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

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ABSTRACT

Background: Dried blood spot (DBS) microsampling has gained interest in different clinical fields, owing to its many advantages compared to conventional blood sampling. However, whilst being applied for decades for screening purposes, some challenges, such as the hematocrit (Hct) effect, hinder further widespread use of DBS for quantitative purposes in clinical practice. Amongst the approaches that were developed to cope with this issue, is the Hct prediction of DBS using near-infrared (NIR) spectroscopy.
Methods: Using left-over EDTA-anticoagulated patient samples, the accuracy and precision, stability, and robustness were assessed. Furthermore, applicability of the method on capillary DBS was evaluated via finger prick samples.
Results: A maximal bias, respectively CV, of 0.012 L/L and 4.5% were obtained. The method was robust towards several aspects, including storage (except for storage at 60°C), measurement location, type of filter paper and spotted volume. Furthermore, the potential to predict the Hct of capillary DBS was demonstrated.
Conclusion: A commercially available NIR set-up was extensively and successfully validated, allowing non-contact Hct prediction of DBS with excellent accuracy and precision. This allows to correct for the Hct-based bias observed in partial-punch DBS analysis and the set-up of blood-plasma conversion factors, increasing the application potential of patient-centric sampling.

1. Introduction

In different clinical fields, and in particular in therapeutic drug monitoring (TDM), the interest in dried blood sampling keeps growing. Capillary dried blood sampling, where samples are obtained from a finger or heel prick, has many advantages over traditional blood sampling. The best-known dried blood sampling technique is the generation of dried blood spots (DBS) on filter paper. Since it was first introduced by Guthrie and Susi for newborn screening [1], DBS have been used for a wealth of applications, both in pediatrics and in many other fields, including toxicology [2].

Evidently, DBS sampling has some specific advantages and challenges, with as key advantage primarily the patient centricity, with a minimally invasive procedure (finger prick) allowing the collection of a minimal volume of a representative matrix (blood), in a non-supervised setting (e.g. at home). This makes the approach particularly attractive for children, where both the sampling procedure (causing anxiety) and the sampling volume may be limiting factors. Amongst the main challenges associated with DBS sampling for quantitative purposes is the so-called hematocrit (Hct) effect [3], which has an analytical and a physiological aspect [4]. For the former, the most important consequence is the differential area over which a drop of blood of the same volume spreads depending on the Hct, thereby possibly affecting analytical results when sub-punches of the DBS are

Abbreviations: CV, Coefficient of variation; DBS, Dried blood spot(s); FT, Freeze-thaw; Hct, Hematocrit; HIL-index, Hemolytic/icteric/lipemic index; ISR, Incurred sample reanalysis; LoA, Limit(s) of agreement; NIR, Near-infrared spectroscopy; PC, Principal component; PLS, Partial least squares; RT, Room temperature; TDM, Therapeutic drug monitoring; UV/Vis, Ultraviolet–visible.

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used. The physiological aspect on the other hand, includes the conversion of measurement results in blood to a concentration in plasma or serum, which are often the standard matrices in which reference intervals are determined [3]. Whilst in a general healthy population the overall impact of the Hct will remain limited, much larger variations may be seen in patients, with the largest variations seen in children [3,5,6].

In recognition of this problem, many alternative sampling devices have been proposed as an alternative to conventional DBS sampling, aiming at overcoming one or more facets of the Hct effect [7]. Nonetheless, conventional DBS cards remain valuable to collect dried blood microsamples, not in the least because DBS sampling is well known in the newborn and pediatrics field, the analysis can easily be automated and the cost is low. Therefore, in conjunction with the development of alternative sampling devices, also methodologies to predict the Hct from non-volumetrically collected DBS have been set up. This predicted Hct allows (i) to compensate for the Hct effect via dedicated algorithms, (ii) to verify whether the Hct of a DBS sample is within a validated range, or (iii) to calculate plasma or serum concentrations based on DBS results [7–15].

Two non-contact approaches, allowing to predict the Hct of DBS via mere scanning of the DBS card, have been proposed: ultraviolet–visible spectroscopy (UV/Vis) and near-infrared spectroscopy (NIR) [8,10,13,14]. While proof-of-concepts of NIR-based Hct prediction have already been reported, these used in-house generated configurations, compromising widespread use and implementation. Here, we used an existing commercially available NIR set-up to perform an extensive validation of the accuracy and precision, as well as an in-depth evaluation of the robustness, of NIR-based Hct prediction. We also evaluated its applicability to assess the Hct of capillary DBS obtained from healthy volunteers.

2. Materials and methods

2.1. Instrumentation

NIR measurements were performed on a NIRFlex N-500 Fourier Transformation spectrometer equipped with a fiber optics solids cell N500-007 (Büchi Labortechnik, Flawil, Switzerland). The instrument was controlled by the NIRWare 1.6 Operator software (Büchi Labortechnik), in which the calibration model was incorporated to predict the Hct values, expressed as L/L. Spectra were recorded between 10,000 and 4500 cm^{-1} at a resolution of 4 cm^{-1} and scanning each sample 12 times.

