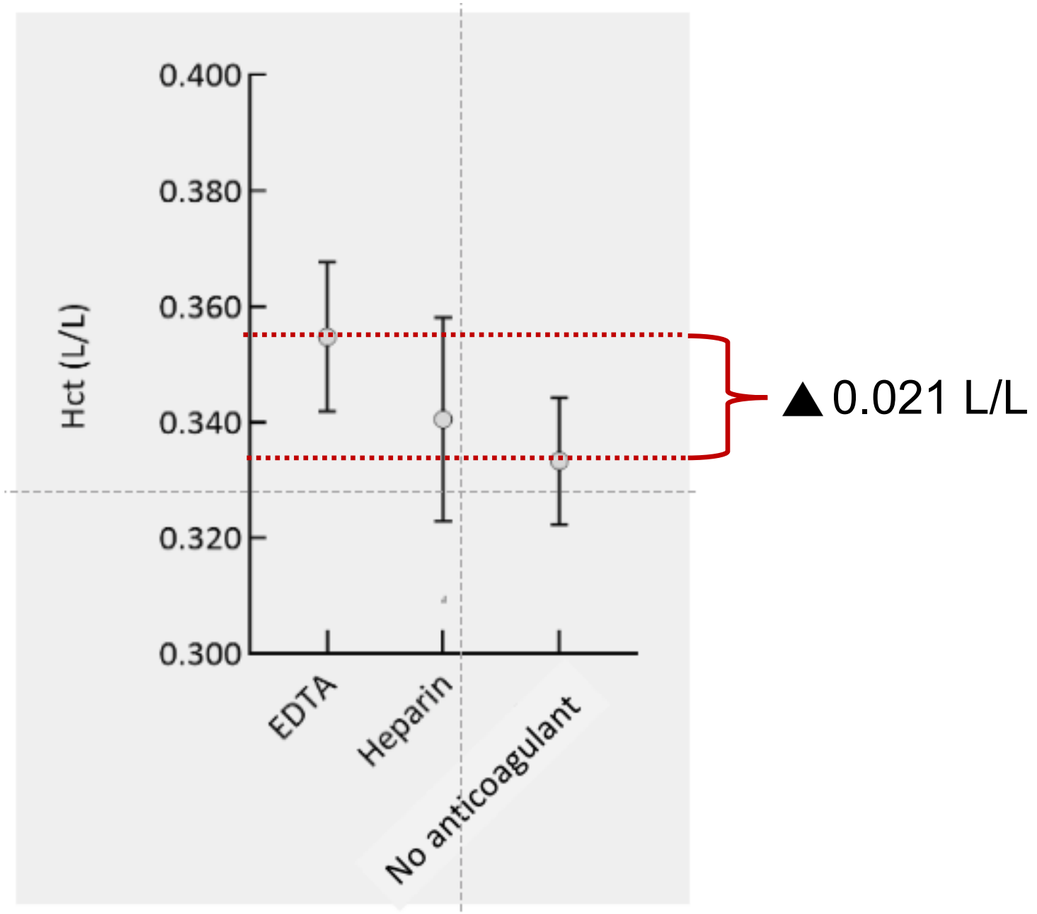
NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT

Method application

- Application of the method on capillary DBS (n=36)
  - NIR-based Hct vs Hct measured with a Hct centrifuge: **underestimation of the Hct of -0.043 L/L**

- What is the difference between capillary and venous patient samples?

