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Letters to the Editor

Measurement of Hematocrit in Dried Blood Spots Using Near-Infrared Spectroscopy: Robust, Fast, and Nondestructive

To the Editor:

Dried blood spot (DBS)[1] collection is an established sampling method for new born screening and is increasingly used in other domains, including therapeutic drug monitoring, toxicology, microbiology, and genetics. Advantages of DBS sampling are the low blood volume requirements, minimally invasive collection, favorable stability of many analytes, and the potential of patient self-sampling at home. Importantly, the introduction of more sensitive techniques in the clinical laboratory has paved the way for analysis of small volume DBS samples in clinical chemistry *(1)*.

A well-recognized concern for accurate analyte quantification using DBS is the hematocrit (Ht) effect *(2)*. First, Ht influences blood viscosity and thereby fluidity of blood on the paper. This can lead to different volumes of blood in equally sized punches and to varying analyte concentrations within the spot, resulting in a potential bias. Second, Ht may influence analyte extraction from the paper. Third, many analytes in clinical chemistry are currently measured in plasma and/or serum, whereas DBS samples are whole blood lysates. Therefore, for many compounds, Ht is necessary to accurately convert DBS measurements to plasma/serum values.

Previously proposed techniques to estimate Ht in DBS quantified intracellular potassium and hemoglobin content as surrogates for Ht *(3–5)*. Although the former can easily be performed on routine chemistry analyzers, it is destructive and may lack the accuracy required for routine application. The latter uses ultraviolet-visible spectrometry, but requires correction for altered coloration caused by hemoglobin oxidation over time.

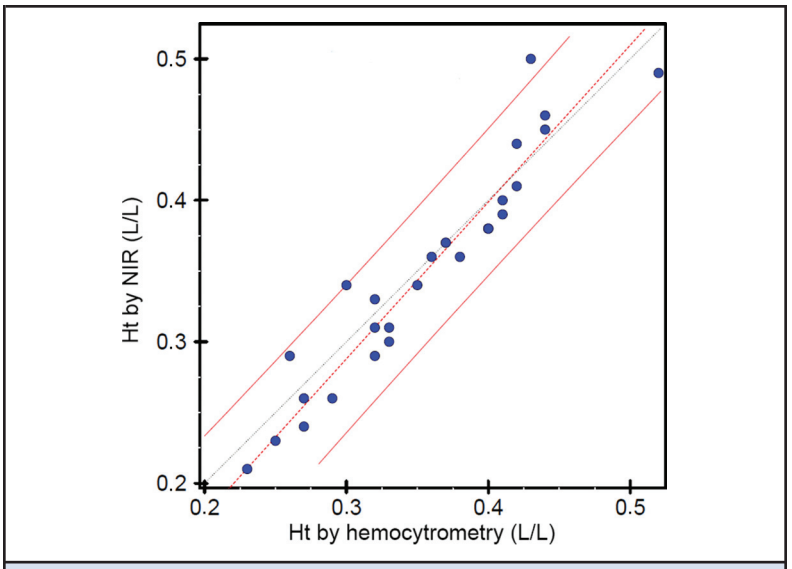

Fig. 1. Scatter plot showing the Ht values as determined using the NIR method vs the reference method on the Celldyn Sapphire hemocytometer.

The Deming regression line with 95% CIs (red) and the identity line (black) are also shown. The slope and intercept were 1.11 (95% CI, 0.97–1.25) and −0.044 (95% CI, −0.095 to 0.007), respectively.

Here, we describe a near-infrared (NIR) spectroscopy method to measure Ht in DBS. NIR is nondestructive, fast, and robust, because it is a spectroscopic technique based on molecular overtone and combination vibrations of covalent bonds. Therefore, NIR is considered a feasible candidate to measure Ht in DBS.

All Ht results are expressed in L/L. NIR measurements were performed on a NIRFlex N-500 spectrometer equipped with a fiber optics solids cell N500–007 (Büχ Labortechnik AG,). The CellDyn Sapphire hemocytometer (Abbott Diagnotics) was used as reference method. DBS samples were prepared by spotting 1 drop of venous EDTA blood in duplicate on Whatman 903 cards. Dry time was 1–10 days. Samples from 261 patients (male/female: 132/129; ages 0–95 years) with variable Ht values (0.15–0.60) were used to design the NIR model. Samples were equally distributed over 9 Ht groups, patient age and sex, and DBS dry time. All specimens underwent a QC step including a visual inspection for homogenous coloration. Spectra were obtained in triplicate at wavelengths of 4500–10000 cm^{-1} with a 4 cm^{-1} resolution. The NIR model was created using a partial least squares algorithm incorporated in the Büχ NIRcal 5.5 software (model characteristics: R^2 calibration = 0.983; R^2 validation = 0.979; SE of calibration = 0.017; standard error of prediction = 0.015). The calibration and validation sets contained 1031 and 509 spectra, respectively.

To evaluate assay reproducibility, DBS with Ht values of 0.20, 0.35, and 0.50 were prepared in 6-fold. Ht was measured using NIR after 1, 2, 6, 7, 8, and 9 days of dry-

[1] Nonstandard abbreviations: DBS, dried blood spots; Ht, hematocrit; NIR, near-infrared spectroscopy.

ing. Assay performance was further investigated by comparing 30 random patient samples (Ht range 0.23–0.52).

The NIR assay showed excellent reproducibility and accuracy with overall coefficients of variation of 6.0%, 3.2%, and 2.8% at mean Hts of 0.20, 0.33, and 0.46, respectively. Fig. 1 shows the Ht in patient samples determined using NIR vs hemocytometry. A good correlation was obtained with a Pearson's correlation coefficient of 0.95. Deming regression showed a slope and intercept of 1.11 (95% interval 0.97–1.25) and −0.044 (95% interval −0.095–0.007) respectively, indicating that NIR results are comparable with the reference method. Drying time, albumin concentration, age, and sex were not identified as significant covariates.

As NIR is based on vibrations of covalent bonds, the NIR signal most likely reflects protein content (vibrations in peptide bonds). Because hemoglobin is the most abundant protein in whole blood lysates, it thereby reflects the Ht level of the sample. Our NIR method indeed allowed accurate measurement of Ht in DBS samples with good reproducibility. Other major advantages of NIR are the short analysis time and the fact that it is nondestructive, allowing measurement of other components from the same spot. Furthermore, actual patient samples were used to create the calibration model. This is contrary to previous studies *(3–5)*, in which Ht series were prepared by centrifuging blood from a single donor and mixing cells and plasma in the desired ratio.

In conclusion, NIR facilitates a fast, robust and nondestructive quantification of Ht in DBS. The proposed NIR method has great promise to tackle the Ht problem of DBS and may help to bring this patient friendly sampling technique closer to routine application in clinical chemistry.

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