The Hct (L/L) determined with a Sysmex XN-5000 hematology analyzer (Sysmex, Kobe, Japan) was used as the reference value. The Hct of liquid capillary blood samples was determined via centrifugation in a Hct-centrifuge (5 min, 12,000 rpm), and then measured using a microhematocrit reader (Hawksley, Lancing, UK). Hemolytic, icteric and lipemic (HIL) indices and protein concentrations were measured with an Abbot Architect c16000 (Abbott, Illinois, US). HbA1c concentrations were measured with a Tosoh G8 HPLC analyzer (Tosoh, Pennsylvania, US).

2.2. Sample collection and preparation

This study was approved by the ethics committee from Ghent University Hospital (EC2018/0519). Left-over venous EDTA-anticoagulated patient samples from Ghent University Hospital were used to generate DBS by pipetting 25 μL of whole blood onto Whatman 903 filter paper (GE Healthcare, Dassel, Germany), unless otherwise specified. After drying at room temperature (RT) for 2 h, the DBS were further dried in resealable bags with desiccant for a minimum of 24 h before analysis. Patient samples were used for the evaluation and update of the calibration model, validation of the method, evaluation of the robustness, and method comparison.

For the application of the method on capillary DBS, blood was collected from 12 healthy volunteers via finger prick. Each volunteer provided 3 capillary DBS and 3 corresponding liquid capillary samples, the latter being collected via heparinized microcapillaries.

2.3. Calibration model

Initially, an existing calibration model for Hct prediction was used, based on a partial least squares (PLS) algorithm set-up with the NIRCal 5.6 software [13], with a calibration range from 0.150 to 0.600 L/L. A more detailed description of the PLS algorithm used can be found in the Supplementary Material Section 1.1. Prior to validation of the method, this 'historic' calibration model was evaluated and updated, using left-over patient samples. A detailed description of these evaluations can be found in the Supplementary Material Section 1.2.

2.4. Validation

The method validation was based on internationally accepted guidelines for bioanalytical method validation, as well as on the IATDMCT guideline for DBS-based method validation [16–18]. The method validation was performed using 49 left-over patient samples and encompassed accuracy, precision, sample storage and incurred sample reanalysis. The set-up of the method validation experiments is described in detail in the Supplementary Material Section 1.3.

2.5. Robustness

After a short (approximately 2′) introduction on the use of the NIR instrument, two additional operators, unacquainted with the system, independently measured the DBS from the validation set (n = 49; singlicate analysis). The results were compared to the Hct predictions of the regular operator on the same day.

To evaluate other robustness aspects of the methodology, 12 DBS were generated from again an independent set of left-over patient samples (Hct range 0.199–0.513 L/L). DBS with different volumes (10, 17, 25, 50, and 100 μL) were generated from these samples, to evaluate the influence of the spotted volume on the Hct prediction, taking the 25 μL DBS as the reference. The 100 μL DBS were also used to evaluate the effect of peripheral vs. central measurement of the DBS.

Next, the impact of some commonly observed 'issues' in the generation of DBS were evaluated: (i) pressing the DBS between the fingertips right after application of the blood, to further spread the blood ('spot pushed'); (ii) double application of 25 μL on the same area, with a 10 s interval ('double spotted'), (iii) holding the filter paper at an angle of approximately 45° when generating the DBS ('angle 45°'). In addition, also DBS generated onto Ahlström 226 filter paper (not shown) and on top of a printed "X" at the center of the DBS were evaluated ('ink'). The results obtained from all these different conditions were compared against those obtained from standard 25 μL DBS. Fig. 1 shows the DBS generated under these circumstances.

Finally, the effect of several clinical parameters on the hematocrit prediction was investigated, including hemolysis, icterus and lipaemia, differing total protein concentration and differing hemoglobin glycation (HbA1c). Hereto, DBS were generated from samples with a hemolytic (n = 10, Hct range 0.314–0.471 L/L), icteric (n = 10; Hct range 0.238–0.523 L/L) and lipemic (n = 10; Hct range 0.232–0.472 L/L) (HIL) index above the cut-off value for which no interferences are expected in routine clinical analyses, respectively (H: 163–351, cut-off = 88; I: 6–29, cut-off = 5; L: 56–97, cut-off = 40). To evaluate the impact of total protein and HbA1c concentrations, DBS were generated from samples with varying total protein concentrations (n = 53, concentration range 32–90 g/L, Hct range 0.289–0.501 L/L) and varying HbA1c concentrations (n = 40, concentration range 4.48–12.9%, Hct range 0.231–0.486 L/L), respectively.