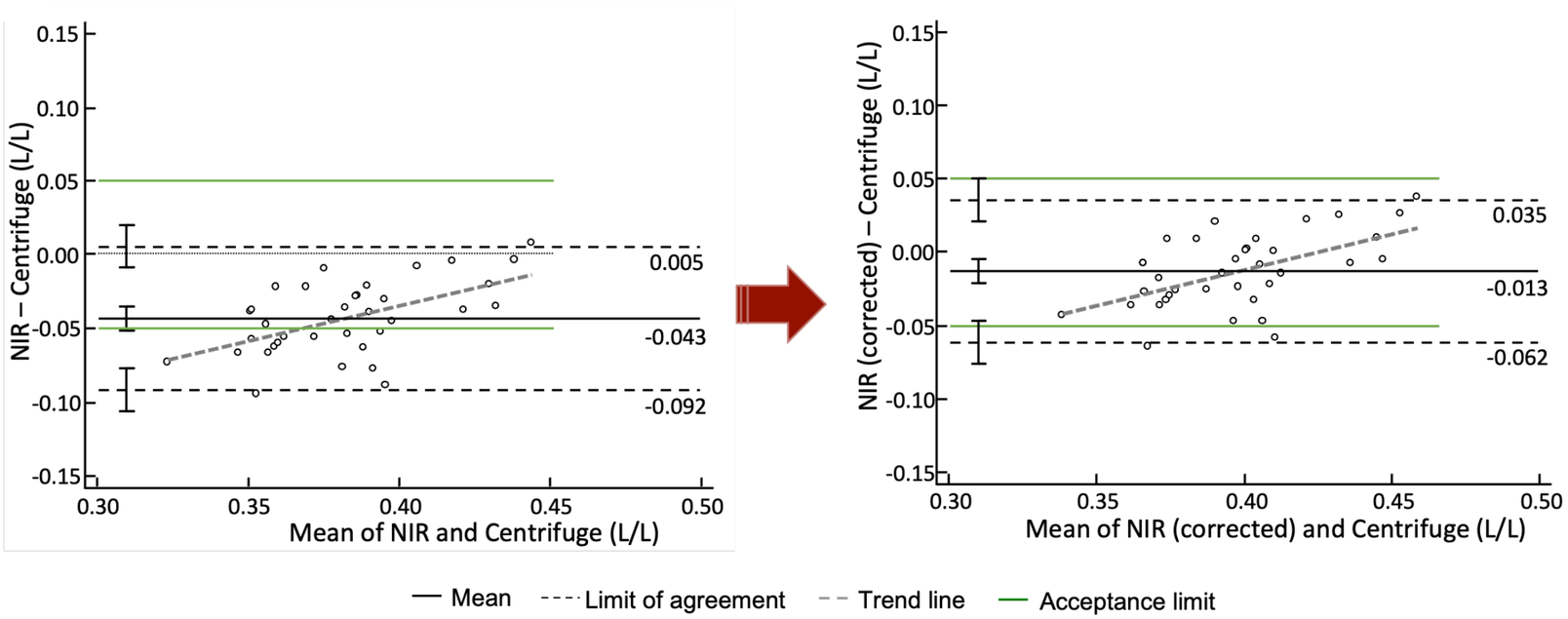
The presence of an anticoagulant



NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT

Method application

- Application of the method on capillary DBS (n=36)
  - NIR-based Hct vs Hct measured with a Hct centrifuge **after correction**



NEAR-INFRARED-BASED PREDICTION OF THE HEMATOCRIT: CONCLUSION

CONCLUSION

NIR – based Hct prediction is:

- Accurate and precise
- Hct can still be predicted after 1 month of storage of the DBS at RT or lower
- Robust

- Applicable on capillary samples, however the predicted Hct is currently underestimated (-0.043 L/L)
  - This may be corrected for by an arbitrary correction factor - future research needed.