When two sample sets were compared, or when the results of the NIR measurements were compared to those obtained by the hematology

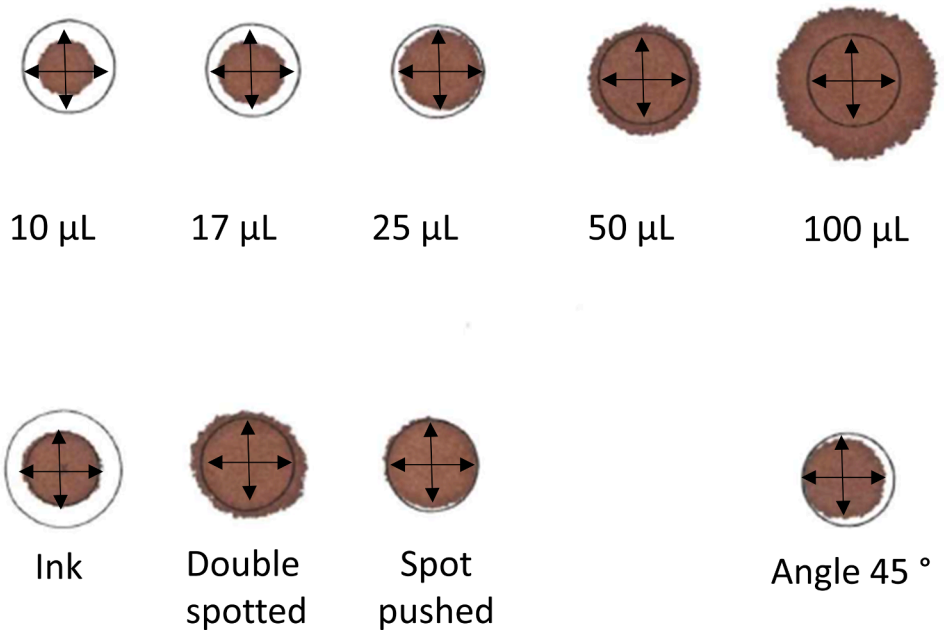

Fig. 1. Visual representation of the different robustness experiments. The first row shows DBS generated using increasing volumes (10–100 µL) of blood (Hct 0.41 L/L). The second row displays some incorrect or non-standard sampled DBS. The black arrows represent the area of the 25 µL reference spot for comparison.

analyzer, a paired sample two-sided *t*-test was performed ($\alpha = 0.05$). When two or more groups were compared to a reference set, repeated measures one-way ANOVA, with Dunnett post-hoc analysis ($\alpha = 0.05$) was used. For the analysis of the impact of varying protein and HbA1c concentrations, trend analysis using linear regression was performed.

2.6. Method comparison and application

The results obtained via NIR for the DBS validation set (n = 49; singlicate analysis), measured at Day 0 and Day 5, were compared to those obtained with the hematology analyzer, being the standard method. A Bland-Altman and mountain plot were constructed, and Deming regression was performed. For the Bland-Altman plot, the maximum allowable difference was set at ±0.050 L/L.

In addition, the method's applicability on capillary DBS (n = 36), containing no anticoagulant, was demonstrated. Here, the Hct of the liquid capillary blood sample, determined via a Hct centrifuge, was considered as the reference value. The results obtained with both methods were compared via a Bland-Altman plot.

3. Results

3.1. Calibration model

The initial model showed two issues: first, there was an unacceptable overall negative bias; second, the predicted Hct increased with longer storage times (Supplementary Fig. 4A). Therefore, additional patient samples were included in the calibration model, and the wavenumber range 5380–4968 cm^{-1} was excluded from the calibration model. In addition, a small though significant difference was found between Hct predictions from EDTA anticoagulated DBS vs non-anticoagulated DBS. Further details on the adaptations and evaluation of the calibration model are described in the Supplementary Material Section 2.1.

3.2. Validation

The method validation amply met the pre-set acceptance criteria, with a maximum total precision of 4.5% and bias of 0.012 L/L. Also storage did not relevantly affect the Hct prediction, except for storage at 60°C. Detailed results concerning the method validation can be found in the Supplementary Material Section 2.2.

3.3. Robustness

No inter-operator variability was observed, as for 2 different operators the mean differences in predicted Hct over the 49 samples were 0.011 L/L (95% CI 0.007–0.015) and 0.002 L/L (95% CI –0.002 to 0.007) compared to the Hct measured by the standard operator.

Neither the measurement location (central vs. peripheral) nor switching from Whatman 903 filter paper to Ahlström 226 paper showed a relevant difference, with respective mean differences of −0.0013 L/L (95% CI −0.015 to 0.012; p = 0.83; Fig. 2A) and −0.004 L/L (95% CI −0.015 to 0.006; p = 0.39; Fig. 2B).

Taking a 25 µL DBS as the reference, no relevant nor significant differences were found for spotted volume, except for 10 µL DBS. Although the observed difference –a decrease in predicted Hct of 0.035 L/L– was still below 0.050 L/L, it was significantly different (95% CI 0.011–0.059; p = 0.0019) (Fig. 2C). Additionally, some incorrectly or non-standard sampled DBS were generated (Fig. 2D). Only the DBS pushed after application of the blood gave a significant and relevant difference compared to the standard application of blood, with a mean difference of 0.064 L/L (95% CI 0.050–0.078 L/L; p < 0.0001).

Finally, the effect of multiple clinical parameters was evaluated. On the one hand, a high HIL-index was investigated. Only for the samples with a high lipemic index (Fig. 3C) a significant difference was found of −0.035 L/L (95% CI −0.051 to −0.019; p = 0.0006). For the icteric and hemolytic samples (Fig. 3A and B) no significant differences were found (p = 0.728 and p = 0.59, respectively). On the other hand, evaluation of varying total protein and HbA1c concentrations did not reveal a significant trend (Supplementary Fig. 9). Detailed results can be found in the Supplementary Material Section 2.3. Furthermore, the NIR spectrum of a dried plasma spot was found to be too different from the NIR spectrum of a DBS (Supplementary Fig. 10) to result in a hematocrit measurement ('out of spec' message). In contrast, the spectra of DBS with similar hematocrits but increasing HbA1c values were essentially identical (Supplementary Fig. 11).

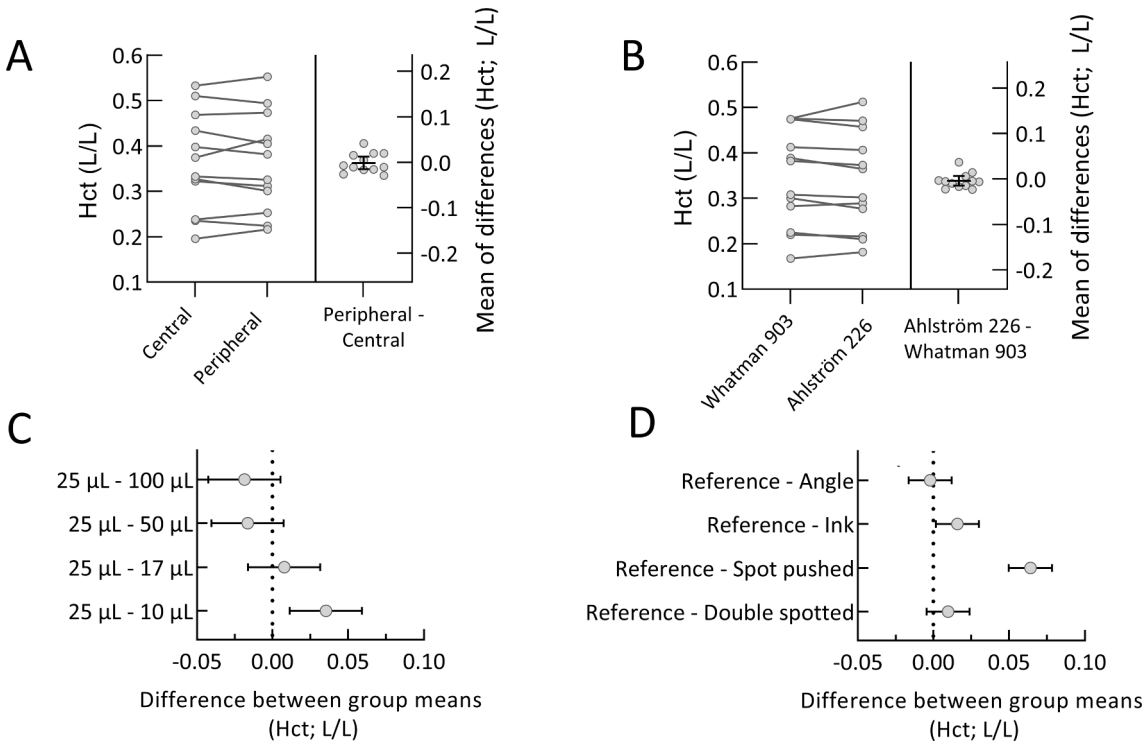

Fig. 2. Evaluation of the robustness. The effect of measurement location (A), filter paper type (B), spotted volume (C), and some non-standard sampled DBS (D) were evaluated. The mean differences and their 95% CI are indicated in graphs C and D and in the right part of graphs A and B.