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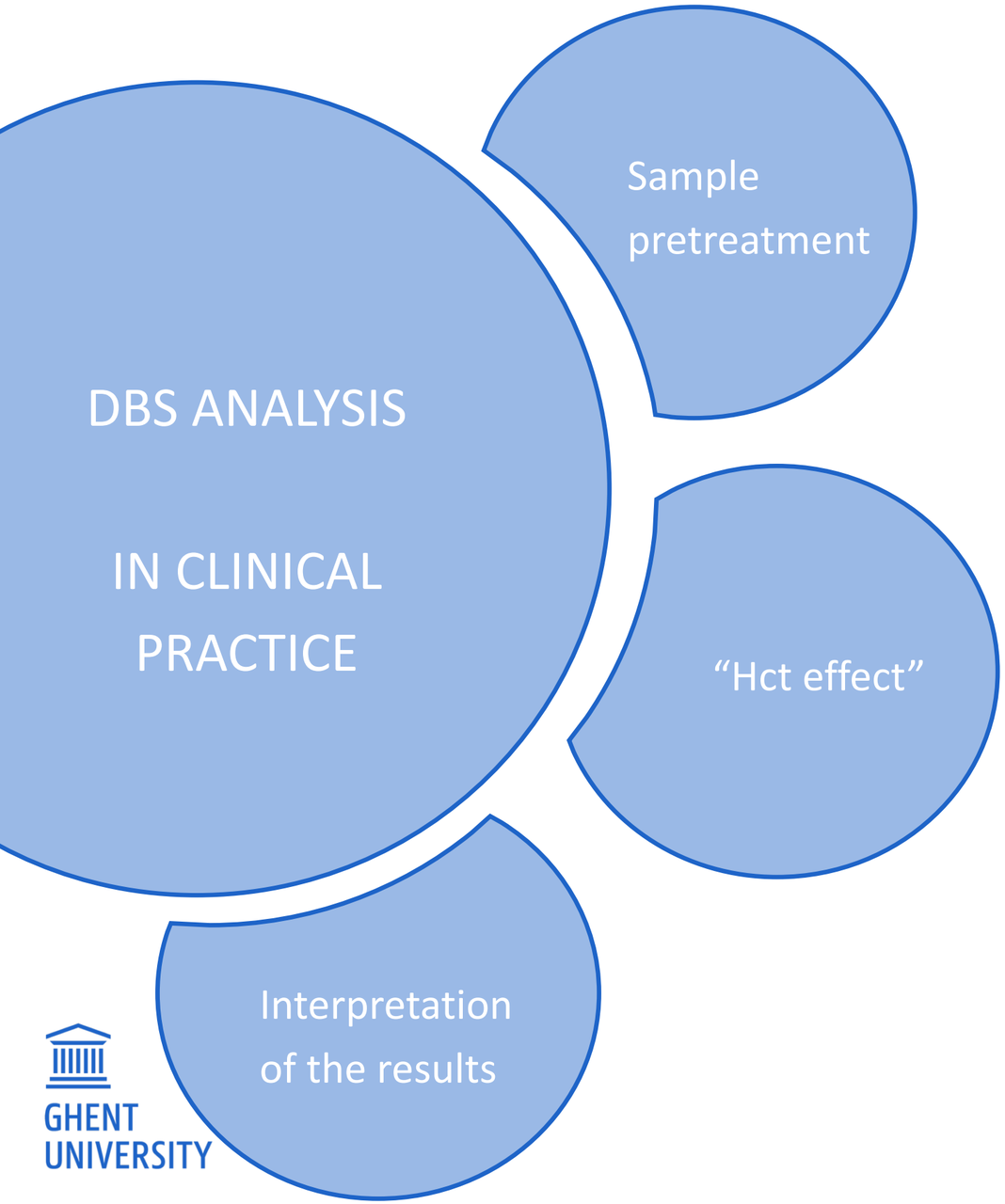
FUTURE OUTLOOK: WHERE ARE WE HEADING?



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FUTURE OUTLOOK: WHERE ARE WE HEADING?