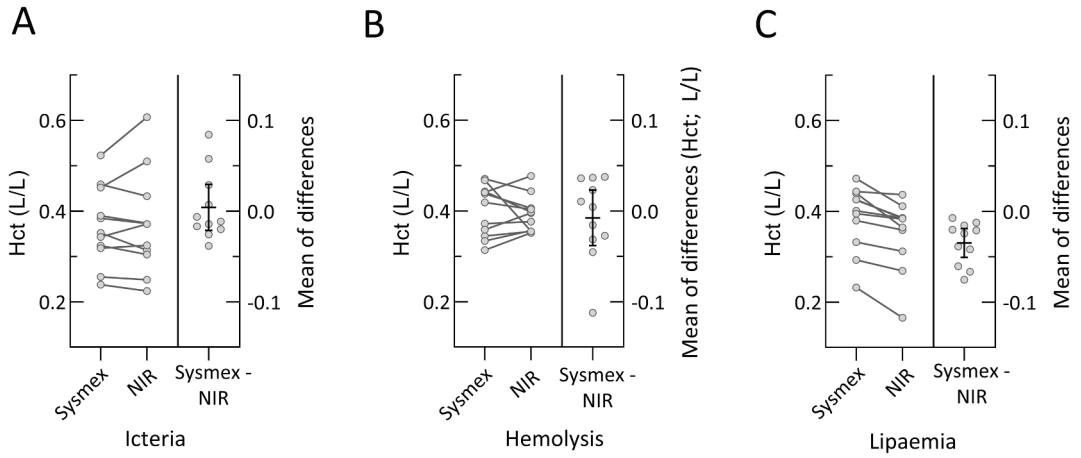

Fig. 3. Evaluation of the effect of icterus (A), hemolysis (B) and lipaemia (C) on the hematocrit prediction (n = 10 for each condition). The Hct predictions (NIR) are compared to the reference value. The black lines in the right part of the graphs indicate the mean differences and their 95% CI.

3.4. Method comparison and application

Fig. 4 shows the results of the Bland-Altman analysis, depicting the difference between the NIR-predicted Hct and the Sysmex-measured Hct. Both for the comparison with fresh (Day 0; Fig. 4A) and 5-day old DBS (Fig. 4D) the limits of agreement (LoA) lay within the maximum allowable difference of ±0.050 L/L, with only minimal mean differences (–0.010 L/L, 95% CI −0.015 to −0.005 L/L, and 0.005 L/L, 95% CI −0.0003 to 0.0094 L/L, respectively). The trend line on Day 0 shows a negative slope, indicating that the bias increases (i.e. becomes more negative, but still within acceptance limits) with an increasing Hct. This trend was less pronounced at Day 5. The mountain plots show an even distribution of the differences around the mean differences on both days, with the differences laying between –0.044 and 0.020 L/L on Day 0 (Fig. 4B) and between –0.059 and 0.033 L/L on Day 5 (Fig. 4E). The Deming regression with the results from Day 0 showed a minimal intercept, albeit significantly different from zero (0.0245; 95% CI 0.0145–0.0344) and also 1 was not included in the 95% CI of the slope (0.916; 95% CI 0.888–0.945) (Fig. 4C). The Deming regression performed with the Hct predictions from Day 5 (Fig. 4F) no longer showed a significant deviation from 1 for the slope (0.980; 95% CI 0.945–1.016). The intercept on the other hand, while still minimal, was still significantly different from 0 (0.0144; 95% CI 0.0012–0.0277).

Finally, a Bland-Altman plot was generated from the results of the analysis of liquid (heparinized) and corresponding dried capillary blood samples (NIR-based Hct prediction from DBS), obtained via finger prick (n = 36, Fig. 5A). Here, the LoA's lay at –0.092 and 0.005 L/L, and the mean difference was –0.043 L/L. This negative bias can be attributed to at least 2 factors. First, our evaluation (to be substantiated by larger panels) of capillary samples revealed that the Hct predicted from non-

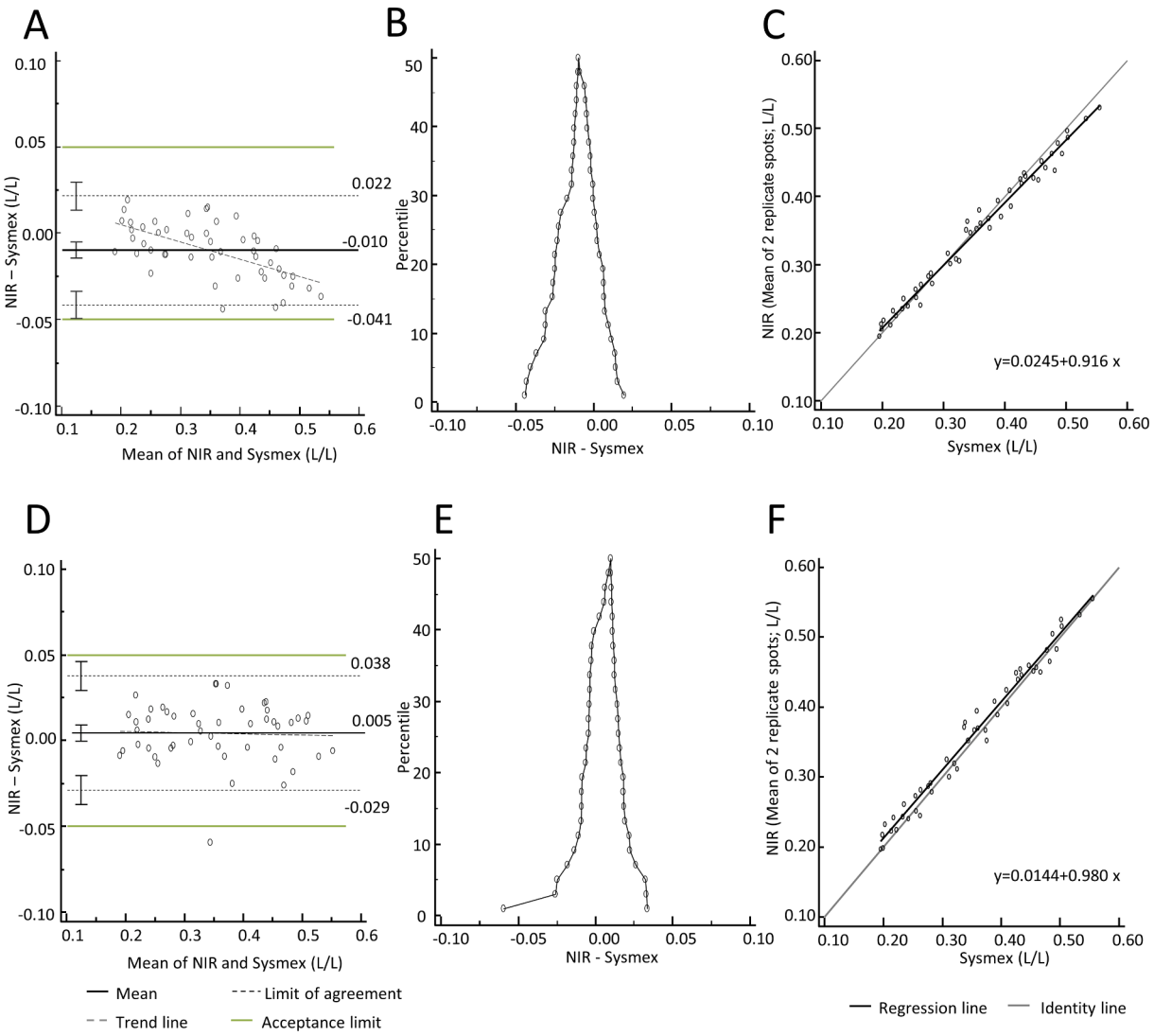

Fig. 4. Method comparison of the NIR-predicted Hct on two validation days with the standard method (Sysmex) Hct (Day 0: A, B, C and Day 5: D, E, F). Bland-Altman plot (A and D) and mountain plot showing the distribution of the differences (B and E). Deming regression analysis (C and F).

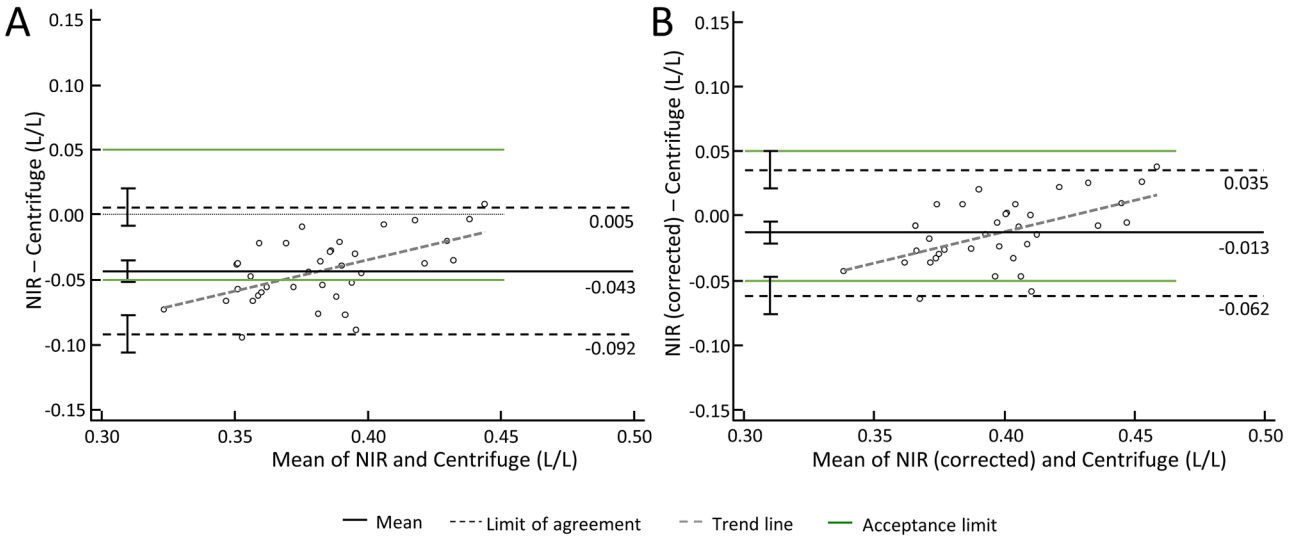

Fig. 5. Bland-Altman comparison of the Hct of capillary DBS samples predicted via NIR and the reference method (centrifugation). In panel A, the uncorrected data are presented, in panel B, a correction of 0.030 L/L was applied to the values obtained for the Hct prediction via NIR (see Section 3.4).

anticoagulated blood is slightly underestimated compared to EDTA-anticoagulated blood with a bias of −0.021 L/L, as discussed in Supplementary Material Section 2.1. The same bias may be expected here, since the calibration model was set up using EDTA-anticoagulated samples, and the samples from healthy volunteers were non-anticoagulated. Second, an additional bias of –0.009 L/L should be considered, to account for the slight overestimation of the Hct via centrifugation-based determination versus the hematology analyzer (historic validation data from the clinical laboratory from Ghent University Hospital). Arbitrarily correcting for these two sources of bias yields LoA's at −0.062 and 0.035 L/L, with a mean difference at –0.013 L/L (Fig. 5B).