DBS ANALYSIS

IN CLINICAL PRACTICE

Sample pretreatment

"Hct effect"

Interpretation of the results

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Labor intensive

Risk of human error

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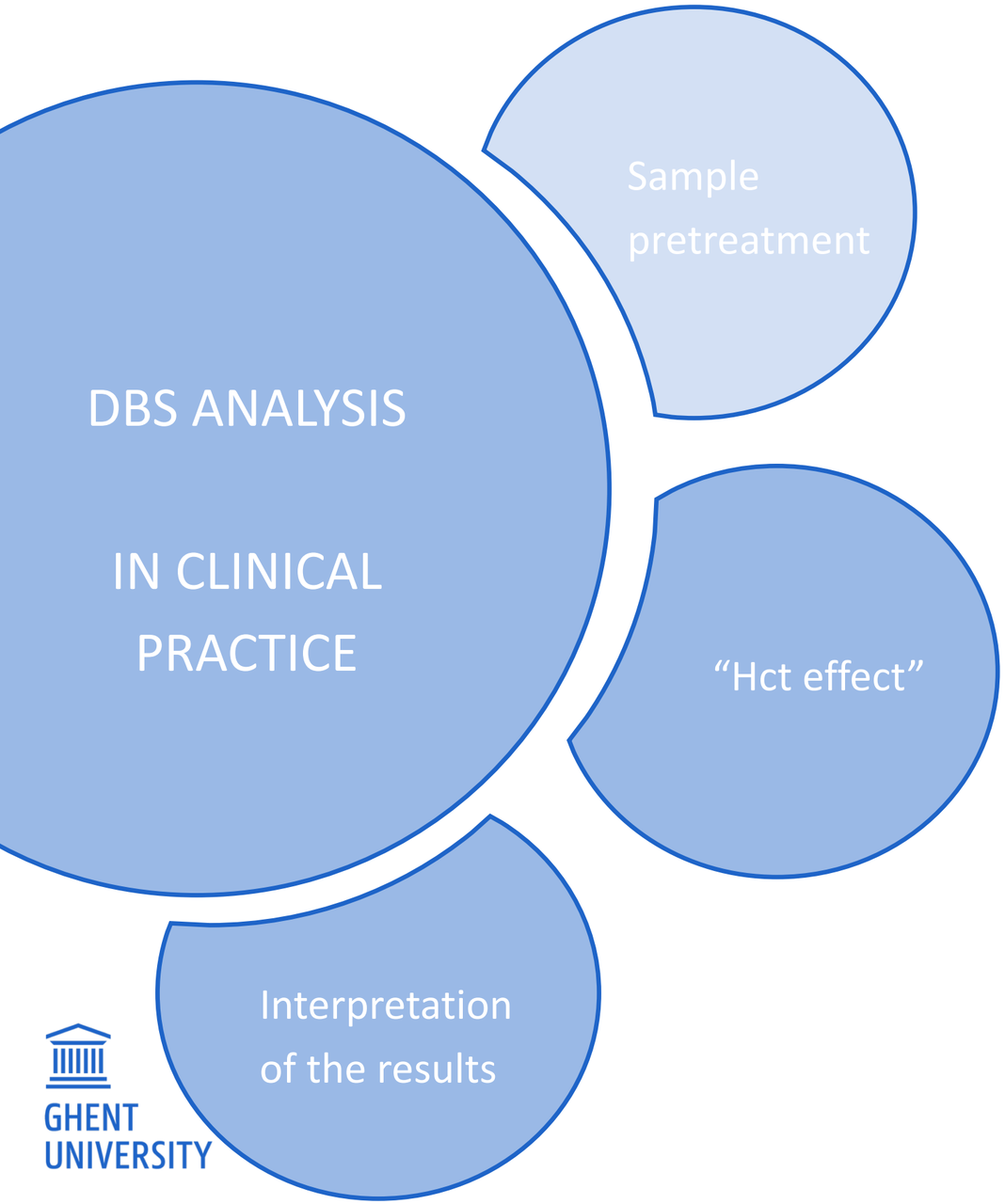
Availability of automated extraction units

DBS-MS 500, CAMAG

DBS Autosampler, Spark Holland

FUTURE OUTLOOK: WHERE ARE WE HEADING?



The predicted Hct (either determined via destructive or non-destructive methods) allows:

- To compensate for the Hct effect via dedicated algorithms
- To verify whether the Hct of a DBS sample is within a validated range
- To calculate plasma or serum concentrations based on DBS results

All tools are available for a successful implementation of DBS-based methods in clinical practice.

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