4. Discussion

A calibration model, developed and evaluated by Oostendorp *et al.* and incorporated in the NIR software, was evaluated prior to validation of the instrument set-up [13]. Initially, an overall unacceptable negative bias was seen, which exceeded the acceptance criterion of ±0.050 L/L, especially in the higher Hct range (>0.50 L/L) (Supplementary Fig. 4A). This bias was not entirely unexpected, since this model was generated on another instrument several years back, and also the primary calibrator used for calibrating the NIR probe by the supplier was different. Hence, the spectra of 300 DBS (150 duplicates) were added to the model. Secondly, a time-dependent bias was observed, both in the evaluation of the initial and the updated model (Supplementary Fig. 4A and B). Analysis of some NIR spectra revealed a wavenumber range (5380–4968 cm^{-1}) with a higher variation in reflectance over time compared to the other ranges. This range reflects a typical range in the NIR spectrum for H_2O/OH^- overtones [19], and hence a differential presence of water in the samples due to their different storage time may explain this variation. In the recent report by van de Velde *et al.* a partially overlapping wavenumber range (5800–5000 cm^{-1}) was also excluded from the calibration model, for the same reason [14]. After exclusion of this wavenumber range, the Hct predictions showed less variation over time, yielding the final calibration model.

Next, the number of samples added to the calibration model was evaluated (Supplemental Tables 2 and 3). Except for the model without additional samples, all models still yielded validation results within the acceptance criteria, and, hence, less samples (30 duplicate DBS) may suffice to update the calibration model and reduce the bias, if needed. In any case, upon implementation of the instrument set-up it should be evaluated if the method performance is fit-for-purpose.

Furthermore, when using spectroscopic methods, re-evaluation of the method performance on a regular basis can be necessary, e.g. after movement of the system to another place, after maintenance/repair, or when transferring the calibration model to another NIR spectrometer. This can easily be done by adding the spectra of a small set of additional patient samples spread over the Hct range.

Finally, prior to starting the method validation, a preliminary evaluation of the effect of the used anticoagulant was performed, which revealed a slight, though significant, difference in predicted Hct between non- and EDTA-anticoagulated DBS, the latter being on average 0.021 L/L higher (Supplementary Material Sections 1.2 and 2.1). However, more samples are needed to substantiate this difference. The same observation (i.e. lower values obtained from non-anticoagulated blood) was made in the preliminary evaluation by Lange *et al.*, who also suggested to further investigate the influence of EDTA on the Hct prediction [20].

The method validation, which was entirely performed using patient samples, demonstrated that accurate and precise (maximal bias was 0.012 L/L, maximal total CV was 4.5%) results can be obtained with a currently commercially available NIR set-up. The performance of the method is comparable to that of other published methods for the non-destructive Hct prediction from DBS [10,14].

Although the Deming regression analysis showed that there were systematic and proportional differences between the evaluated NIR method and the reference method, these differences were small and no correction of the predicted Hct or adaptation of the calibration model is needed. This is also reflected in the Bland-Altman plots, comparing the results from the validation sample set on Day 0 and Day 5 with the standard method which yielded LoA's smaller than the pre-set maximum allowable differences of ±0.050 L/L. These limits were chosen based on the purpose of the method: to give an estimation of the Hct, to then (i) allow correction of an analytical result, (ii) evaluate whether the Hct is within a pre-defined range, or (iii) convert DBS-based results to plasma or serum values.

In the second part of the study, we demonstrated the pre-analytical robustness of the method. First, sample storage did not relevantly affect the Hct prediction of the DBS, except for DBS stored at 60°C. However, for TDM purposes, it is unlikely that samples will be exposed to these extreme conditions. As samples destined for TDM are usually not stored for a long period of time prior to analysis, stability upon storage was limited to 1 month. Importantly, also storage without a desiccant still yielded reliable results, which is relevant in case of home-sampling, where the addition of a desiccant may be forgotten. Also shorter drying times did not markedly influence the Hct prediction, which would allow to analyze samples on the day of the sample collection, as may be the case in a hospital context. However, the drying time is ideally longer than 2 h, otherwise an underestimation may be present for low-Hct DBS.

In addition, the method is very robust towards several aspects including measurement location and filter paper. Also, spotted volumes of 17 μL and more yielded reliable results. Only a volume of 10 μL resulted in a slight underestimation of the Hct, which may be attributed to the filter paper being less saturated, and/or to a suboptimal positioning of the DBS under the NIR probe, due to its small area. For finger prick samples, it is our experience that a typical DBS will exceed a volume of 10 μL, but one could opt to use pre-printed circles defining the minimal area that a DBS should cover. For devices like the Hemaxis DB 10, which volumetrically generates 10 μL DBS on classical DBS cards [21] this underestimation might be relevant, although the mean bias was only 0.035 L/L.

The analysis of samples with a high HIL-index showed that only lipaemia had a significant effect on the Hct predictions. The mean difference was 0.035 L/L, which was considered acceptable.

Since hemoglobin is a protein, we evaluated whether other proteins present in plasma (e.g. albumin and globulin) may affect the recorded spectrum and, consequently, the Hct prediction. However, no relevant trend was observed across a protein concentration range of 32–90 g/L. This was further supported by the observation that the spectrum obtained from a plasma sample is only similar to the spectrum of a whole blood sample to a certain extent (Supplemental Fig. 10).

Furthermore, the effect of hemoglobin glycation on the Hct prediction was also investigated, as in literature it has already been reported that glycation may have an impact on the NIR spectrum obtained from nail clippings (e.g. keratin vs glycated keratin in nails) [22]. In the range of 4.48–12.9% HbA1c no relevant effects were seen. Moreover, the spectra of samples with similar hematocrit but increasing HbA1c values were almost identical, which further supports this result (Supplemental Fig. 11).

Finally, different unconventional applications of blood onto the filter paper were also evaluated. Again, the method proved robust against these elements. Of note, and importantly, the NIR approach did yield a different (i.e. lower) result for DBS which had been pressed after application of the blood. Such tampering is sometimes seen when insufficient instructions or training is given to the person collecting the DBS samples, who are instructed to "fill the pre-printed circle" and do so by increasing the area that is covered by a drop of blood. Evidently, a differential spreading of the blood, which is the case when pressure is exerted to the filter paper, will affect both the amount of analyte per area and the Hct prediction, since the volume of blood spread over a certain

area changes (in this case decreases). Knowledge of the Hct may thus allow correction for this tampering.

The potential for real-life use of the technology, was further revealed by demonstrating its applicability on capillary samples. The original Bland-Altman plot of the capillary DBS samples revealed a mean bias of −0.043 L/L compared to the reference method. Following correction for two sources of bias, this bias was reduced to −0.013 L/L. In an attempt to completely remove the bias, the model was re-evaluated to search for differences in the NIR spectrum between non- and EDTA-anticoagulated DBS, and exclude these from the calibration model. However, no important differences could be identified. Therefore, as from a practical point-of-view it is virtually impossible to set-up a calibration model using non-anticoagulated samples, we propose –and implemented here– an arbitrary correction factor for the Hct prediction in non-anticoagulated DBS. To confirm this correction factor (provisionally set at 0.030 L/L), an even more extensive set of capillary liquid blood samples and corresponding DBS should be collected, covering a Hct range as wide as possible. Besides the observation of a bias in capillary samples, the span between the upper and lower LoA was wider compared to that found in the Bland-Altman plots of the validation sample sets. This increase could be expected, as the validation was performed with DBS generated via pipetting a fixed volume onto the filter paper under well controlled circumstances, while more variation can be expected upon collecting capillary DBS. Nonetheless, eventually, 34/36 Hct predictions from capillary DBS differed less than 0.050 L/L from the reference Hct value, demonstrating the potential of the technology for Hct prediction of DBS generated from capillary, non-anticoagulated blood. In future, more samples, preferably covering a wider Hct range, may be used to further confirm the application on capillary DBS.

5. Conclusion

After updating the initially incorporated calibration model, a commercially available NIR set-up was successfully validated, yielding acceptable accuracy and precision results for NIR-based Hct prediction of DBS. Stability experiments showed that DBS can be stored up till 1 month at RT or lower temperatures without a relevant effect on the Hct prediction, even without the presence of a desiccant. We also demonstrated that the required drying time before analysis can be reduced to 4 h, instead of the currently advised 24 h, which allows to perform Hct prediction on the same day of sampling. Furthermore, the method proved very robust towards several aspects such as the operator, the measurement location, the volume spotted and some incorrectly or non-standard sampled DBS. Finally, the method was applied on capillary DBS samples obtained via finger prick, demonstrating its application potential in real-life settings.

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Competing interests

Dr. Christoph Lühr is an employee of BÜCHI Labortechnik GmbH (Essen, Germany). The research presented here was independently conducted at Ghent University, without any steering by BÜCHI Labortechnik or Dr. Lühr. As Dr. Lühr did provide essential scientific input related to data analysis and troubleshooting, the lead author (C. Stove) invited him to be co-author on this paper, in line with proper scientific conduct. None of the other authors has any competing interest.

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Ethical approval

Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' Institutional Review Board (EC2018/0519).

Informed consent

Informed consent was obtained from all healthy volunteers included in this study. For the use of left-over samples the Ethics Committee granted an exemption for the informed consent.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2021.10.002.

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